BEFORE THE DEPARTMENT OF ENVIRONMENTAL QUALITY
OF THE STATE OF MONTANA

In the matter of ARM 17.30.602(32) and 17.30.632(7)(a) pertaining to the definition of "steady state" and site-specific water column water quality criteria	))))	PETITION FOR RULEMAKING

INTRODUCTION

Pursuant to Mont. Code Ann. § 2-4-315, the Lincoln County Board of County Commissioners ("Lincoln County") submits this Petition for Rulemaking to DEQ in light of new information and data that changes the water column water quality criteria in the water quality standard for Lake Koocanusa under ARM 17.30.632(7)(a) and the definition of "steady state" under ARM 17.30.602(32).[1]

I. Identity of Petitioner Lincoln County

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II. Interests of Petitioner Lincoln County

Lincoln County has a cognizable interest in ensuring fair, balanced, and scientifically defensible water quality standards in Lake Koocanusa. Lake Koocanusa is located in Lincoln County. Lincoln County is a political subdivision of the State of Montana. The Board of County Commissioners of Lincoln County is granted all legislative, executive, and administrative

[1] On April 19, 2022, the Montana Board of Environmental Review decided that ARM 17.30.632(7)(a) violated § 75-5-203, MCA. Notwithstanding, this Petition refers to the water column water quality criteria by reference to ARM 17.30.632(7)(a) for the sake of clarity without regard to its current validity or invalidity.

powers and duties of the local government not specifically reserved by law or ordinance to other elected officials. § 7-3-401, MCA. The Board of County Commissioners has jurisdiction and power to represent the County and has care of the County property and the management of the business and concerns of the County and to perform all other acts and functions which may be necessary to the full discharge of the duties of the chief executive authority of the county government. § 7-5-2101, MCA. Lincoln County is committed to both environmental stewardship and fostering a business-friendly climate. Lincoln County believes these objectives are not mutually exclusive and can be achieved through balanced, science-based regulation.

Lincoln County is comprised of approximately 75% federal and state land, limiting its tax base and local control over regulatory decisions. The County depends on the viability of local industries—particularly mining, timber, ranching, and agriculture—for economic stability and job creation. However, the industries that historically employed many Lincoln County residents have declined sharply, resulting in the loss of quality middle-class jobs while housing costs and other living expenses have simultaneously increased substantially. Overly restrictive environmental regulation compounds these economic challenges by deterring new business investment in the region and placing additional burdens on existing industries. Lake Koocanusa, treasured for its economic and recreational value, is at the center of this regulatory concern.

For example, mining, timber, ranching, and agriculture all involve some component of land disturbing activities that may be regulated through the Clean Water Act ("CWA") and affected by water quality standards in Lake Koocanusa. Any National Pollutant Discharge Elimination System ("NPDES") Permit issued to one of these industries may require strict water quality based effluent limitations rather than the more flexible and less expensive technology based effluent limitations. 33 U.S.C. § 1311(b)(1)(c)-(d). Even without a permitted point source activity, the CWA requires states to manage non-point source discharges that may cause or contribute to violations of state water quality standards. 33 U.S.C. § 1329(a)(1). The state non-point source management plan often results in imposing strict controls on the industries that support Lincoln County's economy as a result of water quality standards in receiving waters. *See e.g.* 2017 Montana Nonpoint Source Management Plan, p. 1-7.

These regulatory mechanisms to implement water quality standards in Lake Koocanusa effect Lincoln County's ability to meet its policy objectives. Accordingly, Lincoln County has a cognizable interest in a fair, balanced, and scientifically defensible water quality standard for Lake Koocanusa.

III. Support for Petition and Reason for the Proposed Action

A. Support for Amendment of 17.30.632(7)(a)

Since DEQ's initial rulemaking to develop a site-specific selenium water quality standard for Lake Koocanusa, the U.S. Environmental Protection Agency ("EPA") has published critical

new technical support and implementation guidance[2] [3], and new fish egg data from Lake Koocanusa confirm that tissue standards are not exceeded. To have the most robust, up-to-date and scientifically-defensible standard, this new information must be accounted for in a new water column water quality criteria for Lake Koocanusa. Copies of the cited reference materials are enclosed with this Petition for Rulemaking as Appendices A-P and incorporated herein by this reference.

The EPA[4] identifies two instances where it recommends that a state develop a site-specific water column criterion element for selenium. The first is for fishless waters (not applicable to Lake Koocanusa); and the second is when ambient water column selenium concentrations are meeting the national CWA section 304(a) recommended water column criterion, but the fish tissue concentrations are greater than the national recommended fish tissue criterion (also not applicable to Lake Koocanusa, as explained below). In such an instance, the elevated fish tissue concentrations may indicate an impact on the aquatic system and a water column criterion element lower than the national CWA section 304(a) recommended water column criterion element is needed to protect aquatic life. Based upon new data and EPA technical guidance, neither of these instances are applicable to Lake Koocanusa. Therefore, a water column water quality criteria lower than the CWA section 304(a) national criteria is not recommended by EPA or appropriate in this instance.

In 2024, the EPA issued technical guidance for fish tissue monitoring and data acceptability when implementing the 2016 selenium criterion. The fish tissue monitoring guidance document published in 2024 underscores the importance of sampling mature eggs for comparison against the national criteria[5] and that the egg/ovary criterion element was derived directly from toxicity data using mature eggs and served as the basis for deriving all the other criterion elements[6] [7]. The 2024 EPA fish tissue monitoring guidance document confirms that egg/ovary tissue as defined within the national criterion refers to "mature eggs, pre-spawn ovary tissue that contains mature eggs, or both"; and that ovary tissue sampled from a female that is not gravid[8] will not be representative of selenium concentrations. Recent peer reviewed literature[9] confirms this approach (*i.e.,* that it is imperative to perform analysis of mature eggs to allow for comparisons with egg selenium guidelines and/or criteria because selenium toxicity thresholds are established based on concentrations measured in mature eggs).

The fish egg/ovary data available to DEQ during rulemaking were not of gravid females. The Montana Department of Fish, Wildlife and Parks has confirmed that they never sampled a

[2] United States Environmental Protection Agency. 2024. Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards. EPA 820-R-24-001. April 2024.
[3] *Id.*
[4] *Id.*
[5] *Id.*
[6] United States Environmental Protection Agency. 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. EPA 822-R-16-006. June 2016.
[7] United States Environmental Protection Agency. 2021. 2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. 21-006. August 2021.
[8] Gravid – a female fish that is distended with or full of eggs.
[9] Palace, V., S. Graves, and J. Brandt. 2024. Guidance on assessing the potential impacts of selenium in freshwater ecosystems. Integrated Environmental Assessment and Management 2024:1–16 DOI: 10.1002/ieam.4969. Accepted: 21 May 2024.

gravid fish from Lake Koocanusa[10]; and the United States Geological Survey has also confirmed that fish reproductive tissue was a data gap[11] during model development in the initial rulemaking.

Review of egg/ovary data available at the time of rulemaking identifies that although egg/ovary data collected were collected within established spawning windows for Lake Koocanusa[12], samples were not collected consistent with DEQ's fish tissue sampling standard operating procedure[13] or the 2018 fish tissue quality assurance project plan for Lake Koocanusa.[14] Consistent with EPA 2024 fish tissue monitoring guidance, ovary data that is not collected from a gravid fish will not be representative of selenium concentrations and should not be compared against the national criterion. However, since the initial rulemaking, 50 individual fish egg samples have been collected from gravid females from Lake Koocanusa and are publicly available from the Water Quality Portal.[15]

Selenium fish egg concentrations measured from Lake Koocanusa in these 50 new individual fish tissue samples are well below the egg/ovary tissue standard of 15.1 mg/kg dw, see below figure. Average selenium egg concentrations in redside shiner are equal to 7.6 mg/kg dw (sample year = 2019; n = 14), and average selenium egg concentrations in northern pikeminnow are equal to 3.7 mg/kg dw (sample years = 2019 and 2022; n = 36). These new data confirm that average egg selenium concentrations are well below the tissue standard of 15.1 mg/kg dw.[16]

In addition to measured egg data, there are new peer reviewed models that can be used to estimate mature egg concentrations using fish muscle tissue data.[17] These models offer the ability to use the over 2,700 individual fish tissue muscle samples collected from Lake Koocanusa since 2002 to estimate egg concentrations and compare against the egg/ovary tissue

[10] Selch, Trevor. 2022. Oral Testimony provided at the February 2022 Water Policy Interim Committee HJ37 Work Group Meeting.
[11] Kilpatrick, John. 2022. Oral Testimony provided at the February 2022 Water Policy Interim Committee HJ37 Work Group Meeting.
[12] Montana Department of Environmental Quality. 2024. Selenium Assessment Method for Lake Koocanusa and the Kootenai River in Montana - Public Comment Draft. WQDMASWQA-01, Version 1. July 2024.
[13] Montana Department of Environmental Quality. 2015. Fish Tissue Sampling Standard Operating Procedure. WQPBWQS-29.
[14] Montana Fish, Wildlife & Parks and Montana Department of Environmental Quality. 2018. FWP/DEQ Fish Tissue Quality Assurance Project Plan for Lake Koocanusa. WQSMQAP-02. April 30, 2018.
[15] The Water Quality Portal (WQP) integrates publicly available water quality data from the USGS National Water Information System (NWIS) and the EPA Water Quality Exchange (WQX) Data Warehouse. https://www.waterqualitydata.us/
[16] At the time of rulemaking, DEQ believed that egg/ovary tissue data were greater than the tissue criterion of 15.1 milligrams per kilograms dry weight basis (mg/kg dw). In 2020, DEQ identified three redside shiner egg/ovary samples as exceeding the EPA 304(a) criterion of 15.1 mg/kg dw along with one peamouth chub sample. In 2022, DEQ stated that it relied on data from nine (9) individual fish spanning three (3) species with egg/ovary selenium concentrations greater than the tissue criterion. The new data and guidance documents confirm that these conclusions are inaccurate.
[17] Detering C, K.V. Brix, M. Adzic, B.A. Fulton, and D.K. DeForest. 2025. Relationships in selenium concentrations among fish tissues to support selenium assessments and regulations. Environmental Toxicology and Chemistry. DOI: 10.1093/etojnl.vgaf045. Advanced access publication February 27, 2025.

criterion element. Consistent with DEQ's 2020 findings, fish tissue selenium concentrations in muscles and whole-body continue to remain below their respective fish tissue criterion elements.

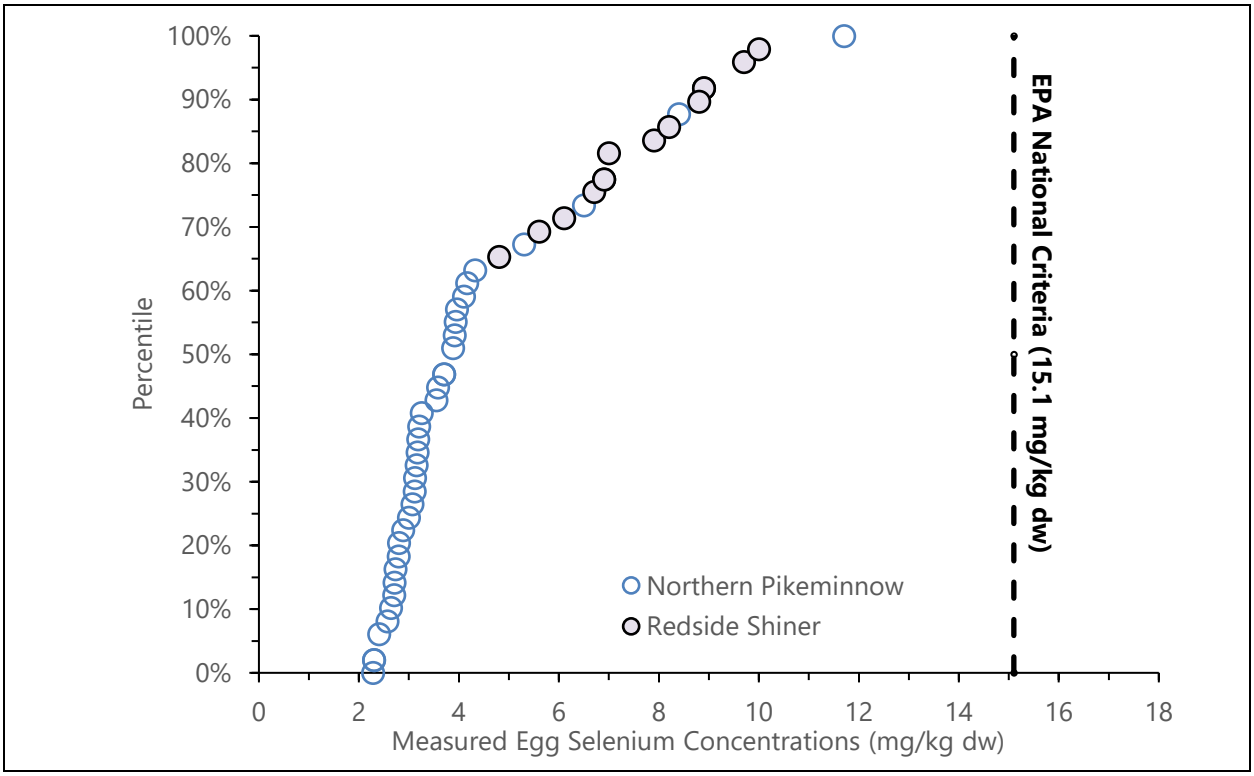


Figure. Measured Egg Selenium Concentrations in Lake Koocanusa Relative to the National Criteria of 15.1 mg/kg dw.

In summary, there are new data that confirm fish tissue selenium concentrations including egg/ovary data are below the national recommended fish tissue criteria elements, while ambient water column selenium concentrations also continue to be below the national CWA section 304(a) recommended water column criterion of 1.5 µg/L. As a result, and consistent with the 2024 EPA guidance,[18] there is no need to develop a site-specific water column criterion element more stringent than the recommended national criterion. A Water Column Standard of 1.5 µg/L for Lake Koocanusa is protective of aquatic resources and can be confirmed by using the empirical bioaccumulation factor approach for deriving a site-specific water column criterion element.

Predicted protective water column values for Lake Koocanusa using empirical data and the bioaccumulation factor approach are all greater than the national CWA section 304(a) recommended water column criterion of 1.5 µg/L, see below figure.

[18] EPA. 2024. Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards. EPA 820-R-24-001. April 2024.

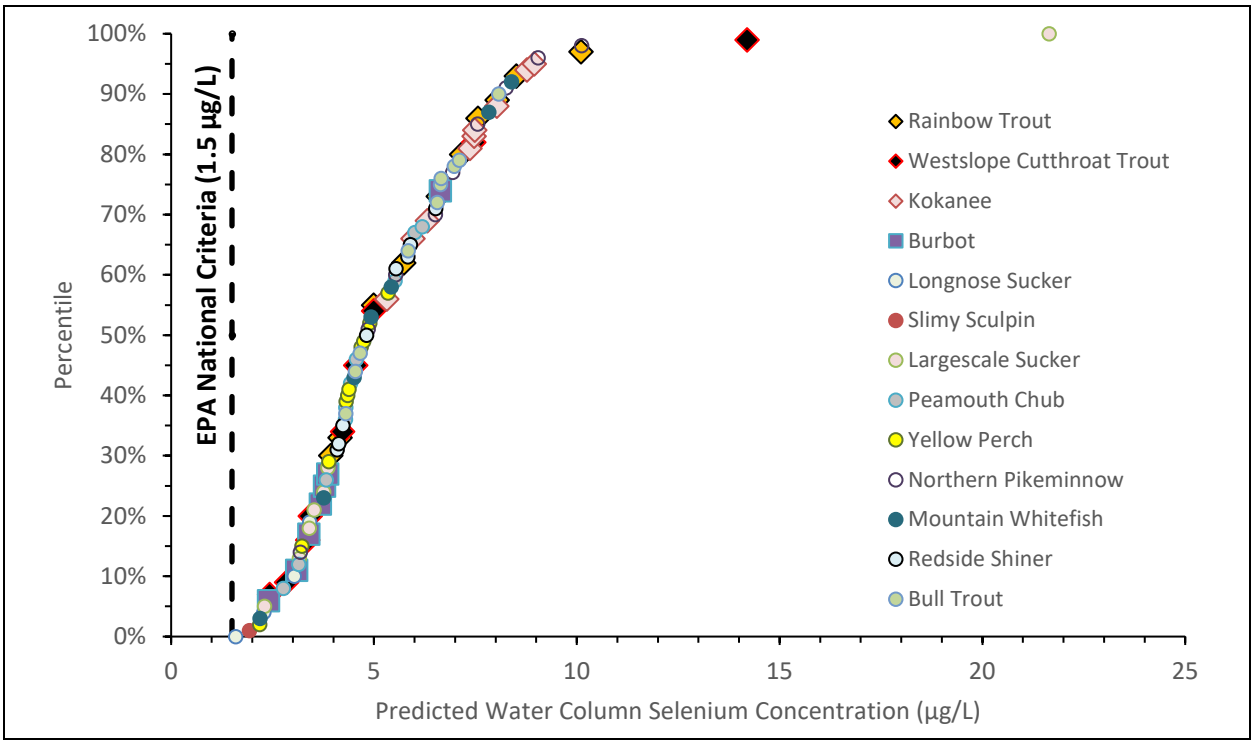


Figure. Predicted Protective Water Column Values for Lake Koocanusa using the Empirical Bioaccumulation Factor Approach.

Note: The minimum calculated protective water column value using the empirical bioaccumulation factor approach is 1.6 µg/L.

B. Support for Amendment of 17.30.602(32)

At the time of initial rulemaking, the 2016 EPA criterion document did not include a definition of "steady state," but did outline that fish tissue elements of the selenium criterion supersede water column elements under steady state conditions because the selenium concentrations in fish tissues are a more sensitive and reliable indicator of the negative effects of selenium in aquatic life.[19] This was further clarified by Ms. Judy Bloom of EPA Region 8 to DEQ, noting that the language from the EPA 2016 criterion document was intended to address the scenario where fish tissue data are not exceeding respective criterion elements, but the water column data are exceeding that element.[20]

During the initial rulemaking and as outlined in Part A above, DEQ was under the impression (based on the data available to it at the time) that fish tissue egg/ovary data exceeded the tissue criterion element, while the other tissue elements and the water column data did not.

[19] EPA. 2021. 2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. 21-006. August 2021.

[20] Judy Bloom, Manager, Clean Water Branch. U.S. Environmental Protection Agency, Region 8. February 25, 2021. Ref: 8WP-CWQ. Letter to Steven Ruffatto, Chair, Montana Board of Environmental Review, Montana Department of Environmental Quality.

Under that scenario, EPA accepted DEQ's definition of steady-state. Since then, EPA has provided further guidance and a definition for steady state.

As outlined within 2024 EPA guidance documents, for the purposes of the national CWA section 304(a) recommended selenium criterion, EPA refers to steady-state conditions "where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing."[21] [22]

EPA guidance further clarifies that "new inputs" as outlined within the 2016 criterion document are "both new and increased inputs of selenium" and refers "to the release of a substantial amount of additional selenium from either anthropogenic point or nonpoint sources." New or increased selenium inputs "do not refer to seasonal variability of selenium that occurs naturally within a system (e.g., spring run-off events or precipitation-driven pulses) or de minimis discharges. Furthermore, the EPA notes that "if selenium inputs are decreasing in a waterbody, the hierarchy of the criterion should apply, and fish tissue criterion elements should take precedence for CWA implementation. Therefore, the EPA recommends using the fish tissue criterion elements for the CWA implementation of WQC based on the national CWA section 304(a) recommended criterion when selenium inputs to a waterbody or the watershed of a waterbody are decreasing."

The inclusion of "or changing" language with the definition of steady state as set out in ARM 17.30.602(32) does not meet the intent of the 2016 EPA criterion document and implementation of the recommended selenium criterion under the CWA. The "or changing" language means both decreasing and increasing, and therefore should be updated to only state "or increasing" to make it consistent with the national criterion and 2024 EPA guidance.

Further, selenium concentrations at the mouth of Elk River are neither new nor increasing.[23] [24] This can be demonstrated by applying the Weighted Regression on Time, Discharge and Season model using the EGRET (version 3.0.7) package, as described in Storb *et al.* (2023) with current (*i.e.*, post-2022) flow and selenium concentration data, see below figure.

[21] EPA. 2024. Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards. EPA 820-R-24-001. April 2024.
[22] EPA. 2024. Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits. EPA-820-R-24-002. April 2024.
[23] Storb, Meryl B., Bussell, Ashley M., Caldwell Eldridge, Sara L., Hirsch, Robert M., and Schmidt, Travis S. 2023. Growth of Coal Mining Operations in the Elk River Valley (Canada) Linked to Increasing Solute Transport of Se, NO_3^-, and SO_4^{2-} into the Transboundary Koocanusa Reservoir (USA–Canada). Environ. Sci. Technol. 2023, 57, 45, 17465–17480.
[24] Terry, Kyle. 2017. Selenium Loading to Lake Koocanusa. Technical memorandum submitted to Mike Sokal of BC Ministry of Environment and Climate Change Strategy. October 20, 2017.

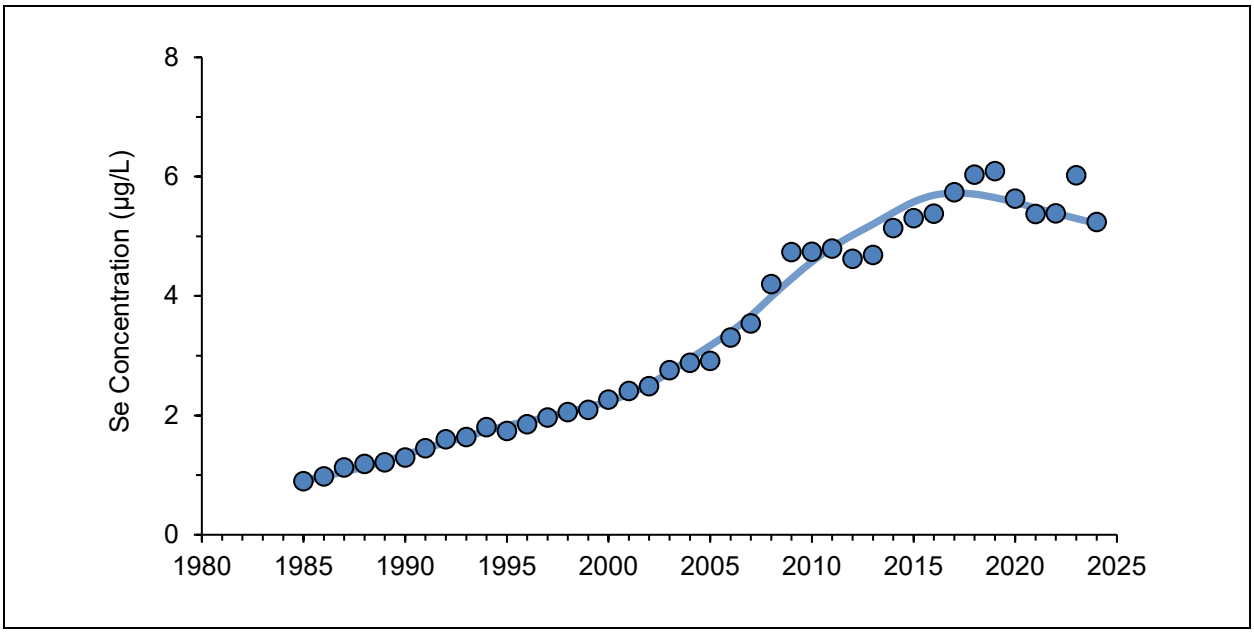


Figure. Flow-normalized selenium concentration trends at the Elk River through 2024.

Recent water quality data and trend analyses using publicly available models (Storb *et al.*, 2023) confirm that selenium concentrations from sources into Lake Koocanusa are decreasing and, consistent with EPA 2024 guidance, fish tissue criterion elements should take precedence for CWA implementation. As a result, the scenario contemplated by DEQ in the initial rulemaking for whether Lake Koocanusa was in steady-state is no longer applicable.

C. Summary

In sum, new data confirm fish tissue selenium concentrations including egg/ovary data are below the national recommended fish tissue criteria elements; while ambient water column selenium concentrations also continue to be below the national CWA section 304(a) recommended water column criterion of 1.5 µg/L. As a result, and consistent with the 2024 EPA guidance,[25] a site-specific water column criterion element more stringent than the recommended national criterion is not necessary to protect aquatic resources. A water column water quality criteria more stringent than the recommended national criterion frustrates Lincoln County's ability to meet its policy objectives. A Water Column Standard of 1.5 µg/L for Lake Koocanusa is protective of aquatic resources and can be confirmed by using the empirical bioaccumulation factor approach for deriving a site-specific water column criterion element.

Furthermore, new guidance from EPA supports an amendment of the definition of "steady state" due to the trend of decreasing selenium concentrations from sources into Lake Koocanusa. An amendment concluding that Lake Koocanusa met steady-state conditions would result in fish tissue elements of the selenium criterion superseding water column elements

[25] EPA. 2024. Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards. EPA 820-R-24-001. April 2024.

because the selenium concentrations in fish tissues are a more sensitive and reliable indicator of the negative effects of selenium in aquatic life[26] [27].

IV. Proposed Rule

The proposed rules are as follows (new matter underlined and deleted matter interlined):

ARM 17.30.602 DEFINITIONS

(32) "Steady state" means, for the purposes of ARM 17.30.632, conditions whereby there are no activities resulting in new~~,~~ or increasing~~, or changing~~ selenium loads to the lake or river aquatic ecosystem, and selenium concentrations in fish living in the aquatic ecosystem have stabilized.

ARM 17.30.632 SELENIUM STANDARDS FOR LAKE KOOCANUSA AND THE KOOTENAI RIVER

(7) Water column standards are the numeric standards for total dissolved selenium computed as a 30-day average, and shall not be exceeded more than once in 3 years, on average.

(a) Lake Koocanusa from the US-Canada international boundary to the Libby Dam: 1.5 ~~0.8~~ µg/L.

(b) Kootenai River mainstem from the outflow below the Libby Dam to the Montana-Idaho border: 3.1 µg/L.

CONCLUSION

Lincoln County respectfully requests DEQ to Grant its Petition for Rulemaking to account for new data and guidance that changes the outcome for the water column water quality criteria in ARM 17.30.632(7)(a) and the definition of steady state in ARM 17.30.602(32).

Dated this 2nd day of June, 2025

Aimee Hawkaluk [signature]

Aimee Hawkaluk [name]

On behalf of the Board of County Commissioners of Lincoln County, Montana

[26] EPA. 2021. 2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. 21-006. August 2021.

[27] EPA. 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. EPA 822-R-16-006. June 2016.

Appendix A

United States Environmental Protection Agency
Office of Water 4304T
EPA 822-R-16-006 June 2016

Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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NOTICE

This document has undergone a contractor-led external expert peer-review, as well as an EPA review process following publication and public comments received on the May 14, 2014, and July 28, 2015 criteria drafts. Final review by the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, has been completed, and the document has been approved for publication.

This document provides guidance to States and Tribes authorized to adopt water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of selenium. Under the CWA, States and Tribes are to adopt water quality criteria to protect designated uses. State and tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. While this document contains EPA's scientific recommendations regarding ambient concentrations of selenium that protect aquatic life, it does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally-binding requirements on EPA, States, Tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. EPA may change this document in the future. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

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FOREWORD

Section 304(a)(l) of the Clean Water Act requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document presents EPA's updated chronic ambient water quality criterion (AWQC) for the protection of aquatic life based upon consideration of all available information relating to effects of selenium on aquatic organisms. EPA has incorporated revisions into this final document based on comments from the general public and an external expert peer review panel on an earlier draft published in the Federal Register in May 14, 2014, and comments from the general public on a second draft published in the Federal Register in July 28, 2015.

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(l) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criterion presented in this document is such a scientific assessment. If water quality criteria associated with specific designated uses are adopted by a state or authorized tribe as water quality standards under section 303, and approved by EPA, they become applicable Clean Water Act water quality standards in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. However, states and authorized tribes may adopt water quality criteria that reflect adjustments to EPA's recommended section 304 criteria to reflect local environmental conditions and human exposure patterns. Alternatively, states and authorized tribes may derive numeric criteria based on other scientifically defensible methods but the criteria must be protective of designated uses. It is not until their adoption as part of state or tribal water quality standards, and subsequent approval by EPA, that criteria become Clean Water Act applicable water quality standards. Guidelines to assist the states and authorized tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994, as updated), which along with additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

This document provides guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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ACRONYMS

AE	Assimilation Efficiency
AWQC	Ambient Water Quality Criteria
BAF	Bioaccumulation Factor
CF	Conversion Factor
CV	Chronic Value (expressed in this document as an EC10)
CWA	Clean Water Act
dw	Dry Weight
ECx	Effect Concentration at X Percent Effect Level
EF	Enrichment Factor
EPA	Environmental Protection Agency
EO	Egg Ovary
FCV	Final Chronic Value
GMCV	Genus Mean Chronic Value
HNOEC	Highest No Observed Effect Concentration
IR	Ingestion Rate
k_e	Rate of selenium loss
k_u	Rate of selenium uptake
LOEC	Lowest Observed Effect Concentration
M	Muscle
MATC	Maximum Acceptable Toxicant Concentration (expressed mathematically as the geometric mean of the NOEC and LOEC)
MDR	Minimum Data Recommendations or Requirements
NPDES	National Pollutant Discharge Elimination System
NOEC	No Observed Effect Concentration
SMCV	Species Mean Chronic Value
SSD	Species Sensitivity Distribution
TMDL	Total Maximum Daily Load
TRAP	EPA's Statistical Program: Toxicity Relationship Analysis Program
TTF	Trophic Transfer Factor
WB	Whole body
WQBELS	Water Quality-based Effluent Limitations
WQC	Water Quality Criteria
WQS	Water Quality Standards
ww	Wet Weight

EXECUTIVE SUMMARY

This document sets forth the basis for and derivation of the Clean Water Act, Section 304(a) water quality criterion for protecting freshwater aquatic life from harmful effects of selenium, a naturally occurring chemical element that is nutritionally essential in small amounts, but toxic at higher concentrations. This assessment provides a critical review of all data identified in EPA's literature search quantifying the toxicity of selenium to freshwater aquatic organisms, and provides a basis for a criterion that will assure protection of populations of fish, amphibians, aquatic invertebrates, and plants, based on available data.

Although selenium may cause acute toxicity at high concentrations, the most deleterious effect on aquatic organisms is due to its bioaccumulative properties; these chronic effects are found at lower concentrations than acute effects. Organisms in aquatic environments exposed to selenium accumulate it primarily through their diets, and not directly through water (Chapman et al. 2010). The best science also indicates that selenium toxicity occurs primarily through transfer to the eggs and subsequent reproductive effects. Consequently, in harmony with the recommendations of expert panels (U.S. EPA 1998; Chapman et al. 2010) and with peer review and public comments on previous U.S. EPA (2004, 2014, 2015) drafts, the Agency developed a chronic criterion reflective of the reproductive effects of selenium concentrations on fish species.

The 2016 "Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater, 2016," is a chronic criterion that is composed of four elements. All elements are protective against chronic selenium effects. Two elements are based on the concentration of selenium in fish tissue and two elements are based on the concentration of selenium in the water column. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent element to account for potential chronic effects from short-term exposures (one value for lentic and one value for lotic aquatic systems). The assessment of the available data for fish, invertebrates, and amphibians indicates that a criterion value derived from fish will protect the aquatic community. All four criterion elements applied together should protect aquatic life from the chronic effects of exposure to total selenium in waters inhabited by fish, as well as "fishless waters."

Because the factors that determine selenium bioaccumulation vary among aquatic systems, site-specific water column criterion element values may be necessary at aquatic sites with high selenium bioaccumulation to ensure adequate protection of aquatic life (Appendix K). Finally, this freshwater chronic selenium criterion applies only to aquatic life, and is not intended to address selenium toxicity to aquatic-dependent wildlife such as aquatic-dependent birds.

The toxicity studies relevant to the derivation of the fish tissue selenium criterion elements involve (a) extended duration dietary exposure, and (b) measurement of total selenium in the tissue of the target organism. Selenium either in fish whole-body or in muscle is usually measured in non-reproductive studies, and selenium in eggs or ovaries is typically measured in reproductive studies. Selenium accumulation in the eggs of the exposed adult female prior to spawning has been shown to yield the most robust relationship (statistically significant) with occurrence of deformities and reduced survival of the offspring.

The outcome of assessing both reproductive and non-reproductive studies under laboratory and field conditions led EPA to the conclusion, consistent with expert consensus (Chapman et al. 2009, 2010), that reproductive effects, linked to egg-ovary selenium concentrations, provide the most sound basis for the criterion compared to non-reproductive (e.g., survivorship, growth) endpoints. Reproductive effects have been linked to observed reductions in the populations of sensitive fish species in waterbodies having elevated concentrations of selenium (Young et al. 2010). EPA applied the sensitivity distribution concepts from the U.S. EPA *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses* (Stephan et al. 1985) to derive the national selenium criterion. Based on the available data, expressed as EC_{10} values, the egg-ovary criterion element concentration is 15.1 milligrams selenium per kilogram dry weight (mg Se/kg dw), based primarily on 17 reproductive studies representing 10 fish genera.

EPA recommends states and tribes adopt all four elements of the criterion into their water quality standards. Two elements are based on the concentration of selenium in fish tissue (eggs or ovaries, and whole-body or muscle) and two elements are based on the concentration of selenium in the water column (a 30-day chronic element and an intermittent exposure element). Both water column elements are further refined into values for lentic waters (e.g., lakes and impoundments) and lotic waters (e.g., rivers and streams). The difference between lentic and lotic water column values reflect the observed difference in selenium bioaccumulation in these

two categories of aquatic systems (ATSDR 2003; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). EPA derived the intermittent exposure element based on the chronic 30-day water column element and the fraction of any 30-day period during which elevated selenium concentrations occur. EPA recommends the intermittent element to address short-term exposures that contribute to chronic effects through selenium bioaccumulation (e.g., releases from storage ponds or other intermittent releases). EPA derived the values for the water-column criterion elements from the egg-ovary element by assessing food-chain bioaccumulation based on available data collected at lentic and lotic systems in the continental United States. Thus, all four criterion elements are based on reproductive effects in freshwater fish.

EPA primarily used field studies in freshwater systems to provide quantitative estimates of selenium bioaccumulation in particulate material (algae, detritus, and sediment) from water, and used field observations and laboratory data to quantify and model the trophic transfer of selenium from particulate material into invertebrates, and from invertebrates into fish. EPA additionally used field and laboratory observations to assess species-specific selenium partitioning between different tissues within a fish (whole-body, eggs and/or ovaries, and muscle). EPA developed food web models of fish in aquatic systems with a range of bioaccumulation potentials and used the food web models with the species-specific estimates of trophic transfer (or the most proximate taxonomic surrogate when species-specific data was not available) to develop water column criterion elements from the egg-ovary criterion element for lotic and lentic aquatic systems. EPA validated this approach using selenium measurements from aquatic systems with a range of bioaccumulation potentials. Similar approaches could be used in the derivation of selenium criteria in saltwater or estuarine systems with selenium data and food webs relevant to those systems.

While more than half the available chronic studies were fish studies, available field data and laboratory toxicity studies suggest that a criterion based on fish will protect amphibians, aquatic invertebrates, and plants since these taxa appear to be less sensitive to selenium than fish (see **Sections 3.1.4** and **6.1.4**).

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg/Ovary [2]**	**Fish Whole Body or Muscle [3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. Water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and fint is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

The recommended chronic selenium criterion is expected to protect the entire aquatic community, including fish, amphibians, and invertebrates, based on available data. Because fish are the most sensitive to selenium effects, EPA recommends that selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) data take precedence over the criterion elements based on water column selenium data due to the fact, noted above, that fish tissue concentrations provide a more robust and direct indication of potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium

bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) "fishless" waters, and 2) areas with new selenium inputs.

For purposes of this document, EPA defines "fishless waters" as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (e.g., extirpation) due to temporary or permanent changes in water quality (e.g., selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas. Appendix K of this criterion document discusses approaches to develop a site-specific water column criterion element in such situations.

For purposes of this document EPA defines "new inputs" as new activities resulting in the release of selenium into a lentic or lotic aquatic system. New inputs will likely result in a greater concentrations of selenium in the food web and a relatively slow increase in the selenium concentration in fish until the new selenium release achieves a quasi-"steady-state" balance in the aquatic system. EPA estimates that the concentration of selenium in fish tissue will not reach steady state for several months in lotic systems and longer time periods (e.g., 2 to 3 years) in lentic systems. Achievement of steady state in an aquatic system also depends on the hydrodynamics of the aquatic system, (particularly reservoirs with multiple riverine inputs), the location of the selenium input and the particular food web. EPA expects the time needed to achieve steady state with new or increased selenium inputs to be site specific. Thus, EPA recommends that fish tissue criterion elements not take precedence over the water column criterion elements until the aquatic system achieves steady state. In the interim, EPA recommends sampling and using site-specific data to determine steady state in the receiving water to gain a better understanding of the selenium bioaccumulation dynamics in a given system.

EPA recommends states and tribes adopt into their water quality standards a selenium criterion that expresses the four elements as a single criterion composed of multiple parts in a manner that explicitly affirms the primacy of the whole-body or muscle element over the water column elements, and the egg-ovary element over any other element. Adopting the fish whole-

body or muscle tissue element into water quality standards ensures the protection of aquatic life when measurements from fish eggs or ovary are not available, and adopting the water column element ensures protection when fish tissue measurements are not available.

EPA recommends that when states adopt a four-part criterion for selenium reflecting EPA's recommended criterion, states use the default monthly average exposure water column elements of the criterion, adopted as part of the state's water quality criterion when implementing the criterion under the National Pollutant Discharge Elimination System (NPDES) permits program and to assist with implementation of other Clean Water Act programs. Alternatively, states may want to develop adopt, and submit for EPA approval, either a site-specific water column criterion element (or set of lentic/lotic criterion element values), or a set of procedures to facilitate the translation of the fish tissue criterion concentration elements into site-specific water concentration values. A site-specific water column criterion element or set of lentic/lotic criterion element values can be developed using a mechanistic modeling approach (Presser and Luoma 2010) or using the empirical bioaccumulation factor approach, both described in Appendix K, for the specific waterbody or waterbodies. Any translation procedure must be scientifically defensible, produce repeatable, predictable outcomes, and result in criterion element values that protect the applicable designated use. Examples of such procedures include the mechanistic modeling approach and the empirical BAF approach described in Appendix K.

This recommended selenium criterion applies to freshwater lentic and lotic systems, as it is based on the toxicity of selenium to freshwater organisms. A similar approach may be appropriate for deriving criteria for selenium in estuarine and marine waters if appropriate data are available.

1 INTRODUCTION AND BACKGROUND

The objective of the Clean Water Act (CWA) is to "restore and maintain the chemical, biological and physical integrity of the Nation's waters." One of the tools that EPA uses to meet this objective is the development of recommended ambient water quality criteria (AWQC) under section 304(a)(1) of the Act. As provided for by the Clean Water Act, EPA reviews and from time to time revises 304(a)(1) AWQC to ensure the criteria are consistent with the latest scientific knowledge. Section 304(a) aquatic life criteria serve as recommendations to states and authorized tribes for defining ambient water concentrations that will protect against adverse ecological effects to aquatic life resulting from exposure to a pollutant found in water from direct contact or, ingestion of contaminated water and/or food. Aquatic life criteria address the Clean Water Act goals of providing for the protection and propagation of fish and shellfish. When adopted into state or tribal water quality standards (WQS), these criteria can become a basis for establishing National Pollutant Discharge Elimination System (NPDES) program permit limits and, the basis for listing impaired waters under Section 303(d) and establishing Total Maximum Daily Loads (TMDLs).

1.1 HISTORY OF THE EPA RECOMMENDED SELENIUM AWQC FOR AQUATIC LIFE

In 1980 EPA first published numeric aquatic life criteria for selenium in freshwater. These criteria were based on water-only exposure (no dietary exposure). In order to address the lack of consideration of bioaccumulation in the 1980 selenium criteria, in 1987 EPA published updated selenium criteria to address field-based toxicity observed in aquatic ecosystems at levels below the existing criteria values. The 1987 criteria were field-based and accounted for both the water column and dietary uptake pathways manifested at Belews Lake, North Carolina (USA), a cooling water reservoir where water quality and fish communities had been affected by selenium loads from a coal-fired power plant. At that time EPA also provided an acute criterion of 20 µg/L derived from a reverse application of an acute-chronic ratio obtained from conventional water-only exposure toxicity tests applied to the 5 µg/L chronic value based on dietary and water column exposure in Belews Lake.

In 1998-1999 EPA published a revised acute criterion, a formula that recognized that the two oxidation states, selenate and selenite, appeared to have substantially different acute

toxicities. This acute criterion assumed toxicity was based on water-only exposure. Subsequent research has demonstrated that sulfate levels influence selenate toxicity in water-only exposures.

In 1998 EPA held a peer consultation workshop (EPA-822-R-98-007) to evaluate new science available for selenium relevant to the selenium aquatic life criterion. EPA concluded, and the peer reviewers agreed, that fish-tissue values more directly represent chronic adverse effects of selenium than the conventional water concentration approach used by EPA to protect aquatic life, because chronic selenium toxicity is primarily based on the food-chain bioaccumulation route, not on a water column route of exposure.

In 2004 EPA published a draft chronic whole-body fish-tissue criterion with a water-based monitoring trigger in the summer and fall. The critical effect considered at that time was the impact on survivorship based on overwintering stress to bluegill sunfish. An acute criterion was estimated at that time that addressed concerns with the species of selenium present and adjusted for sulfate levels; however, it did not address the dietary uptake pathway.

Further refinement of the fish tissue approach occurred in 2009 based on the findings of a Pellston scientific workshop on the ecological risk assessment of selenium (Chapman et al. 2009, 2010). As presented by Chapman et al. (2009), some key findings resulting from that workshop are:

- Diet is the primary pathway of selenium exposure for both invertebrates and vertebrates.
- Traditional methods for predicting toxicity on the basis of exposure to dissolved [water column] concentrations do not work for selenium because the behavior and toxicity of selenium in aquatic systems are highly dependent upon site-specific factors, including food web structure and hydrology.
- Selenium toxicity is primarily manifested as reproductive impairment due to maternal transfer, resulting in embryotoxicity and teratogenicity in egg-laying vertebrates.

In this 2016 final recommended freshwater chronic criterion for selenium, EPA includes revisions based on the public and external expert peer reviews of the 2014 draft, public comments on the 2015 draft, data and information from additional studies provided by the public and peer reviewers, and additional scientific analyses. EPA also conducted a new literature review and reanalyzed data considered in the 2004 and 2009 draft criteria documents. This final criterion reflects the latest scientific consensus (e.g., Chapman et al. 2010) on the reproductive

effects of selenium on aquatic life and their measurement in aquatic systems and supersedes all previous national aquatic life water quality criteria for selenium.

EPA is recommending a national selenium criterion expressed as four elements. All elements are protective against chronic selenium effects, and account for both short term and longer term exposure to selenium. Two elements are based on the concentration of selenium in fish tissue (eggs and ovaries, and whole-body or muscle) and two elements are based on the concentration of selenium in the water-column (two 30-day chronic values and an intermittent value). EPA derived the 30-day chronic water column element from the egg-ovary element by modeling selenium bioaccumulation in food webs of lotic and lentic aquatic systems. EPA is recommending the intermittent value to address short-term exposures that could contribute to chronic effects through selenium bioaccumulation in either lotic or lentic systems. EPA derived the intermittent element based on the chronic 30-day water column element and the fraction of any 30-day period during which elevated selenium concentrations occur. These water column criterion elements apply to the total of all oxidation states (selenite, selenate, organic selenium, and any other forms) (See Appendix L for Analytical Methods for Measuring Selenium). Aquatic communities are expected to be protected by this chronic criterion from any potential acute effects of selenium.

2 PROBLEM FORMULATION

Problem formulation provides a strategic framework for water quality criteria development by focusing the effects assessment on the most relevant chemical properties and endpoints. The structure of this effects assessment is consistent with EPA's Guidelines for Ecological Risk Assessment (U.S. EPA 1998).

This ecological effects assessment defines a scientifically-defensible water quality criterion for selenium under section 304(a)(1) of the Clean Water Act. Clean Water Act Section 304(a)(1) requires EPA to develop criteria for water quality that accurately reflect the latest scientific knowledge. These criteria are based solely on high quality data and best professional scientific judgments on toxicological effects. Criteria are developed following overarching guidance outlined in the Agency's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985), hereafter referred to as "EPA Ambient Water Quality Criteria Guidelines." States and authorized tribes may adopt EPA's recommended criteria into their water quality standards to protect designated uses of water bodies, they may modify EPA's criteria to reflect site-specific conditions, or they may derive criteria using other scientifically-defensible methods, all subject to EPA review and approval.

2.1 OVERVIEW OF SELENIUM SOURCES AND OCCURRENCE

Selenium is a naturally occurring element present in sedimentary rocks and soils. It is also present in the aquatic environment as methyl derivatives of selenium, naturally occurring in freshwaters through methylation by bacteria (Ranjard et.al. 2003). Selenium's occurrence in surficial soils and aquatic sediments in the United States is illustrated in **Figure 2.1**. There are around 40 known selenium-containing minerals, some of which can have as much as 30% selenium, but all are rare and generally occur together with sulfides of metals such as copper, zinc and lead (Emsley 2011). Sedimentary rocks, particularly shales, have the highest naturally occurring selenium content (Burau 1985). The distribution of organic-enriched, sedimentary shales, petroleum source rocks, ore deposits, phosphorites, and coals, in which selenium typically co-occurs, is well characterized in the United States (Presser et al. 2004). Natural weathering of selenium-bearing geologic strata containing selenium can lead to selenium leaching into groundwater and surface water. Two major anthropogenic activities are known to

cause increased selenium mobilization and introduction into aquatic systems. The first is the mining of metals, minerals and refinement and use of fossil fuels; the second is irrigation of selenium-rich soils.

Mining activities bring selenium-enriched deposits to the surface, where they are exposed to physical weathering processes. The release of selenium related to resource extraction activities is most common in the phosphate deposits of southeast Idaho and adjacent areas of Wyoming, Montana, and Utah, and in coal mining areas in portions of West Virginia, Kentucky, Virginia, and Tennessee (Presser et al. 2004). Where selenium-containing minerals, rocks, and coal are mined, selenium can be mobilized when rock overburden and waste materials are crushed, increasing the surface area and exposure of material to weathering processes. Selenium contamination of surface waters can also occur when sulfide deposits of iron, uranium, copper, lead, mercury, silver, and zinc are released during the mining and smelting of these metal ores. Where coal is burned for power production, selenium can enter surface waters as drainage from fly-ash ponds and fly-ash deposits on land (Gillespie and Baumann 1986). Fly ash deposits have a high surface area to volume ratio, resulting in rates of selenium in leachate several times higher than from the parent feed coal (Fernández-Turiel et al. 1994). The refining of crude oil containing high levels of selenium can also be a major source of loading in certain water bodies (Maher et al. 2010).

Irrigation of selenium-rich soils for crop production in arid and semi-arid regions of the country can mobilize selenium and move it off-site in surface water runoff or via leaching into ground water. Where deposits of Cretaceous marine shales occur, they can weather to produce high selenium soils; such soils are present in many areas of the western U.S. (Lemly 1993c). Selenium is abundant in the alkaline soils of the Great Plains, and some ground waters in California, Colorado, Kansas, Oklahoma, South Dakota and Wyoming contain elevated concentrations of selenium due to weathering of and leaching from rocks and soils. In semi-arid areas of the West, irrigation water applied to soils containing soluble selenium can leach selenium. The excess water (in tile drains or irrigation return flow) containing selenium can be discharged into basins, ponds, or streams. For example, elevated selenium levels at the Kesterson Reservoir in California originated from agricultural irrigation return flow collected in tile drains that discharged into the reservoir (Ohlendorf et al. 1986).

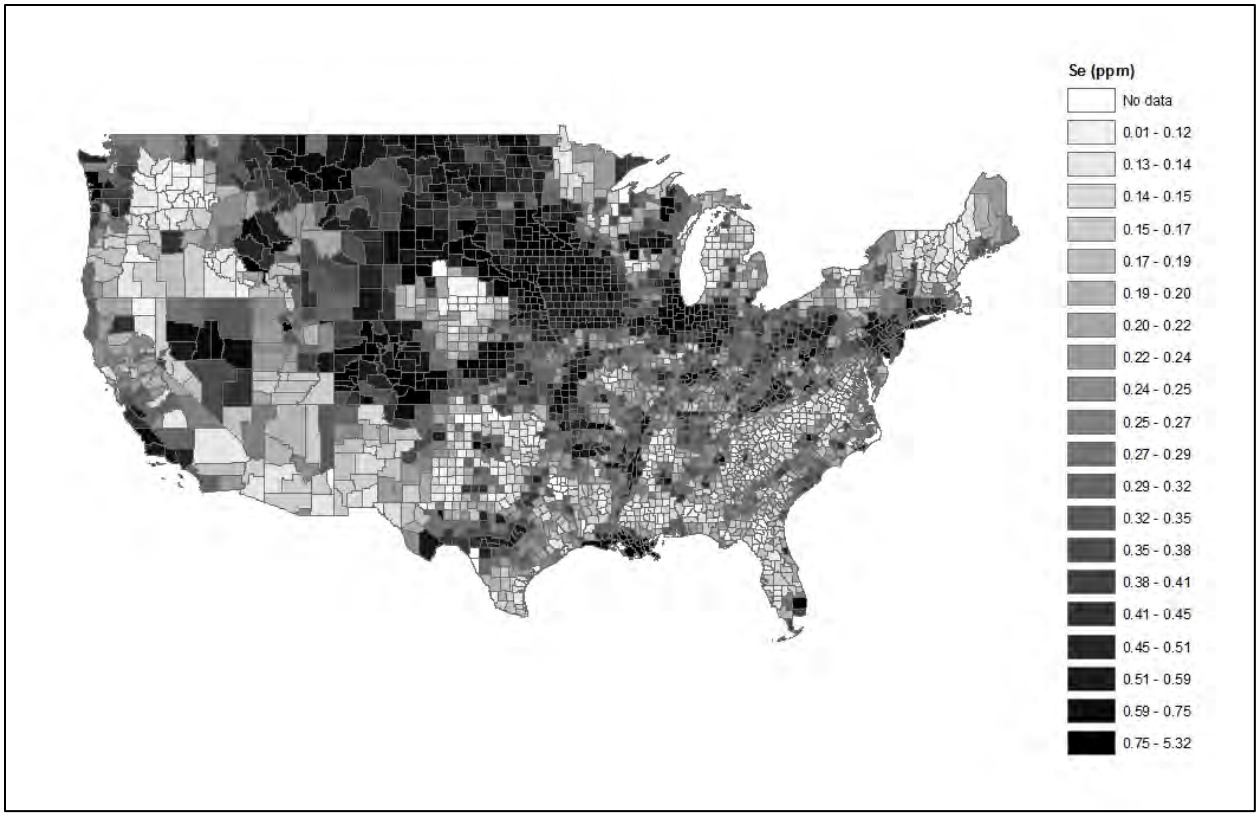


Figure 2.1. Selenium in Surficial Soils and Aquatic Sediments in counties of the Conterminous United States.
U.S. Geological Survey Open-File Report 2004-1001. URL: http://mrdata.usgs.gov/geochem/doc/averages/countydata.htm. Data are available from: http://mrdata.usgs.gov/geochem/doc/groups-cats.htm.

Atmospheric emissions of selenium can originate from several sources including power plants and other facilities that burn coal or oil, selenium refineries that provide selenium to industrial users, base metal smelters and refineries, resource extraction industries, milling operations, and end-product manufacturers (e.g., semiconductor manufacturers) (ATSDR 2003). Airborne selenium particles can settle either on surface waters or on soils from which selenium can be further transported and deposited into water bodies through ground or surface water conveyances or runoff.

The chemical form of selenium that dominates a location is usually dependent on its sources, effluent treatments, and biogeochemical processes in the receiving waters. Irrigation activities in areas with seleniferous soils typically mobilize selenate (SeO_4^{2-}, or Se[VI]) (Seiler et al. 2003). Combustion of coal for power generation creates predominantly selenite (SeO_3^{2-}, or Se[IV]) in the fly ash waste due to the temperatures, pH, and redox conditions involved with the

process (Huggins et al. 2007). Similar conditions during refinement of crude oil can also result in high concentrations of selenite relative to selenate, as was observed in the San Francisco Bay estuary in the 1980s (Cutter 1989). Although selenite is the dominant species in the discharges resulting from crude oil refining and coal burning using conventional technologies, the implementation of alternative treatment technologies can alter the relative concentrations of selenate and selenite. For example, in scrubbers with forced oxidation systems that produce strong oxidizing conditions and high temperatures, the majority of discharged selenium is in the form of selenate (Maher et al. 2010). However for flue gas desulfurization systems that are the inhibited oxidation type, the selenium chemistry is more complex, and selenite may not be the primary form emitted (Petrov et al. 2012). **Table 2.1** shows the predominant form of selenium that is associated with different activities and industries.

EPA's Office of Water and Office of Research and Development conducted the first statistically based survey of contaminants in fish fillets from U.S. rivers from 2008 through 2009. This national fish survey was conducted under the framework of EPA's National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers (Lazorchak et al. 2014). During June through October of 2008 and 2009, field teams applied consistent methods nationwide to collect samples of fish species commonly consumed by humans at 541 randomly selected river locations ($\geq 5^{th}$ order based on 1:100,000-scale Strahler order) in the lower 48 states. They collected one composite fish sample at every sampling location, with each composite consisting of five similarly sized adult fish of the same species from a list of target species. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. Samples were collected from both non-urban (379 sites) and urban locations (162 sites). Each fillet composite sample was homogenized and analyzed using an ICP-MS (Inductively Coupled Plasma- Mass Spectrometry) method for total selenium, and results were reported as wet weight. Three of the 541 samples (approximately 0.6%) exceeded the 2016 criterion for muscle tissue, 11.3 mg/kg dw. The maximum value detected was 17.75 mg Se/kg dw muscle, the median was 1.90 mg Se/kg dw, and the minimum 0.41 mg Se/kg dw.

Table 2.1. Predominant Chemical Forms of Selenium in Discharges Associated with Different Activities and Industries.

Selenium Form	Sources
Selenate	Agricultural irrigation drainage Treated oil refinery effluent Mountaintop coal mining/ valley fill leachate Copper mining discharge
Selenite	Oil refinery effluent Fly ash disposal effluent Phosphate mining overburden leachate
Organoselenium	Treated agricultural drainage (in ponds or lagoons)

Source: Presser and Ohlendorf 1987; Zhang and Moore 1996; Cutter and Diego-McGlone 1990.

2.2 Environmental Fate and Transport of Selenium in the Aquatic Environment

The fate and transport of selenium in aquatic systems is affected by the distribution of selenium species and their transformations in water, sediment, and biota. These transformations include the assimilation and conversion of inorganic selenium to organic selenium species in plants and microbes that are transferred to higher trophic level consumer species throughout the aquatic food web.

2.2.1 Selenium Species in Aquatic Systems

Aquatic organisms are exposed to a combination of predominantly organic selenium species present in the food web throughout their life history; reproductive effects integrate these exposures to transformed inorganic and organic species of selenium. The bioavailability and toxicity of selenium depend on both its concentration and speciation (Cutter and Cutter 2004; Meseck and Cutter 2006; Reidel et al. 1996). Selenium exists in four oxidation states (VI, IV, 0, -II) and in a wide range of chemical and physical species across these oxidation states (Doblin et al. 2006; Maher et al. 2010; Meseck and Cutter 2006). Therefore, in the effects assessment that follows, we have correlated the adverse effects on aquatic life with total dissolved selenium.

In oxygenated surface waters, the primary dissolved selenium species are selenate (SeO_4^{2-}, or Se[VI]) and selenite (SeO_3^{2-}, or Se[IV]), as well as dissolved organic selenides (-II) formed from fine particulate organic matter (e.g., Doblin et al. 2006; Meseck and Cutter 2006). The relative abundance of selenate and selenite depends on relative contributions from the

geologic and anthropogenic sources of selenium to the receiving waters, as there is negligible inter-conversion between the two species (e.g., Maher et al. 2010). Aqueous selenite is more abundant than selenate when the majority of selenium originates from discharges from coal fly ash tailings or oil refineries (e.g., Cutter 1989; Huggins et al. 2007). Particulate species in the water column include selenate, selenite, and elemental selenium (Se(0)) bound to resuspended sediments and organic particles, as well as particulate organic selenium species incorporated into suspended detritus (e.g., Cutter and Bruland 1984; Meseck and Cutter 2006).

In sediments, selenate and selenite can be reduced to iron selenides or elemental selenium under abiotic or biotic processes; elemental selenium and selenides can be converted to selenate under oxidizing conditions (Maher et al. 2010). For example, selenate can be reduced to elemental selenium in sediments (e.g., Oremland 1990) in the presence of iron oxides (Chen et al. 2008) and iron sulfides (Breynaert et al. 2008). Elemental selenium and organic selenides are produced by selenate-reducing microbes in sediments. Overall, the reduction of selenate and particularly selenite in sediments increases with increasing sediment organic matter (Tokunaga et al. 1997). Selenite in particular is readily bound to iron and manganese oxy-hydroxides (Maher et al. 2010), and is readily adsorbed to inorganic and organic particles, particularly at a lower pH range (e.g., McLean and Bledsoe 1992; Tokungawa et al. 1997). Microbial reduction of selenite to organic forms (via methylation) increases the solubility and bioavailability of selenium (Simmons and Wallschlägel 2005). Plants and algae produce volatile selenium species by biomethylation of excess selenium, which upon reaching the sediment can be transformed to a more bioavailable species, or deposited in the sediments and effectively removed from the system (Diaz et al. 2009). Depending on environmental conditions, the reduction processes described above are largely reversible, as elemental selenium and selenides in sediments can be oxidized to selenate through microbial or abiotic transformations (e.g., Maher et al. 2010; Tokunaga et al. 1997).

The most important transformation of selenium, with respect to its toxicity to aquatic organisms, is in the uptake of dissolved inorganic selenium into the tissues of primary producers at the base of the food web. The main route of entry of selenium into aquatic foodwebs is from the consumption of particulate selenium of primary producers, and to a lesser degree, from the consumption of sediments (Doblin et al. 2006; Luoma and Presser 2009). For algae, selenite and organic selenides are similarly bioavailable, and both dissolved species are more bioavailable

than selenate (e.g., Baines et al. 2001; Luoma et al. 1992). In vascular plants, selenate uptake is greater than for the other dissolved species, as the majority of selenium uptake occurs in the roots, and selenate is more easily transported to the shoots and leaves than selenite or organic selenides (Dumont 2006). Following uptake, selenium is metabolized into a variety of organic species that are assimilated into plant tissues. Selenium metabolism in plants is analogous to sulfur metabolism (e.g., Dumont et al. 2006; Ouerdane et al. 2013). Selenate is reduced to selenite, which is then reduced to selenide in a process involving reduced glutathione (Dumont et al. 2006). Selenide is converted to selenocysteine (SeCys), which is then converted to selenomethionine (SeMet) (Dumont et al. 2006). In addition to SeCys and SeMet, a variety of other organic selenium species can be formed; however, SeCys, and particularly SeMet are toxicologically important because these amino acids nonspecifically replace cysteine and methionine in proteins, and are more bioavailable to higher trophic level consumers (Fan et al. 2002; Freeman et al. 2006).

2.2.2 Bioaccumulation of Selenium in Aquatic Systems

Dissolved selenium uptake by animals is slow, whatever the form, such that under environmentally relevant conditions, dissolved selenium in the water column makes little or no direct contribution to bioaccumulation in animals (Lemly 1985a; Ogle and Knight 1996), but does influence the concentration of selenium in particulate matter. Selenium bioaccumulation in aquatic organisms occurs primarily through the ingestion of food (Fan et al. 2002; Luoma et al. 1992; Maher et al. 2010; Ohlendorf et al. 1986; Presser and Ohlendorf 1987; Presser et al. 1994; Saiki and Lowe 1987). However, unlike other bioaccumulative contaminants such as mercury, the single largest step in selenium accumulation in aquatic environments occurs at the base of the food web where algae and other microorganisms accumulate selenium from water by factors ranging from several hundred to tens of thousands (Luoma and Presser 2009; Orr et al. 2012; Stewart et al. 2010). Bioaccumulation and transfer through aquatic food webs are the major biogeochemical pathways of selenium in aquatic ecosystems. Dissolved selenium oxyanions (selenate, selenite) and organic selenides are assimilated into the tissues of aquatic primary producers (trophic level 1 organisms), such as periphyton, phytoplankton, and vascular macrophytes; and subsequently biotransformed into organoselenium. These organisms, together with other particle-bound selenium sources, constitute the particulate selenium fraction in the water column. Selenium from this particulate fraction is then transferred to aquatic primary

consumers such as zooplankton, insect larvae, larval fish, and bivalves (trophic level 2), and then to predators such as fish and birds (trophic level 3 and above). In addition to the water concentration of selenium, the process of selenium bioaccumulation in aquatic life residing in freshwater systems depends on several factors specific to each aquatic system. These factors include:

Water residence time. Residence time is a measure of the average time a water molecule will spend in a specified region of space. Residence time influences both the proportion of selenium found in particulate and dissolved forms and the predominant form of selenium. Organisms in waters with long residence times such as lakes, ponds, reservoirs, wetlands or estuaries will tend to bioaccumulate more selenium than those living in waters with shorter residence times such as rivers and streams (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). Several interrelated factors underlie selenium's greater bioaccumulation potential in slow moving systems, such as food web complexity and the organic content and reduction/oxidation potential of sediments. Finally, selenium toxicity in flowing waters with shorter residence times may only be apparent downstream of their selenium sources, whereas waters with longer residence times are more likely to exhibit selenium toxicity near their sources (Presser and Luoma 2006).

Distribution of selenium between particulate and dissolved forms. Selenium is found in both particulate and dissolved forms in water, but direct transfer of selenium from water to animals is only a small proportion of the total exposure. The proportion of selenium found in particulate matter (algae, detritus, and sediment) is important because it is the primary avenue for selenium entering into the aquatic food web (Luoma et al. 1992; Luoma and Rainbow 2005; Ohlendorf et al. 1986; Presser and Ohlendorf 1987; Presser et al. 1994; Presser and Luoma 2006; Saiki and Lowe 1987).

Bioaccumulation in prey. Trophic level 1 organisms such as periphyton and phytoplankton, as well as other forms of particulate material containing selenium, such as detritus and sediment, are ingested by trophic level 2 organisms such as mollusks, planktonic crustaceans, and many insects, increasing the concentration of selenium in the tissues of these higher-level organisms. Differences in the physiological characteristics of these organisms result in different levels of bioaccumulation. Also, selenium effects on invertebrates typically occur at concentrations higher than those that elicit effects on the vertebrates (e.g., fish and birds) that

prey upon them. Additionally, certain molluscan taxa such as mussels and clams can accumulate selenium to a much greater extent than planktonic crustaceans and insects (although the levels do not seem to be toxic to the mussels) due to higher ingestion rates of both particulate-bound (algae) and dissolved selenium from the water column through filter feeding, as well as the lower rate at which they eliminate selenium (Luoma and Rainbow 2005; Stewart et al. 2013). Because egg-laying (oviparous) vertebrates such as fish and birds are most sensitive to selenium effects, (Janz et al. 2010), these vertebrate consumers are also the most vulnerable groups to selenium poisoning and the focal point of most selenium environmental assessments (Ogle and Knight 1996; Stewart et al. 2010).

Trophic transfer to predators. Bioaccumulation of selenium by higher trophic level organisms, such as trophic level 3 and 4 fish, is highly influenced by the food web of the aquatic environment. For example, fish that primarily consume freshwater mollusks (e.g., redear sunfish) will exhibit greater selenium bioaccumulation than fish that consume primarily insects or crustaceans from waters with the same concentration of dissolved selenium because mollusks tend to accumulate selenium at higher concentrations than other trophic level 2 organisms, as noted above (Luoma and Presser 2009; Stewart et al. 2004).

2.3 Mode of Action and Toxicity of Selenium

Selenium is a naturally occurring chemical element that is also an essential micronutrient. Trace amounts of selenium are required for normal cellular function in almost all animals. However, excessive amounts of selenium can also have toxic effects, with selenium being one of the most toxic of the biologically essential elements (Chapman et al. 2010). Egg-laying vertebrates have a lower tolerance than do mammals, and the transition from levels of selenium that are biologically essential to those that are toxic occurs across a relatively narrow range of exposure concentrations (Luckey and Venugopal 1977; U.S. EPA 1987, 1998; Haygarth 1994; Chapman et al. 2009, 2010). Selenium consumed in the diet of adult female fish is deposited in the eggs, when selenium replaces sulfur in vitellogenin, which is transported to the ovary and incorporated into the developing ovarian follicle (Janz et al. 2010), the primary yolk precursor.

Selenium is a member of the sulfur group of nonmetallic elements, and consequently, the two chemicals share similar characteristics. Selenium can replace sulfur in two amino acids, the seleno-forms being selenomethionine and selenocysteine. It has been a long-standing hypothesis

that the cause of malformations in egg-laying vertebrates is due to the substitution of selenium for sulfur in these amino acids and their subsequent incorporation into proteins, which causes disruption of the structure and function of the protein. When present in excessive amounts, selenium is erroneously substituted for sulfur, resulting in the formation of a triselenium linkage (Se-Se-Se) or a selenotrisulfide linkage (S-Se-S), either of which was thought to prevent the formation of the normal disulfide chemical bonds (S-S). The end result was thought to be distorted, dysfunctional enzymes and protein molecules that impaired normal cellular biochemistry (Diplock and Hoekstra 1976; Reddy and Massaro 1983; Sunde 1984).

Recent research, however, suggests that selenium's role in oxidative stress plays a role in embryo toxicity, whereas selenium substitution for sulfur does not. The substitution of selenomethionine for methionine does not appear to affect either the structure or function of proteins (Yuan et al. 1998; Mechaly et al. 2000; Egerer-Sieber et al. 2006). The reason is apparently due to selenium not being distally located in selenomethionine, which insulates the protein from an effect on its tertiary structure. Although the incorporation of selenomethionine into proteins is concentration-dependent (Schrauzer 2000), selenocysteine's incorporation into proteins is not (Stadtman 1996). This suggests that neither selenomethionine nor selenocysteine affect protein structure or function. In fact, Se as an essential micronutrient is incorporated into functional and structural proteins as selenocysteine.

The role of selenium-induced oxidative stress in embryo toxicity and teratogenesis appears to be related to glutathione homeostasis. A review of bird studies by Hoffman (2002) showed exposure to selenium altered concentrations and ratios of reduced to oxidized glutathione thereby increasing measurements of oxidative cell damage. Palace et al. (2004) suggested oxidative stress due to elevated selenium levels results in pericardial and yolk sac edema in rainbow trout embryos. Evidence for the role of oxidative stress in selenium toxicity is growing, but mechanistic studies are needed to better understand its effects on egg-laying vertebrates. For a more in depth discussion on the mechanism of toxicity at the cellular level including the evidence against sulfur substitution as a cause and the role of oxidative stress, see Janz et al. (2010).

The most well-documented, overt and severe toxic symptoms in fish are reproductive teratogenesis and larval mortality. Egg-laying vertebrates appear to be the most sensitive taxa, with toxicity resulting from maternal transfer to eggs. In studies involving young organisms

exposed through transfer of selenium from adult female fish into their eggs, the most sensitive diagnostic indicators of selenium toxicity in vertebrates occur when developing embryos metabolize organic selenium that is present in egg albumen or yolk. It is then further metabolized by larval fish after hatching.

A variety of lethal and sublethal deformities can occur in the developing fish exposed to selenium, affecting both hard and soft tissues (Lemly 1993b). Developmental malformations are among the most conspicuous and diagnostic symptoms of chronic selenium poisoning in fish. Terata are permanent biomarkers of toxicity, and have been used to identify impacts of selenium on fish populations (Maier and Knight1994; Lemly 1997b). Deformities in fish that affect feeding or respiration can be lethal shortly after hatching. Terata that are not directly lethal, but distort the spine and fins, can reduce swimming ability, and overall fitness. Because the rate of survival of deformed young would be less than that for normal young, the percentage of deformed adults observed during biosurveys will likely understate the underlying percentage of deformed young, although quantitation of the difference is ordinarily not possible.

In summary, the most sensitive indicators of selenium toxicity in fish larvae are effects modulated through the reproductive process and exhibited in fish larvae as teratogenic deformities such as skeletal, craniofacial, and fin deformities, and various forms of edema that result in mortality (Lemly 2002). The toxic effect generally evaluated is the reduction in the number of normal healthy offspring compared to the starting number of eggs. In studies of young organisms exposed to selenium solely through their own diet (rather than via maternal transfer), reductions in survival and/or growth are the effects that are generally evaluated.

2.4 Narrow Margin between Sufficiency and Toxicity of Selenium

Selenium has a narrow range encompassing what is beneficial for biota and what is detrimental. Selenium is an essential nutrient that is incorporated into functional and structural proteins as selenocysteine and selenomethionine. Several of these proteins are enzymes that provide cellular antioxidant protection. Selenomethionine is readily oxidized, and its antioxidant activity arises from its ability to deplete reactive oxygen species. Selenomethionine is required as a mineral cofactor in the biosynthesis of glutathione peroxidases. All of the classic glutathione peroxidases contain selenium and are found to be involved in the catalytic reaction of these many enzymes (Allan 1999). The major function of the glutathione peroxidases involves the reduction

of hydrogen peroxide to water at the expense of the oxidation of glutathione, the enzyme's cofactor, an important antioxidant process at normal dietary levels.

Aquatic and terrestrial organisms require low levels of selenium in their diet to sustain metabolic processes, whereas excess concentrations of selenium that are only an order of magnitude greater than the required level have been shown to be toxic to fish, apparently due to generation of reactive oxidized species, resulting in oxidative stress (Palace et.al. 2004). Dietary requirements in fish have been reported to range from 0.05 to 1.0 mg Se/kg dw (Watanabe et al. 1997). Selenium requirements for optimum growth and liver glutathione peroxidase activity in channel catfish were reported as 0.25 mg Se/kg dw (Gatlin and Wilson 1984). Estimated selenium dietary requirements in hybrids of striped bass, based on selenium retention, were reported as 0.1 mg Se/kg dw (Jaramillo 2006). Selenium deficiency has been found to affect humans (U.S. EPA 1987), sheep and cattle (U.S. EPA 1987), deer (Oliver et al. 1990), fish (Thorarinsson et al. 1994; Wang and Lovell 1997; Wilson et al. 1997; U.S. EPA 1987), aquatic invertebrates (Audas et al. 1995; Caffrey 1989; Cooney et al. 1992; Cowgill 1987; Cowgill and Milazzo 1989; Elendt 1990; Elendt and Bais 1990; Harrison et al. 1988; Hyne et al. 1993; Keating and Caffrey 1989; Larsen and Bjerregaard 1995; Lim and Akiyama 1995; Lindstrom 1991; U.S. EPA 1987; Winner 1989; Winner and Whitford 1987), and algae (Doucette et al. 1987; Keller et al. 1987; Price 1987; Price et al. 1987; Thompson and Hosja 1996; U.S. EPA 1987; Wehr and Brown 1985). The predominance of research on selenium deficiency in invertebrates and algae is related to optimizing the health of test organisms cultured in the laboratory. A summary of several studies that evaluated the deficiency and/or the sufficiency of selenium in the diet of fish is provided in Appendix E.

2.5 Interactions with Mercury

The most well-known interactions with selenium occur with both inorganic and organic mercury, and are generally antagonistic (Micallef and Tyler 1987; Cuvin and Furness 1988; Paulsson and Lundbergh 1991; Siegel et al. 1991; Southworth et al. 1994; Ralston et al. 2006), with the most likely mechanism being the formation of metabolically inert mercury selenides (Ralston et al. 2006; Peterson et al. 2009). However, other studies have found interactions between mercury and selenium to be additive (Heinz and Hoffman 1998) or synergistic

(Huckabee and Griffith 1974; Birge et al. 1979). The underlying mechanism for these additive and synergistic interactions between mercury and selenium are unknown.

2.6 ASSESSMENT ENDPOINTS

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" and are defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (U.S. EPA 1998). Assessment endpoints may be identified at any level of organization (e.g., individual, population, community). In the context of the Clean Water Act, aquatic life criteria for toxic pollutants are typically determined based on the results of toxicity tests with aquatic organisms in which unacceptable effects on growth, reproduction, or survival occurred. The goal of criteria is to protect the diversity, productivity, and stability of aquatic communities. To achieve this goal, the endpoint of criteria assessment is the survival, growth, and reproduction of a high percentage of species of a diverse assemblage of freshwater aquatic animals (fish, amphibians, and invertebrates) and plants. Toxicity data are aggregated into a sensitivity distribution that indicates the impact of the toxicant under study to a variety of genera representing the broader aquatic community. Criteria are designed to be protective of the vast majority of aquatic animal species in an aquatic community (i.e., approximately 95^{th} percentile of tested aquatic animals representing the aquatic community). As a result, health of the aquatic community may be considered as an assessment endpoint indicated by survival, growth, and reproduction. Assessment endpoints are the ultimate focus in risk characterization and link the measurement endpoints to the risk management process (e.g., policy goals). When an assessment endpoint can be directly measured, the measurement and assessment endpoints are the same. In most cases, however, the assessment endpoint cannot be directly measured, so a measurement endpoint (or a suite of measurement endpoints) is selected that can be related, either qualitatively or quantitatively, to the assessment endpoint. For example, a decline in a sport fish population (the assessment endpoint) may be evaluated using laboratory studies on the mortality of surrogate species, such as the fathead minnow (the measurement endpoint) (EPA/630/R-92/001 February 1992). The assessment endpoint for selenium is the protection of freshwater aquatic life; because we know that fish are the most sensitive aquatic taxon to the toxicological effect of selenium, the criterion is expressed in terms of fish tissue using eggs and ovarian tissue as the most representative element related to selenium toxicity.

To assess potential effects on the aquatic ecosystem by a particular stressor, and develop 304(a) aquatic life criteria under the CWA, EPA typically requires the following, as outlined in the EPA Ambient Water Quality Criteria Guidelines: acute toxicity test data (mortality, immobility, loss of equilibrium) for aquatic animals from a minimum of eight diverse taxonomic groups; as well as chronic toxicity data (e.g., survival, growth and reproduction) for aquatic animals from 8 eight taxonomic groups (described in more detail below). The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. In the case of bioaccumulative compounds like selenium, these acute toxicity studies do not address risks that result from exposure to chemicals via the diet (through the food web). They also do not account for the slow accumulation kinetics of many bioaccumulative compounds such as selenium and may underestimate effects from long-term accumulation in different types of aquatic systems (SAB 2005).

Because the most sensitive adverse effects of selenium are reproductive effects (larval deformities and mortality) on the offspring of exposed fish, chronic effects from long term exposure are the focus of this selenium assessment. In addition to continuous discharges, shorter-term intermittent or pulsed exposures to elevated levels of selenium may also result in bioaccumulation through the aquatic food web and may subsequently adversely affect fish reproduction, and such measures of effect are therefore also estimated from chronic assessment endpoints. Selenium toxicity in the water body could potentially threaten fecundity and recruitment in fishes, resulting in extirpation of sensitive species in a waterbody, and potentially shifting the trophic dynamics of the system. Therefore, the assessment endpoint for selenium is the protection of fish populations. In some waters where ESA-listed fish species occur, a protection goal oriented to protection of individuals may be more appropriate. This should be reflected using site-specific data to derive an SSC for the site.

Chronic toxicity test data (longer-term survival, growth, or reproduction) for aquatic animals are needed from a minimum of eight diverse taxonomic groups (or less generically, [minimum of three taxa] if the derivation is based on an acute to chronic ratio). The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. Specific minimum data recommendations or requirements (MDRs) identified for development of criteria in the EPA Ambient Water Quality Criteria Guidelines require aquatic animal toxicity data from:

1. the family Salmonidae in the class Osteichthyes,
2. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.),
3. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.),
4. a planktonic crustacean (e.g., cladoceran, copepod, etc.),
5. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.),
6. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.),
7. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.), and
8. a family in any order of insect or any phylum not already represented.

Acceptable quantitative chronic values for selenium are available for six of the eight MDRs (requirements 1, 2, 3, 6, 7, and 8). Acceptable chronic values for selenium are not available for two of the MDRs (requirements 4 and 5: planktonic and benthic crustaceans, respectively). Following the approach of U.S. EPA (2008b), which was reviewed by the Science Advisory Board, if information is available to demonstrate that an MDR is not sensitive, then a surrogate value can be used in place of actual toxicity data to represent the missing MDR. Based on the data estimating the sensitivity of insects (*Centroptilum triangulifer*), rotifers (*Brachionus calyciflorus*), and oligochaetes (*Lumbriculus variegatus*), EPA determined that invertebrates (e.g., insects and crustaceans) are generally less sensitive to selenium than fish, based on the characteristics of selenium toxicity to aquatic life. Therefore, the available fish data were used in the genus-level sensitivity distribution to derive the chronic selenium criterion (Note: invertebrate data were included in the sensitivity distribution for the whole body criterion element to demonstrate that the derivation of the criterion element based on the fish egg-ovary to whole body translated values protected invertebrates given the sensitivity range of the available species).

The EPA Ambient Water Quality Criteria Guidelines also require at least one acceptable test with a freshwater alga or vascular plant. If plants are among the aquatic organisms most sensitive to the stressor, results of a plant in another phylum should also be available. A

relatively large number of tests from acceptable studies of aquatic plants were available for possible derivation of a Final Plant Value. However, the relative sensitivity of freshwater plants to selenium (Appendix F) is less than fish, so plant criterion elements were not developed.

The available scientific evidence indicates that for selenium, critical assessment endpoints for aquatic species are offspring mortality and severe development abnormalities that affect the ability of fish to swim, feed and successfully avoid predation, resulting in impaired recruitment of individuals into fish populations. Selenium enrichment of reservoir environments (e.g., Belews Lake, NC (Lemly 1985), Hyco Reservoir (DeForest 1999), and Kesterson Reservoir, CA (Ohlendorf 1986)) are well documented and demonstrate that adverse effects resulted from bioaccumulative processes at different levels of biological organization, resulting in population-level reductions of resident species.

2.7 Measures of Effect

Each assessment endpoint requires one or more "measures of ecological effect", which are defined as changes in the attributes of an assessment endpoint itself or changes in a surrogate entity or attribute in response to chemical exposure. Ecological effects data are used as measures of direct and indirect effects to growth, reproduction, and survival of aquatic organisms.

The toxicity testing data available for any given pollutant vary significantly, depending primarily on whether any major environmental issues are raised. An in-depth evaluation of available data for selenium has been performed by EPA to determine data acceptability and quality, based on criteria established in the EPA Ambient Water Quality Criteria Guidelines.

In traditional chronic tests used in many EPA aquatic life criteria documents, organisms are exposed to contaminated water but fed a diet grown in uncontaminated media not spiked with the toxicant prior to introduction into the exposure chambers. Such tests are not suitable for deriving a criterion for a bioaccumulative pollutant unless (1) effects are linked to concentrations measured in appropriate tissues, and (2) the route of exposure does not affect the potency of residues in tissue. For selenium, the first condition might be met, but the second condition is not, because the route of selenium exposure appears to influence the potency of a given tissue residue (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978). Consequently, toxicity tests with water-only exposures (and any tests not relying on dietary exposure) are not included in this assessment.

Selenium toxicity is primarily manifested as reproductive impairment due to maternal transfer, resulting in embryo mortality and teratogenicity. Measurements of selenium in fish tissue are most closely linked to the chronic adverse effects of selenium (Chapman et al. 2010), since chronic selenium toxicity is based on the food-chain bioaccumulation route, not a direct waterborne route. In this selenium criterion document, water column criterion element concentrations for selenium were derived from fish tissue concentrations by modeling selenium transfer through the food web. The next sections describe approaches used to establish selenium effects concentrations in fish tissue and to relate the concentrations in fish tissue to concentrations in water.

2.7.1 Fish Tissue

Chronic measures of effect concentrations are the $EC_{10,}$ $EC_{20,}$ No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), and Maximum Acceptable Toxicant Concentration (MATC). The EC_{10} is the concentration of a chemical that is estimated to result in a 10 percent effect in a measured chronic endpoint (e.g., growth, reproduction, or survival); the EC_{20} corresponds to 20 percent effect. The NOEC is the highest chemical concentration at which none of the observed effects are statistically different from the control, as determined by hypothesis testing. The LOEC is the lowest test concentration at which observed effects are found to be statistically different from the control. For selenium, in all cases the effect endpoint used in the estimation of chronic values (e.g., EC_{10}s) is an effect on offspring (with exposure via maternal transfer) from parents exposed to selenium via diet. Selenomethionine was used exclusively in dietary exposures in the lab, whereas field-exposed females would be exposed to a combination of forms of selenium as a function of the selenium in their prey items.

Whenever possible, estimates of selenium concentrations associated with a low level of effect (i.e., EC_{10}) were calculated for each study using the computer program TRAP (version 1.30a), Toxicity Relationship Analysis Program (U.S. EPA 2013). The program is based on a regression approach that models the level of adverse effects as a function of increasing concentrations of the toxic substance. With the fitted model it is possible to estimate the contaminant concentration associated with a small effect. TRAP was used when there are sufficient data for EC_{10} estimation. For studies with binary data, the analysis proceeded by tolerance distribution analyses using the log-triangular distribution, unless there was substantial

extrabinomial variability, in which case regression analysis was used. For regression analysis, the threshold sigmoidal model was used, exposure variables were log-transformed, and effects variables were weighted appropriately to address their relative uncertainties.

When there were insufficient data for TRAP to fit an effects/exposure curve (no treatments with clear effects near the EC_{10} and/or significant background variability), the EC_{10} was based on interpolation. To ensure that the interpolations were comparable to the TRAP analyses, threshold sigmoidal equation was used. This equation is fitted to two points, and constrained so that 3 equation parameters can be set. The first set-point was treated as the EC_0 with a second associated set-point being the threshold for background effects values, based on the highest NOEC (HNOEC) datum and other NOEC data. The final set-point was the LOEC. If the LOEC is a partial effect, then this point was used to estimate the equation slope. If the LOEC was a 100% effect, it was specified as the EC_{100}; with the EC_0 specified, then this relationship dictated the equation slope. It should be noted that despite the superficial resemblance of these analyses to TRAP they are also subject to the uncertainties associated with the interpolation method.

It should be noted that TRAP involves the assumption that (a) there is a single underlying relationship of the effects variable to the exposure variable which follows the specified equation and (b) the exposure variable is known with negligible error, with uncertainty being predominantly in the effects variable. Some of the reproductive data for selenium involved multiple sources of variability that led to both multiple relationships across different cohorts of offspring and uncertainty in the exposure variable, so that the resulting TRAP curves were more approximate, and TRAP error estimates were generally not useful. These issues can also affect the interpolation protocol. It should also be noted that estimating a concentration associated with a low effects level, such as an EC_{10}, is especially uncertain when treatments yielding partial effects values are lacking in the concentration response data produced by a study. These two issues prevented the use of TRAP in some datasets. When the data are insufficient to provide any meaningful EC_{10} by the first two approaches, the study should either not be used for criteria development or a chronic value should be set by other means than an estimated EC_{10} if possible.

Only studies with a reference site (field surveys) or control treatment(s) (experimental studies) were included in the analysis, because response levels at these low selenium concentrations were the most influential points for calculating the estimated response level at a

selenium concentration of zero (y_0). When considering the use of the EC_{10} versus the EC_{20}, an EC_{10} was determined to be a more appropriate endpoint for tissue-based criteria given the nature of exposure and effects for this bioaccumulative chemical. EC_{20}s have historically been used in the derivation of EPA criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually over time. Thus, where concentrations of selenium in fish tissue are used as an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that are not as bioaccumulative. Furthermore, it was found that the dose-response curves for selenium across a broad range of fish genera are very steep, such that a small change in selenium tissue concentration yielded a large increase in observed adverse effect. In many cases, the selenium data indicated a change from control effect levels to effects in excess of 50% for larval mortality or deformity over a few mg/kg dry weight increase in selenium detected in fish tissue. These issues call for use of a lower level of effect to attain sufficient protection. The EC_{10} was also preferred over the NOEC or LOEC as these measures of effect are influenced by study design, specifically the concentrations tested, the number of concentrations tested, the number of replicates for each concentration, and the number of organisms in each replicate. As noted by Campbell (2011), EC_{10}s and NOECs are generally of similar magnitude, but EC_{10}s have the advantage of being more reproducible than NOECs (Van der Hoeven et al. 1997; Warne and van Dam 2008). NOECs and MATCs are generally presented if calculated by the original investigators, but were not used where an EC_{10} could be calculated. The four lowest egg-ovary Genus Mean Chronic Values (GMCVs), whose exact values influence the calculation of the egg-ovary criterion element, are all based solely on EC_{10}s. NOECs contribute to some of the GMCVs for less sensitive species.

In this document, chronic values are presented as tissue concentrations of total selenium in units of mg/kg dry weight (dw). Studies of chronic toxicity of selenium to aquatic organisms measure concentrations in distinct tissues (e.g., whole body, ovaries, eggs, muscle, and liver) and report these values as either wet weight (ww) or dw. Studies reporting tissue concentrations only based on wet weight were converted to dry weight using tissue-specific and species-specific conversion factors. When wet to dry weight conversion factors were not available for a given species, conversion factors for a closely related taxon were used. In deriving the egg or ovary tissue criterion element, chronic values are for those tissues directly measured in the study.

Tissue-to-tissue conversions (e.g., to estimate concentrations in an unmeasured tissue from a study's measured tissue) involve some uncertainty because of variability in tissue concentration ratios (deBruyn et al. 2008; Osmudson et al. 2007). Tissue-to-tissue conversions were needed for calculating the reproductive toxicity-based whole-body and muscle chronic criterion element and water criterion concentration elements.

The overall assessment evaluates both reproductive and non-reproductive studies. Selenium concentrations measured directly in eggs or ovaries from reproductive (maternal transfer) studies are used to derive the egg/ovary criterion element, and corresponding selenium concentrations in whole body or muscle tissue resulting in reproductive effects are estimated using conversion factors. Direct measurements of selenium concentrations in whole-body or muscle from non-reproductive studies are used to examine non-reproductive, chronic effects, such as impairments to growth.

2.7.2 Water

While state monitoring programs may sample ambient waters for selenium, widespread measurements of selenium in fish tissue are relatively rare. Therefore, EPA is providing estimated chronic measures of effect for water column data. The chronic criterion element for the water column is the 30-day average concentration that corresponds to the concentration of selenium in fish tissue estimated to result in a 10 percent effect in fish for a specific water body type (lotic or lentic water bodies as described below in **Section 3.2.4**). The chronic criterion element for the water column is derived by modeling trophic transfer of selenium through the food web resulting in the fish tissue concentration that yields the chronic reproductive effects of concern.

EPA collaborated with the United States Geological Survey (USGS) to develop a model (later published in Presser and Luoma 2010) that relates the concentration of selenium in fish tissue to the water column. The approach is based on bioaccumulation and trophic transfer through aquatic system food-webs. Model parameters are calculated using both field and laboratory measurements of selenium in water, particulate material (algae, detritus and sediment), invertebrates, fish whole-body, and fish egg-ovary. Although EPA and USGS use the same model to relate the concentration of selenium in fish tissue to water, EPA starts with selenium in the egg/ovary (reproductive criterion) whereas USGS starts with selenium in the fish's whole body. The EPA approach therefore has the additional step of converting the

concentration of selenium in the egg/ovary to whole body. This model (which is a set of equations) is described in more detail in **Section 3.2.1**.

2.7.3 Summary of Assessment Endpoints and Measures of Effect

The typical assessment endpoints for aquatic life criteria are based on effects on growth, reproduction, or survival of the assessed taxa. These measures of effect on toxicological endpoints of consequence to populations are provided by results from toxicity tests with aquatic plants and animals. The toxicity values (i.e., measures of effect expressed as genus means) are used in the genus sensitivity distribution of the aquatic community to derive the aquatic life criteria. Endpoints used in this assessment are listed in **Table 2.2**.

Table 2.2. Summary of Assessment Endpoints and Measures of Effect Used in Criterion Derivation for Selenium.

Assessment Endpoints for the Aquatic Community	**Measures of Effect**
Survival, growth, and reproduction of freshwater fish, other freshwater vertebrates, and invertebrates	For effects from chronic exposure: 1. EC_{10} concentrations in egg and ovary, for offspring mortality and deformity. 2. Measured or estimated reproductive EC_{10} in whole body and muscle. 3. Estimated concentrations (µg/L) in water linked to egg-ovary EC_{10}s by food web-modeling. 4. Intermittent water concentrations yielding exposure equivalent to the above. For acutely lethal effects: Acute toxicity effects based on standard water column-only toxicity testing are not provided here for selenium, due to the dominant significance of chronic effects. Note: Chronic criterion is expected to be protective of acute effects.

2.7.4 Conceptual Model of Selenium Effects on Aquatic Life

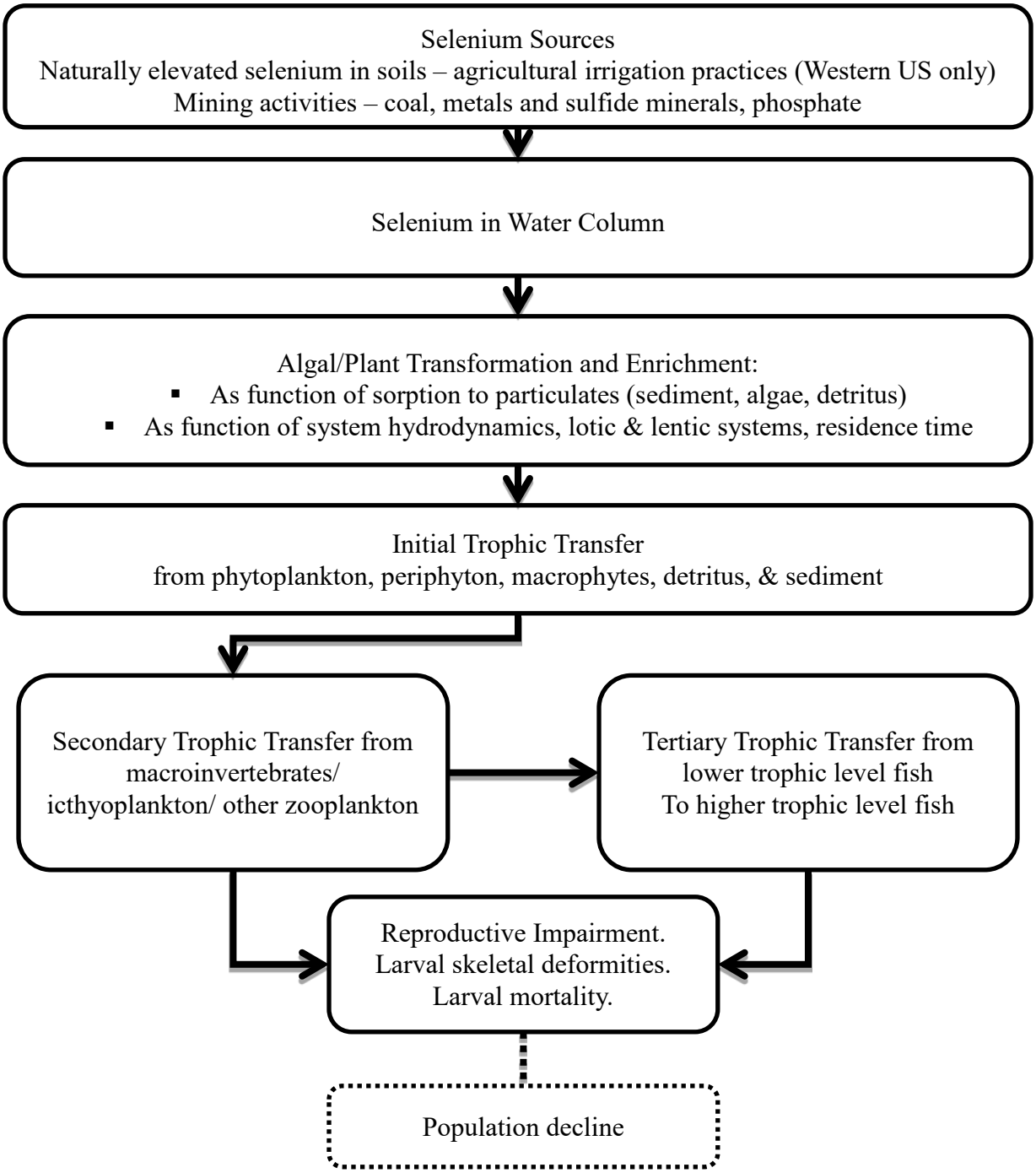


Figure 2.2. Diagram of Selenium Partitioning, Bioaccumulation, and Effects in the Aquatic Environment.

The conceptual model links sources, transformation and uptake through media phases, and consumer transfer and dynamics reflective of the movement of selenium through ecosystems (**Figure 2.2**). Diet is the dominant pathway of selenium exposure for both invertebrates and

vertebrates. Selenium moves from water to particulates, a collection of biotic and abiotic compartments that includes primary producers, detritus, and sediments, which form the base of aquatic food webs. Transfer from particulates to primary consumers (e.g., macroinvertebrates) to fish is species specific. Knowledge of the food web is one of the keys to determining which biological species or other ecological characteristics will be affected.

During the development of CWA section 304(a) criteria, EPA assembles all available test data and considers all the relevant data that meet acceptable data quality and test acceptability standards. This criterion update document is specific to selenium in fresh water. Chronic criterion elements for selenium are protective concentrations measured in fish tissue and related to protective water concentrations generated using food-web modeling. Further modeling is used to estimate short-term concentrations in water from intermittent or pulsed exposures that are protective against the chronic effect.

2.7.5 Analysis Plan for Derivation of the Chronic Fish Tissue-Based Criterion Elements

Data for possible inclusion in the selenium dataset were obtained primarily by search of published literature using EPA's public ECOTOX database (up to July 2013). These studies were screened for data quality as described in the EPA Ambient Water Quality Criteria Guidelines, and adjusted for factors related to dietary lab or field exposure, which were not considered at the time the Guidelines were written. Additional data were considered and reviewed for inclusion in this criterion based on the public and peer review comments on the 2014 "External Peer Review Draft" criterion document, and public comments on the 2015 draft.

Chronic toxicity studies (both laboratory and field studies) were further screened to ensure they contained the relevant chronic exposure conditions of selenium to aquatic organisms (i.e., dietary, or dietary and waterborne selenium exposure), measurement of chronic effects, and measurement of selenium in tissue(s). The criterion derivation uses only those studies in which test organisms were exposed to selenium in their diet, because such studies most closely replicate real-world exposures (diet and/or diet plus water). This approach accords with findings and recommendations of the 2009 SETAC Pellston Workshop (Chapman et al. 2009, 2010).

EPA grouped studies based on whether the effects were chronic reproductive (e.g., effects on offspring survival or morphology) or chronic non-reproductive (e.g., juvenile growth and survival). At the 2009 Pellston workshop (Chapman et al. 2009, 2010), a group of 46 experts in the area of ecological assessment of selenium in the aquatic environment agreed that the most

important toxicological effects of selenium in fish arise following maternal transfer of selenium to eggs during vitellogenesis, resulting in selenium exposure when hatched larvae undergo yolk absorption. Such effects include larval mortality or permanent developmental malformations, such as skeletal and craniofacial deformities. Therefore, the chronic fish-tissue-based criterion elements are based on reproductive effects only.

The egg-ovary Species Mean Chronic Values (SMCVs) were calculated from the chronic values (EC_{10}s and occasionally NOECs) obtained from the relevant toxicity tests. Genus Mean Chronic Values (GMCVs) were calculated from the SMCVs and then rank-ordered from least to most sensitive. The four lowest egg-ovary Genus Mean Chronic Values (GMCVs), whose exact values influence the calculation of the egg-ovary criterion element, are all based solely on EC_{10}s. The egg-ovary Final Chronic Value (FCV) was calculated from regression analysis of the four most sensitive GMCVs, in this case extrapolating to the 5^{th} percentile of the distribution represented by the tested genera. The FCV directly serves as the fish tissue egg-ovary criterion concentration element without further adjustment because the underlying EC_{10}s represent a low level of effect (per the EPA Ambient Water Quality Criteria Guidelines).

For the whole-body and muscle criterion element concentrations, CVs were either measured directly using the relevant tissue or the egg-ovary CVs were converted to estimated equivalent whole-body or muscle CVs. The criterion concentration element expressed as whole-body or as muscle concentration was calculated in a manner similar to the egg-ovary criterion element using a combination of directly calculated CVs or CVs that used conversion factors described below.

2.7.6 Analysis Plan for Derivation of the Fish Tissue Criterion Elements Duration

Duration of the averaging periods in national criteria restrict allowable fluctuations in the concentration of the pollutant in the receiving water and restrict the length of time that the concentration in the receiving water can be continuously above a criterion concentration, in order to protect aquatic life. A numerical value for the fish tissue criterion elements averaging period, or duration, is specified as instantaneous, because fish tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of selenium over time and space in the fish population(s) at a given site. Selenium concentrations in fish tissue are generally expected to change only gradually over time (**Section 3.2.6** and Appendix J) in response to environmental fluctuations; thus, there would be relatively little difference in tissue concentrations with

different averaging period durations if the average selenium concentrations in water are relatively stable over time. Generally fish collected to calculate average tissue concentrations for a site are collected in one sampling event, or over a short time interval due to logistical constraints and costs for obtaining samples incurred by state monitoring programs.

2.7.7 Analysis Plan for Derivation of the Fish Tissue Criterion Elements Return Frequency

Frequency is the number of times an excursion can occur over time without impairing the aquatic community or other use. The current recommendation (1985 Guidelines – EPA PB85-227049) for return frequency of once in 3 years on average is based on the ability of an aquatic ecosystem to recover from a toxic insult when pollutant impacts are associated exclusively with a water column exposure. This recommendation is based on the variability of water concentrations that aquatic life will be exposed to, and is set at a low level such that the water concentrations would mostly be below the criteria concentration. Selenium, however, is a bioaccumulative pollutant, and elevated levels in various ecological compartments (e.g., biota, surficial sediments) require a long period to decrease and the associated aquatic community requires a long time to recover following reduction or removal of an elevated selenium exposure to a given system (e.g., Belews Lake, NC, and Hyco Lake, NC).

Cumbie and Van Horn (1978) first reported young of the year losses in Belews Lake quickly followed by dramatic decreases in standing stocks of many species, and particularly game species like bluegill and largemouth bass. Fish communities were reduced to selenium-tolerant species including cyprinids (e.g., fathead minnow) and green sunfish in both lakes. Selenium reduction in Belews Lake (1985) and Hyco Lake (1990), resulted in rapid decreases in [Se] in the water column, but reductions in fish tissue took much longer. Finley and Garrett (2007) show that concentrations in bluegill and largemouth decreased from 19 and 17 mg/kg dw, respectively in 1992-1994 to ~8.0 mg/kg dw in both species sampled between 2003-2005. In Belews Lake, where Se contamination was higher, [Se] in crappie and redear sunfish averaged 18 and 17 mg/kg dw respectively in 1994-1996, and decreased to ~9-10 mg/kg dw in both species based on sampling in 2004-2006, twenty years later.

Chapman et al. (2010) also reported a similar scenario for Hyco Lake where “fish recruitment failure and the a massive fish kill in 1980 led to a decimated fishery. Selenium concentrations in the reservoir were reduced beginning in 1990 and gradual reductions in Se exposure via the food web led to the reestablishment of a diverse Hyco Lake fish community

similar to the period prior to Se impact." The Belews and Hyco Lake examples indicate that a protracted period of time (in excess of 10 years) would be necessary for fish communities to recover once a selenium in fish tissue reached concentrations associated with reproductive impacts. Thus, the typical "once-in-three years on average" criteria return frequency is not appropriate for selenium, as this could lead to sustained ecological impacts.

2.7.8 Analysis Plan for Derivation of Chronic Water-based Criterion Element

The relationship between the ambient concentration of selenium in water and the concentration of selenium in the eggs or ovaries of fish is primarily through trophic transfer of selenium, which is greatly affected by site-specific conditions. EPA used a peer-reviewed model to derive water concentrations from the egg-ovary criterion element that explicitly recognizes partitioning of selenium in water and particulate material (algae, detritus, and sediment), and trophic transfer from particulate material to aquatic invertebrates, from invertebrates to fish, and partitioning in fish whole-body and fish eggs and ovaries. The method is composed of five main steps:

1. Formulate a mathematical equation relating the concentration of selenium in the eggs and ovaries of fish to the ambient concentration of selenium in the water column.
2. Develop parameters needed to use the mathematical equation formulated in step 1 from available empirical or laboratory data related to selenium bioaccumulation in aquatic systems and aquatic organisms.
3. Classify categories of aquatic systems where a single water column concentration would be adequately protective by evaluating the bioaccumulation potential at the base of the aquatic food web.
4. Translate the egg-ovary criterion element to an equivalent water column concentration at each aquatic site.
5. Apply a statistical threshold to the distribution of translated water column concentrations for each aquatic system category to yield a water column concentration value that would be protective of each aquatic system category.

EPA worked with USGS to derive a translation equation to estimate the site-specific concentration of selenium in the water column corresponding to the egg-ovary criterion element concentration. This equation utilizes a mechanistic model of bioaccumulation previously

published in peer-reviewed scientific literature (Luoma et. al. 1992; Wang et. al. 1996; Luoma and Fisher 1997; Wang 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006, 2010; Presser 2013). The equation uses site-specific food web models, species-specific Trophic Transfer Factor (*TTF*) values, egg-ovary to whole-body conversion factor (*CF*) values, and a site-specific enrichment factor (*EF*) values to calculate a site-specific water column concentration element from the egg-ovary criterion element.

Empirical or laboratory data related to selenium bioaccumulation in aquatic organisms are needed to calculate species-specific *TTF* and *CF* parameters and a site-specific *EF* parameter. EPA obtained these data by reviewing its extensive selenium library of published papers and reports, by searching published literature using EPA's public ECOTOX database and other publically available data received through solicitation of public comments on the 2014 "External Peer Review" draft, through the external peer reviewers of the 2014 draft, and through public comments on the 2015 draft criterion document. Studies were screened using the same data quality guidelines described above. Relevant studies contained selenium measurements from field studies (water, particulate material, and aquatic organisms) or contained laboratory data on physiological parameters of selenium bioaccumulation in aquatic organisms. Literature searches for information on selenium associated with particulate matter included searches for data on all forms of algae, detritus, inorganic suspended material, and sediment.

EPA compiled a collection of selenium concentration measurements from acceptable field studies. Measurements were accepted if the study indicated the samples were collected in the field, and the study identified the unit of measure, the media from which the measurement was made, the location from where the sample was taken, and the date the sample was collected. EPA only used data from studies with adequately described field collection protocols and where concentrations were within the bounds of concentrations found using modern, rigorous protocols in similar systems (Sañudo-Wilhelmy et al. 2004). The spatial precision of field data sample collection locations were generally at the site level, although aggregate measurements were also included if exposure conditions were considered similar (e.g., averages of single or composite measurements from several locations in the same aquatic system). The temporal precision of sample collection times were usually at the level of the day they were collected, although some studies only provided enough information to determine the week, month, or year. If the day a series of samples were collected was not reported but the study provided information that

indicated the samples were taken concurrently, EPA noted sample precision, but assigned a single effective collection date to all the samples.

EPA also compiled a collection of physiological coefficients for food ingestion rate (*IR*), selenium assimilation efficiency (*AE*), and rate of selenium loss (k_e) from published literature. Coefficients were accepted if the studies provided either the actual measurements or sufficient information to derive them, and were reported in standard units (k_e: /d; *AE*: %; *IR*: g/g-d) or could be converted to standard units. Even though IR can be highly variable (Whitledge and Hayward 2000), IR values of surrogate species were occasionally used.

EPA accounted for bioaccumulation variability across aquatic sites by evaluating the parameter *EF* (representing the partitioning of selenium between the dissolved and particulate state) from representative aquatic systems. The parameter *EF* is a measure of bioaccumulation potential because it quantifies the transfer of selenium from the water column to particulate material, which is the single most influential step in selenium bioaccumulation (Chapman et al. 2010). EPA calculated *EF* values for a set of aquatic systems using data from published literature and applied statistical methods to distinguish categories with similar bioaccumulation characteristics. On this basis, a single water column concentration is deemed adequately protective when it is derived using data from aquatic sites in the same category. EPA translated the egg-ovary criterion element into a set of water concentration values and derived a water column criterion element for each aquatic system category using a percentile of the water column concentrations for each category. To ensure adequate protection, EPA selected the 20th percentile of the distribution of median water column values as the statistical cut-off (see **Section 3.2.5**). **Figure 2.3** diagrams the conceptual framework EPA used to derive water column criterion element values from the egg-ovary criterion element.

2.7.9 Analysis Plan for Derivation of the Water Criterion Elements Duration

A numerical value for the lentic and lotic water criterion elements averaging period, or duration, is specified as a 30-day average, because the presence of selenium in water is the initial step in the process of bioaccumulation from the water column to fish tissue. The bioaccumulation process for selenium takes place over a longer term than typically observed for acute and chronic effects on aquatic life based on water concentrations. The derivation of a protective water averaging period from kinetic modeling considerations is described in **Section 3.2.6** and in Appendix J. Because the intermittent criterion element values are based on the water

criteria chronic magnitudes and duration, the kinetic analysis of Appendix J also controls the intermittent criterion element values.

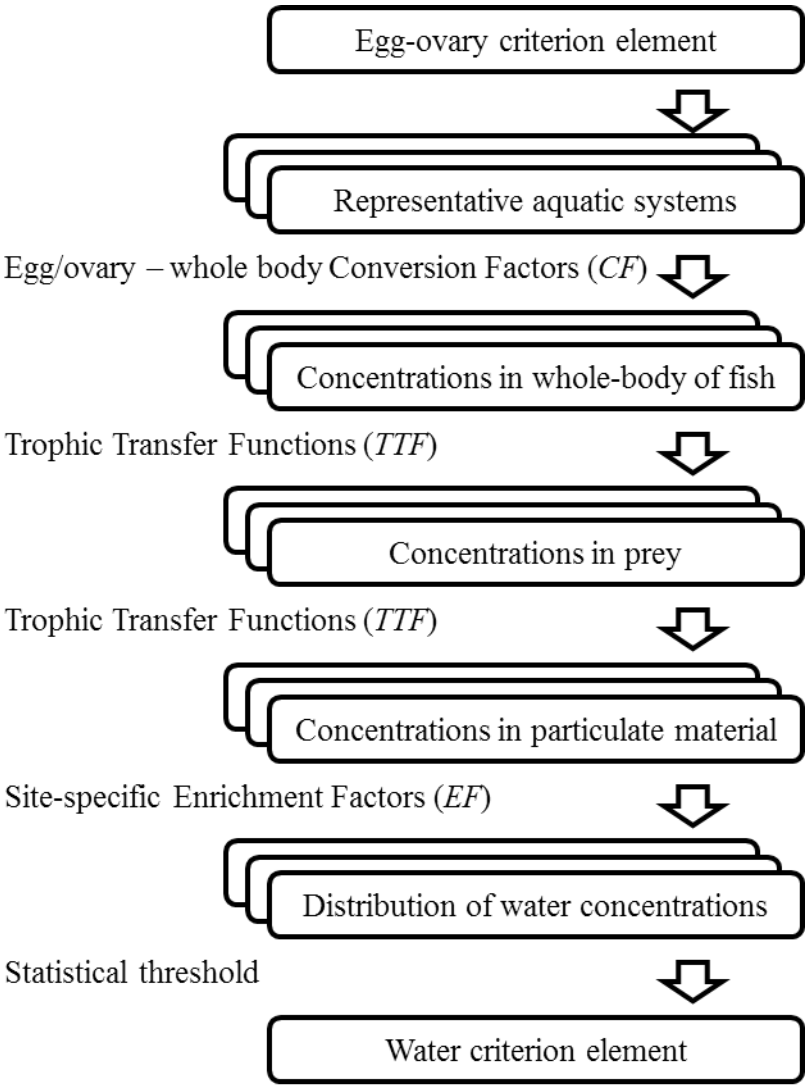


Figure 2.3. Conceptual Model for Translating the Selenium Egg-Ovary Concentration to a Water Column Concentration.

2.7.10 Analysis Plan for Intermittent-Exposure Water-based Criterion Element Derivation

Like the chronic water criterion element, the intermittent-exposure criterion element protects against cumulative exposure of selenium from multiple short-term discharges that may cause an excursion of the fish tissue criterion element. EPA derived the intermittent exposure criterion element directly from the chronic water criterion element by algebraically rearranging

the chronic water criterion element to establish a limit on an intermittent elevated concentration occurring over a specified percentage of time, while simultaneously accounting for background concentrations (see **Section 3.3**).

3 EFFECTS ANALYSIS FOR FRESHWATER AQUATIC ORGANISMS

3.1 CHRONIC TISSUE-BASED SELENIUM CRITERION ELEMENT CONCENTRATION

Data were obtained primarily by search of published literature using EPA's public ECOTOX database. The most recent ECOTOX database search extended to July 2013; this document also reflects data either gathered or received by EPA based on information from the 2014 public comment period and 2014 external expert peer review of the "External Peer Review Draft" published in May 2014, as well as information gathered based on public comments on the 2015 draft criterion. All available, relevant, and reliable chronic toxicity values were incorporated into the appropriate selenium AWQC tables and used to recalculate the FCV, as outlined in detail in the EPA Ambient Water Quality Criteria Guidelines.

The chronic values determined from acceptable chronic toxicity studies were separated into reproductive endpoint and non-reproductive endpoint categories. Although both sets of endpoints assess effects due to selenium on embryo/larval or juvenile development and survival and growth, the fundamental difference is exposure route (inherent in test design). That is, the fundamental difference is whether the aquatic organisms (e.g., fish) were directly exposed to selenium in the diet and water column or exposed via maternal transfer of selenium to the eggs/ovaries prior to reproduction. In studies with reproductive endpoints, parental females are exposed to selenium and the contaminant is transferred from the female to her eggs. In the selenium-exposed females, selenium replaces sulfur in vitellogenin, the primary yolk precursor, which is transported to the ovary and incorporated into the developing ovarian follicle (Janz et al. 2010). In most but not all of these studies, progeny from these females were not additionally exposed to aqueous selenium. The chronic values derived for the reproductive effects (survival, deformities, and edema) are based on the concentration of selenium in the eggs or ovary, the tissues most directly associated with the observed effects. In contrast, in studies grouped under non-reproductive effects (usually larval and/or juvenile survival or growth), the tested fish had no maternal pre-exposure to selenium. Chronic values for non-reproductive effects are based on the concentration of selenium in tissues measured in the study: muscle, liver and/or whole body.

The reproductive endpoint studies applied to the derivation of the chronic criterion elements are described below. Less definitive reproductive studies that are not directly applied to

the criterion derivation are described in **Section 6.1.2** and in Appendix C. Nonreproductive studies are described in **Section 6.1.9**.

3.1.1 Acceptable Studies of Fish Reproductive Effects for the Four Most Sensitive Genera

Below is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the four sensitive genera that drive the calculation of each specific chronic value. The studies in this section involve effects on the offspring of exposed female fish. Data are summarized in **Table 3.1**. Details of these studies and other chronic studies considered for criterion derivation are contained in Appendix C.

3.1.1.1 Acipenseridae

Acipenser transmontanus (white sturgeon)

Linville (2006) evaluated the effect of elevated dietary selenium on the health and reproduction of white sturgeon. Adult female white sturgeon (approximately 5 years old) were fed either a control diet (no added selenium, 1.4 mg/kg Se) or a diet spiked with selenized yeast (34 mg/kg Se) for six months in a freshwater flow through system. At the end of the dietary exposure, females were induced to spawn and fertilized with non-exposed male milt. Large cohorts of fertilized eggs from individual females (two from control and three from the treatment) were collected and separately hatched. After hatching (stage 36), n=500 sets of larvae were randomly distributed into each of six flowthrough chambers, three for stage 40 assessment and three for stage 45 assessment. Length, weight, mortality, abnormalities (edema, skeletal deformities) and selenium were measured at stages 36, 40 and 45. The mortality and abnormality observations from oldest stage (45) were used for effects analysis because these measurements showed the greatest response.

No selenium effects were observed for length or weight of larvae but effects were observed for both abnormalities (edema and skeletal deformities) and survival. Selenium concentrations in eggs from the control fish were 1.61 and 2.68 mg/kg dry weight (dw), and were 7.61, 11 and 20.5 mg/kg dw in eggs from the treatment fish. As stated above larval survival and abnormality frequency was evaluated at stage 45. Because the mortalities for each cohort were recorded up to the sample collection time for abnormalities, a combined effects variable was derived based on the total proportion of hatched larvae which were both alive and without any abnormalities at stage 45. This can be calculated as PS*(1-PA), where PS is the proportion

survival in the test chambers and PA is the proportion of the sample of surviving larvae with abnormalities. The larvae hatched from the batches of eggs with selenium concentrations of 1.61, 2.68, 7.61, 11 and 20.5 mg Se/kg dw had 0.3, 0.3, 13.6, 0.3 and 33.8% combined survival and abnormal (edema and deformities) effects, respectively.

Estimation of the EC_{10} was conducted using weighted nonlinear regression analysis with the threshold sigmoid model equation (TRAP version 1.30a). The binary data (i.e., survival and abnormalities) available in this study would normally be analyzed using the tolerance distribution analysis in TRAP; however, the combined survival/abnormalities effects variable in this study precludes its use because of the different sample sizes for survival and abnormality evaluation. When there are insufficient data for TRAP to fit an effects/exposure curve, an interpolation is conducted with the same TRAP equation, but constrained to provide interpolation between two points.

Since the study yielded only one definite partial effect, TRAP cannot be used to estimate a concentration-response curve. Instead, TRAP was used to interpolate between the last two points to estimate the EC_{10} (see Linville summary in Appendix C for detail). The resultant TRAP slope is 2.96 and the interpolated EC_{10} is 15.6 mg/kg.

The white sturgeon EC_{10} of 15.6 mg/kg egg dw is important to include in the criterion analyses because this species a commercially and recreationally important fish species in the Pacific Northwest, and also serves as a surrogate for other sturgeon species in the United States (see **Section 6.4**, Protection of Threatened or Endangered Species), and has a population listed as endangered in the Kootenai River in Idaho and Montana.

3.1.1.2 Salmonidae

Acceptable studies were available for three salmonid genera, *Oncorhynchus*, *Salvelinus* and *Salmo*. All of these studies evaluated the effects of selenium on salmonid embryo/larval survival and deformity and used wild-caught adults taken from selenium contaminated streams and spawned for effects determination. Exposure for all studies was therefore through the parents. Summaries of the studies with *Salvelinus* are discussed in **Section 6.1.2.3**; *Oncorhynchus* and brown trout (*Salmo trutta*) are discussed below.

Oncorhynchus mykiss (rainbow trout) Holm (2002) and Holm et al. (2005) obtained eggs and milt from ripe rainbow trout collected from reference streams and streams containing elevated selenium from an active coal mine in Alberta, Canada. In 2000, 2001 and 2002 eggs

were fertilized and monitored in the laboratory until swim-up stage, for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. No significant differences among sites were observed for percent fertilization and mortality. Percentages of embryonic deformities and edema were significantly different among streams, but rates of deformities at Wampus Creek, one of the reference streams, were often similar to or higher than deformities at streams with elevated concentrations of selenium (see Holm summary in Appendix C). The measurement of selenium in the otolith layers of rainbow trout collected in this watershed showed low selenium exposure in the fish's early life and a higher exposure to selenium during the fish's adult years (Palace et al. 2007), suggesting that individuals that reach adulthood tend not to start their lives in streams with elevated selenium concentrations, even though they may reside there later.

Craniofacial deformities, skeletal deformities and edema in rainbow trout embryo, as a function of selenium in egg wet weight (ww), were fitted to a curve using a weighted regression and a threshold sigmoidal equation (TRAP) from which EC_{10} values were calculated (see Appendix C for tables and figures). EC estimates for finfold deformities, length and weight of rainbow trout embryos could not be made because of inadequate dose-response data. The most sensitive endpoint was edema with an EC_{10} value of 9.5 mg Se/kg egg ww or 24.5 mg Se/kg egg dw. The conversion of ww to dw used the average percent moisture of 61.2% for rainbow trout eggs reported by Seilor and Skorupa (2001).

Oncorhynchus clarkii lewisi (Westslope cutthroat trout)

In a field study similar to those conducted by Holm et al. (2005), Rudolph et al. (2008) collected eggs from Westslope cutthroat trout from Clode Pond (exposed site) and O'Rourke Lake (reference site). Clode Pond is on the property of Fording River Coal Operations in Southeast British Columbia with reported selenium concentrations of 93 µg/L. O'Rourke Lake is an isolated water body into which Westslope cutthroat trout were stocked in 1985, 1989 and 1992 and has selenium levels reported as <1 µg/L. Eggs with the four highest Se concentrations (86.3 to 140 mg/kg dw) collected from Clode Pond fish died before reaching the laboratory. Of those eggs from both ponds that survived, there was no correlation between egg selenium concentration and frequency of deformity or edema in the fry. The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs; the EC_{10} estimate for alevin survival based on the concentration of selenium in the eggs is 24.7 mg Se/kg

dw. See Appendix C for details on the statistical analysis and how it differed from the previous draft(s).

As a follow-up to the study by Rudolph et al. (2008), Nautilus Environmental (2011) conducted a more extensive study with Westslope cutthroat trout at the same site. Adult Westslope cutthroat trout were collected from lentic and lotic environments from locations near the mining operations. The lentic fish were primarily captured in Clode Pond, a settling area used to improve water quality of the mining discharge. Lotic fish were collected from the Fording River and its tributaries near the mining operation. Reference females were obtained from Connor Lake which is located within the watershed but not exposed to mining discharges. The researchers reared fertilized eggs from the caught females in the laboratory until they reached swim-up fry stage. A subset of fry surviving at swim-up were reared for an additional 28 days. The most sensitive endpoint was larval survival at swim-up with an EC_{10} of 27.7 mg/kg egg dw determined by interpolation between the one partial effect (20.8% survival at 34.2 mg Se/kg and average NOEC of 87.2% survival at 24.8 mg Se/kg; see Appendix C for detail and how this statistical analysis differed from the previous draft(s)). This result is very similar to the EC_{10} of 24.7 mg/kg egg dw determined for the data generated by Rudolph et al. (2008). See Appendix C for more details on the Nautilus Environmental (2011) study.

The GMCV for *Oncorhynchus* reproductive endpoints is 25.3 mg Se/kg EO. This GMCV is the geometric mean of the *O. mykiss* EC_{10} of 24.5 mg (Holm 2002 and Holm et al. 2005) and the SMCV of 26.2 mg Se/kg EO dw for *O. clarkii.* The *O. clarkii* SMCV was based on the EC_{10} values of 24.7 mg Se/kg EO from Rudolph et al (2008) and 27.7 mg Se/kg EO dw from Nautilus Environmental (2011).

Salmo trutta (brown trout)

Formation Environmental (2011) collected adult female and male brown trout from sites with low and high selenium exposure in the vicinity of a phosphate mine located in Southeastern Idaho in November 2007. Eggs were collected from 26 gravid females across three sampling locations, fertilized with milt collected from several males from the same site and taken to the laboratory for hatching and observation of larval malformations and survival. In addition to the field collected fish, fertilized eggs of 14 females from two separate hatcheries were used in the study.

The study had two phases, hatch-to-swim up, and swim up-to-15 days post swim-up. There are two experimental complications that affect the interpretation of these data: (a) elevated deformity rates among the offspring that were to serve as hatchery-originated method controls (very low selenium exposure) and among some of the low exposure field-collected organisms, and (b) the accidental loss of a number of individuals from several treatments during the 15-day post swim up portion of the test due to overflow of the tank water. The current criterion document's analysis is still based on the revised count data from AECOM (2012), which built upon and superseded EPA's 2012 analysis (Taulbee et al. 2012), peer reviewed by ERG (2012).

Approximately 600 eggs/female were obtained from the majority of the field and hatchery-collected females; however, the numbers of eggs per female ranged from 20 to 609. Selenium concentrations were measured for a subsample of eggs taken from each field and hatchery-collected female. EPA's primary evaluation in this document is of the survival of larvae from hatch to swim-up. Hatching success and larval survival were monitored to swim-up, at which time the fry were thinned to a maximum of 100 individuals for monitoring survival for 15 days post swim-up. Larvae from 24 field collected and 14 hatchery collected females were assessed for survival, as no larvae hatched from the eggs of two of the 26 field collected females.

Because of uncertainties regarding how best to address the loss of fish during the overflow event during the second phase of the test, and also because of the preferential selection of healthy fish during the thinning process prior to the post-swim-up portion of the test, where only those individuals presumed to be healthy were retained for assessment of deformities, EPA used survival during only the first portion of the test (hatch to swim-up), as it provides a more reliable chronic value.

The dataset of percent survival from hatch to swim-up versus the selenium concentration in eggs is an excellent dataset and provides a good foundation for setting numeric effect concentrations for selenium. There is a narrow range between the NOEC (20.5 mg/kg) and a LOEC with severe effects (26.8 mg/kg, 61% mortality) that leaves little uncertainty in what an appropriately protective effects concentration should be. There are sufficient data for TRAP to estimate a curve, using weighted least-squares nonlinear regression with the threshold sigmoidal model. The weighting factor for the 33 no-effect points is their standard deviation, and the weighting factor for the 5 effect points is their residual standard deviation. The TRAP parameter values are 96.2% for background survival, 1.45 for the logEC_{50} (EC_{50}=28.2 mg/kg), and 4.28 for

the slope. The EC_{10} is estimated to be 21.0 mg/kg, slightly higher than the NOEC of 20.5 mg/kg. The weighted TRAP model curve fits the 5 higher effects data well, which forces the EC_0 estimate down to 16.4 mg/kg, below two of the points in the background range. In particular, the fitted curve goes through the NOEC data point at 20.5 mg/kg, so that this point is considered to be an EC_8. This is reasonable because the response is so steep at concentrations above this point that some effect at this point is plausible, and also provides further support of an EC_{10} at 21.0 mg/kg. **Section 6.1.6** provides a summary of the analysis that led to the final selection of the EC_{10} for larval survival during the first portion of the test. Appendix C presents details of the study and analysis.

3.1.1.3 Centrarchidae

Lepomis macrochirus (bluegill sunfish)

In a laboratory study, Doroshov et al. (1992a) exposed adult bluegill for 140 days to three dietary treatments of seleno-L-methionine (nominal dietary concentrations of 8, 18 and 28 mg Se/kg) added to trout chow. Near the end of the exposure, ripe females were induced to ovulate and ova were fertilized *in vitro* with milt stripped from males. Fertilized eggs were sampled for fertilization success and selenium content. They were also used in two tests, (a) a larval development study during the first 5 days after hatching, and (b) a 30-day embryo-larval test. In the 30-day larval survival test, statistical difference from the control was only found in the highest test treatment for survival and growth (length and weight) measurements. In the 5-day larval test, the average proportion of larvae with edema was 0% at an egg concentration of 8.33 mg Se/kg (8 mg/kg dietary treatment), 5% at an egg concentration of 19.46 mg Se/kg dw (18 mg/kg dietary treatment), and 95% at an egg concentration of 38.39 mg Se/kg dw (28 mg/kg dietary treatment). The latter two were statistically different from the control (0% edema). All edematous larvae died in the high treatment.

This analysis focuses on the available data for the individual replicates for the 4-day data (5-day were not used because of almost complete mortality at the highest treatment). Of the 33 edema measurements, only 15 could be used because not all the replicate egg concentrations were reported. Table 4 in the Doroshov et al. (1992a) summary in Appendix C shows both individual replicates and the treatment averages, which are only slightly different than the 5-day data (averages) previously used in the selenium document. Individual replicates rather than

treatment means were used because the exposure concentrations vary substantially and effects are correlated with exposure within the treatment (illustrated by nominal dietary treatments of 18 mg/kg (with corresponding Se concentrations in eggs at that nominal treatment level ranging from 8.55 to 30.20 mg/kg) and 28 mg/kg (with corresponding Se concentrations in eggs ranging from 25.21 to 52.18 mg/kg; see Appendix C for details).

TRAP was fitted to the available data based on the individual replicates and the treatment means using the tolerance distribution option with the log-triangular distribution. In both cases, the TRAP program indicates that the dataset contains inadequate partial responses because the partial responses are less than 10% or greater than 90%, and there are no data (responses) between 10 and 90%. However, for this dataset, these partial responses at both ends of the concentration response curve are sufficiently informative based on multiple lines of evidence (e.g., same response on both days 4 and 5, other endpoints that show effects at dietary treatment of 18 mg/kg, and several instances of edema at dietary treatment 18 mg/kg in contrast to absolutely none for many observations at any lower concentration). Also, because dietary treatment 18 mg/kg does have an effect of several percent or so, estimating the EC_{10} near these points is defensible. Therefore, the EC_{10} estimated using separate replicates is 22.6 mg/kg dw.

A similar study with similar results was done by Coyle et al. (1993) in which two year old pond-reared bluegill sunfish were exposed in the laboratory and fed (twice daily *ad libitum*) Oregon moist™ pellets containing increasing concentrations of seleno-L-methionine. Water concentrations were nominal 10 µg Se/L. The fish were grown under these test conditions for 140 days. Spawning frequency, fecundity, and percentage hatch were monitored for 60 days from the initiation of spawning. There was no effect from the highest dietary selenium concentration (33.3 mg Se/kg dw) on adult growth, condition factor, gonadal somatic index, or other endpoints (Appendix C). The effect of interest in this study was 30-d larval survival after hatch (deformities weren't examined and other reproductive endpoints showed no effect at the highest exposure). For this survival endpoint, there was complete mortality after one week at the highest exposure and no significant differences in survival at lower concentrations.

Previously, the day 5 data were used in the analysis described in Appendix C because this was the only day in which control survival was greater than 90%, with the control and all the treatments showing substantial and increasing toxicity over the next 4 days. However, upon closer analysis, EPA asserts that this is not sufficient cause to base the assessment, because from

day 6 through day 30, survival at the fifth treatment was greater than survival in the first and third treatments, indicating this is not an effect level. These later data (day 6-30) establish that the highest treatment is best considered an EC_{100} and the fifth treatment an EC_0. Therefore, the TRAP interpolation was redone using 42 mg/kg as an EC_{100} rather than an EC_{93}, resulting in a slope of 7.6 and an EC_{10} of 26.3 mg Se/kg dw in eggs.

Hermanutz et al. (1992), and Hermanutz et al. (1996) exposed bluegill sunfish to sodium selenite spiked into artificial streams (nominal test concentrations: 0, 2.5, 10, and 30 µg Se/L) which entered the food web, thus providing a simulated field exposure (waterborne and dietary selenium exposure). In an effort originally intended to improve the rigor of the statistical analysis of the Hermanutz et al. (1996) data, Tao et al. (1999) re-examined the raw data records and made corrections to the counts. This criterion document considers the Hermanutz et al. (1992) data and the Tao et al. (1999) re-examination of Hermanutz et al. (1996).

These data come from a series of three studies lasting from 8 to 11 months, conducted over a 3-year period. All three studies began with exposure of adult bluegill sunfish in the fall, and with respective studies ending in the summer of the following year. Temperatures averaged 4.6, 4.1 and 4.5°C during the winter months, and averaged 26.4, 23.9 and 22.4°C during the spawning months (June-July) for Studies I, II and III, respectively. Spawning activity was monitored in the stream, and embryo and larval observations were made *in situ* and from fertilized eggs taken from the streams and incubated within egg cups in the laboratory. None of the adult bluegill exposed to the highest concentration of selenium in the water (Study I, mean measured concentration equal to 29.4 µg/L) survived the entire exposure period (although a few did survive to spawn). Reduced survival and increased deformities on offspring were observed in the selenium-dosed streams in both Study I and Study II, but were not found during Study III (recovering from contamination, no active selenium input/treatment). The incidence of edema, lordosis, hemorrhage and larval survival in the one stream concentration common to both Study I and II, 10 µg/L, ranged from 80 to 100 percent, 5 to 18 percent, 27 to 56 percent, and 29 to 58 percent, respectively over the three years (combined egg cup and nest observations). Edema, lordosis, and hemorrhage in the lowest stream concentration in Study II, 2.5 µg/L, ranged from 0 to 4 percent, 0 to 25 percent, and 3.6 to 75 percent, respectively (combined egg cup and nest observations); larval survival was 71.6 percent (72 and 75 percent in the control streams). (See Hermanutz 1996 and 1992 in Appendix C for more detail). The effects were not observed in

larvae from fish that were not exposed to elevated concentrations of selenium (control treatment). The mean concentrations of selenium in bluegill ovaries, measured at the end of each study, ranged from 2.2 to 5.0 mg/kg dw in controls, 7.6 to 14 mg/kg dw in the 2.5 µg/L treatments, 34 to 52 mg/kg dw in the 10 µg Se/L treatments, and 16 to 55 mg/kg dw in recovering 30 µg/L treatments. Muscle and whole-body measurements were also available. For all three tissue types, concentrations measured during the spring of each exposure period were not used because they were not sufficiently co-occurrent with the observation of effects. It should also be noted that in contrast to more recent field studies, the tissue concentrations cannot be linked from particular adult females to effects on her offspring, but only from an aliquot of females in the treatment to all offspring observed in the treatment.

The egg-cup data for all streams of Studies I, II, and III of this experiment were combined and analyzed in response to measured selenium concentration in the maternal ovaries (mg/kg dw) using TRAP. That is, data for streams receiving water-borne selenium were combined with data for streams recovering from the previous year's contamination. The absence of effects at high tissue levels (55 mg Se/kg ovary dw) in the recovering stream of Study II did not affect the EC_{10} estimate because it was outweighed by three other points showing severe effects at concentrations as low as 16.7 mg Se/kg ovary dw. However, this one observation is suggestive but not definitive corroboration for the field observations of biological recovery in Belews Lake and Hyco Reservoir after selenium loads were reduced, but while tissue concentrations remained relatively high (Lemly 1997a; Crutchfield 2000; Finley and Garrett 2007).

Several egg-cup endpoints were analyzed by TRAP independently (% edema, % lordosis, and % hemorrhage) and in combination (% normal and surviving). The best fit and most sensitive was the combined percent normal and surviving larvae. Due to inadequate partial effects for the ovary analysis, a threshold sigmoidal model was used to interpolate an EC_{10} estimate between the first interpolation point set to the highest no observed effect concentration (HNOEC) of 14.0 mg/kg and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.7 mg/kg and a survival/normal of 5.76%. The resulting EC_{10} is 14.7 mg/kg ovary dw. Chronic values for muscle and whole body based on percentage surviving and free of deformities are 13.4 mg Se/kg muscle dw and 10.6 mg Se/kg whole body dw. (See Appendix C for more discussion of this study).

The SMCV for bluegill reproductive endpoints based on EC_{10} values is 20.6 mg Se/kg dw in egg/ovary, based on the EC_{10} values of 22.6 mg/kg dw in the Doroshov et al. (1992a) study, 26.3 mg/kg dw in the Coyle et al. (1993) study, and 14.7 mg/kg dw for Hermanutz et al. (1992, and 1996 as corrected by Tao et al. 1999).

3.1.2 Summary of Acceptable Studies of Fish Reproductive Effects

Table 3.1 summarizes the effect concentrations obtained from all acceptable reproductive studies with fish. Summaries of the remainder of the reproductive studies (beyond the four most sensitive genera described above) can be found in **Section 6.1.2** below.

Table 3.1. Maternal Transfer Reproductive Toxicity Studies.

Species	Reference	Exposure Route	Toxicological Endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Salvelinus malma Dolly Varden	Golder 2009	dietary and waterborne (field: Kemess Mine NW British Columbia)	EC_{10} for total deformities	56.2 E	56.2 E	56.2 E
Esox lucius northern pike	Muscatello et al. 2006	dietary and waterborne (field: Saskatoon, Sask.)	EC_{24} larval deformities	34.0 E	34.0 E	34.0 E
Cyprinodon macularius desert pupfish	Besser et al. 2012	dietary and waterborne (lab)	Estimated EC_{10} for offspring survival	27 E	27 E	27 E
Micropterus salmoides largemouth bass	Carolina Power & Light 1997	dietary (lab)	EC_{10} for larval mortality & deformity	26.3 O	26.3 O	26.3 O
Pimephales promelas fathead minnow	Schultz and Hermanutz 1990	dietary and waterborne (mesocosm: Monticello)	LOEC for larval edema and lordosis	<25.6 E[b]	NA[c]	NA
Oncorhynchus mykiss rainbow trout	Holm 2002; Holm et al. 2003, 2005	dietary and waterborne (field: Luscar River, Alberta)	EC_{10} for skeletal deformities	24.5 E[b]	24.5 E	25.3 E
Oncorhynchus clarkii lewisi Westslope cutthroat trout	Rudolph et al. 2008	dietary and waterborne (field: Clode Pond, BC)	EC_{10} for alevin mortality	24.7 E	26.2 E	
Oncorhynchus clarkii lewisi Westslope cutthroat trout	Nautilus Environmental 2011	dietary and waterborne (field: Clode Pond & Fording River, BC)	EC_{10} for survival at swim-up	27.7 E		
Salmo trutta brown trout	Formation Environmental 2011; AECOM 2012	dietary and waterborne (field: Lower Sage Creek & Crow Creek, ID)	EC_{10} for larval survival	21.0 E	21.0 E	21.0 E

Species	Reference	Exposure Route	Toxicological Endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Lepomis macrochirus bluegill	Doroshov et al. 1992a	dietary (lab)	EC_{10} larval edema	22.6 E	20.6 E	20.6 E
Lepomis macrochirus bluegill	Coyle et al. 1993	dietary and waterborne (lab)	EC_{10} for larval survival	26.3 E		
Lepomis macrochirus bluegill	Hermanutz et al. 1992, 1996	dietary and waterborne (mesocosm: Monticello)	EC_{10} for larval edema	14.7 O[b]		
Acipenser transmontanus white sturgeon	Linville 2006	dietary (lab)	EC_{10} for combined edema and deformities	15.6 E	15.6 E	15.6 E

E–Concentration reported in egg; O– concentration reported in ovary

[a] All chronic values reported in this table are based on the measured concentration of selenium in egg/ovary tissues.

[b] Tissue value converted from ww to dw. See Appendix C for conversion factors.

[c] SMCV not calculated due to variability in the observations among replicates in Schultz and Hermanutz (1990). The chronic value is presented in this table to show it is in the range of selenium effect concentrations. See Appendix C for detail. Also, see Appendix E for an additional study with fathead minnow.

In order of their sensitivity to selenium, **Table 3.2** presents the Genus Mean Chronic Values from acceptable fish reproductive-effect studies that have been measured in terms of egg-ovary concentrations.

Table 3.2. Ranked Genus Mean Chronic Values for Fish Reproductive Effects Measured as Egg or Ovary Concentrations.

Rank	GMCV* (mg Se/kg dw EO)	Species	SMCV (mg Se/kg dw EO)
8	56.2	Dolly Varden, *Salvelinus malma*	56.2
7	34**	Northern pike, *Esox lucius*	34
6	27	Desert pupfish, *Cyprinodon macularius*	27
5	26.3	Largemouth bass, *Micropterus salmoides*	26.3
4	25.3	Cutthroat trout, *Oncorhynchus clarkii*	26.2
		Rainbow trout, *Oncorhynchus mykiss*	24.5
3	21.0	Brown trout, *Salmo trutta*	21.0
2	20.6	Bluegill sunfish, *Lepomis macrochirus*	20.6
1	15.6	White sturgeon, *Acipenser transmontanus*	15.6

* This table excludes *Gambusia*, which has a reproductive chronic value expressed as adult whole-body rather than egg-ovary, because it is a live bearer.
** The Northern Pike SMCV is an EC_{24} based on larval deformities (**Table 3.1**). The EC_{10} is less than 34 mg/kg.
This table excludes GMCV for *Pimephales* due to uncertainty in the chronic value for the Schultz and Hermanutz (1990) study; the estimate of <25.6 mg/kg egg dw in **Table 3.1** shows it is in the range of reproductive effect levels for selenium (See Appendix C for study details).

3.1.3 Derivation of Tissue Criterion Element Concentrations

Data used to derive the final chronic value were differentiated based on the effect (reproductive and non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for 10 fish genera. Acceptable chronic toxicity data on non-reproductive effects are available for 7 fish genera and 3 invertebrate genera. The fish non-

reproductive effects data were not used to calculate tissue criterion elements because they were more variable and less reproducible than the data on reproductive effects. The genus sensitivity distribution is predominantly populated with data on fish species because field evidence demonstrated that fish communities were affected in situations having no observable change in the accompanying diverse array of invertebrate communities. As a result, decades of aquatic toxicity research have focused primarily on fish. The studies that have been done with invertebrates (**Table 3.8**, **Section 3.1.4**) have shown them to be more tolerant than most of the tested fish species.

Also, while amphibians are potentially sensitive due to physiologic similarities to fish, effects clearly attributable to selenium are largely unknown (Unrine et al. 2007; Hopkins et al. 2000; Janz et al. 2010). Hopkins et al. (2000) reported that amphibian larvae at sites receiving coal combustion wastes appear to efficiently accumulate selenium in their tissues and possibly due to selenium have exhibited axial malformations. In a recent laboratory exposure, Massé et al. (2015) determined an EC_{10} of 44.9 mg/kg Se for the African clawed frog (*Xenopus laevis*) suggesting that amphibians are similar to the less sensitive fish species (see **Section 6.1.4**).

3.1.3.1 Fish Egg-Ovary Concentration

The lowest four GMCVs from **Table 3.2** are shown below in **Table 3.3**.

Table 3.3. Four Lowest Genus Mean Chronic Values for Fish Reproductive Effects.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw egg-ovary)
4	*Oncorhynchus*	25.3
3	*Salmo*	21.0
2	*Lepomis*	20.6
1	*Acipenser*	15.6

With N=15 GMCVs (see **Section 3.1.6**), the 5th percentile projection yields an egg/ovary criterion element concentration of 15.1 mg Se/kg dw egg/ovary, lower than the most sensitive fish species tested, white sturgeon (*A. transmontanus*). The egg/ovary criterion element concentration is compared to the distribution of egg/ovary chronic values in **Figure 3.1**.

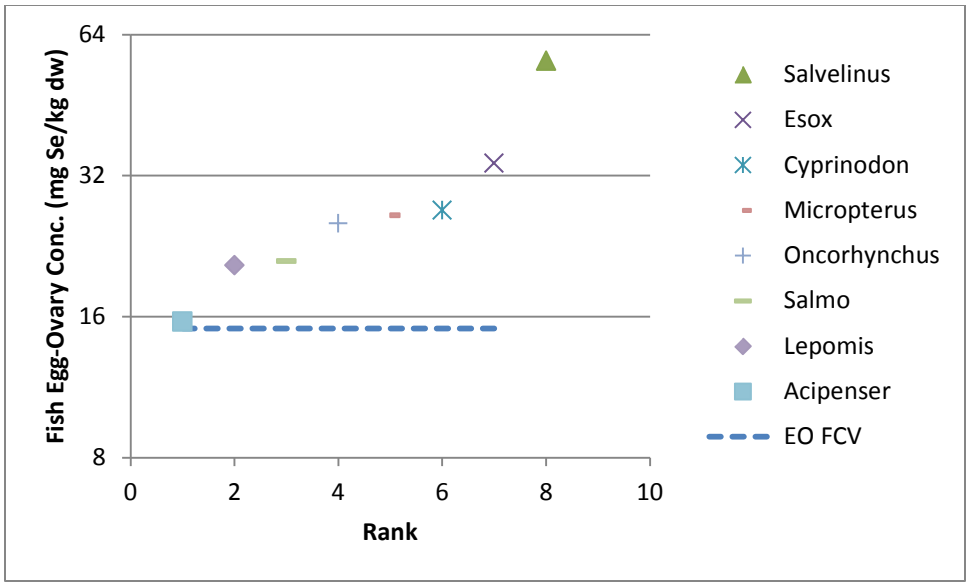


Figure 3.1. Distribution of Reproductive-Effect GMCVs for Fish Measured as Egg-Ovary Concentrations.

3.1.3.2 Fish Whole-Body Criterion Element Concentration

Whole body reproductive chronic values were calculated directly from whole body tissue concentrations measured in the study or by applying an egg-ovary (EO) to whole-body (WB) conversion factor (CF) presented subsequently in **Section 3.2.2.2**. Direct calculations were done when whole body measurements were available in the study and the data were amenable to an effect level determination. **Table 3.4** provides the chronic values for each fish genus and whether it was calculated directly or converted from the reproductive-effect egg-ovary concentrations to whole-body concentrations using a CF. The final EO/WB CF applied to each taxon was determined using a hierarchical approach based on taxonomic relatedness, and is described in **Section 3.2.2**, and in greater detail in Appendix B. The four most sensitive reproductive-effect fish whole-body GMCVs are shown in **Table 3.5**.

Table 3.4. Tested Reproductive-Effect Whole Body (WB) Concentrations Measured Directly or Converted to WB Concentrations from Egg-Ovary (EO) Concentrations.

Taxon*	EO Chronic Value	EO/WB CF	Direct or Calculated WB Repro Chronic Value	Direct Calculation or Basis for EO/WB CF (from Appendix B)
Salvelinus	56.2	1.61	34.9	Dolly Varden EO/M (1.26) x all fish M/WB (1.27)
Esox	34.0	2.39	14.2	Northern pike EO/M (1.88) x all fish M/WB (1.27)
Cyprinodon	27.0	1.20	22.6	Desert pupfish EO/WB
O. mykiss	24.5	2.44	10.0	Rainbow trout EO/M (1.92) x all fish M/WB (1.27)
Rudolph et al. 2008	24.7	1.96	12.6	*Oncorhynchus* EO/WB
Nautilus 2011	27.7	1.96	14.1	*Oncorhynchus* EO/WB
O. clarkii	26.2	NA	13.3	Geometric mean of two studies
Oncorhynchus	25.3	NA	11.6	Geometric mean of *O. mykiss* and *O. clarkii* WB SMCVs
Micropterus	26.3	1.42	18.5	*Micropterus* EO/WB
Salmo	21.0	NA	13.2	Directly calculated EC_{10}
Coyle et al. 1993	26.3	NA	8.6	Directly calculated EC_{10}
Doroshov et al. 1992a	22.6	2.13	10.6	Bluegill sunfish EO/WB
Hermanutz et al. 1992, 1996	14.7	NA	10.6	Directly calculated EC_{10}
Lepomis	20.6	NA	9.9	Geometric mean of three studies
Acipenser	15.6	1.69	9.2	White sturgeon EO/M (1.33) x all fish M/WB (1.27)

* The GMCV for *Gambusia*, a live bearer, not included in the conversion table, was originally measured as adult WB, not EO, and is >13.38 mg Se/kg dw WB. The "greater than" sign signifies that no effects were found at the highest observed concentrations. This table also excludes *Pimephales* due to uncertainty in the chronic value for the Schultz and Hermanutz (1990) study (See Appendix C for details).

Table 3.5. The Lowest Four Reproductive-Effect Whole-Body GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw whole-body)
4	*Salmo*	13.2
3	*Oncorhynchus*	11.6
2	*Lepomis*	9.9
1	*Acipenser*	9.2

Because the factors used to convert egg-ovary to whole-body concentrations vary across species, the whole-body rankings differ from the egg-ovary rankings. With N=15 GMCVs, the 5th percentile projection yields a whole body criterion element concentration of 8.5 mg Se/kg dw whole-body, slightly lower than the most sensitive fish species tested, white sturgeon (*Acipenser transmontanus*). The fish whole body criterion element is compared to the distribution of fish whole-body reproductive chronic values in **Figure 3.2**.

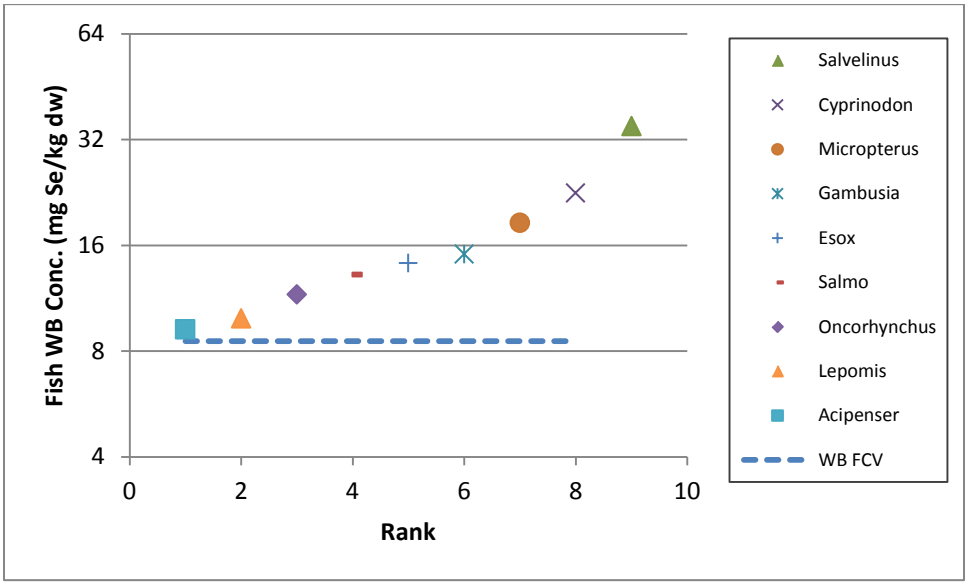


Figure 3.2. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Whole-Body Concentrations in the Original Tests, or Measured as Egg-Ovary Concentrations but Converted to Whole-Body.
(As shown in **Table 3.4**).

3.1.3.3 Fish Muscle Criterion Element Concentration

Reproductive chronic values for muscle tissue were calculated directly from muscle tissue concentrations measured in the study or from the egg-ovary to muscle conversion factors of the bioaccumulation modeling approach (presented in **Section 3.2**). Direct calculations were made when muscle measurements were available in the study and the data were amenable to an effect level determination. The final EO/M CF applied to each taxon was determined using a hierarchical approach based on taxonomic relatedness, consistent with the approach used to calculate EO/WB CFs described in **Section 3.2.2**.

Table 3.6 provides the chronic values for each fish taxa and whether it was calculated directly or converted from reproductive-effect egg-ovary concentrations to muscle concentrations. The four most sensitive reproductive-effect fish muscle GMCVs are shown in **Table 3.7**.

Table 3.6. Tested Reproductive-Effect Muscle (M) Concentrations Measured Directly or Converted to M Concentrations from Egg-Ovary (EO) Concentrations.

Taxon	EO Chronic Value	EO/M CF	Direct or Calculated Muscle Repro Chronic Value	Direct Calculation or Basis for EO/M CF (from Appendix B)
Salvelinus	56.2	1.26	44.5	Dolly Varden EO/M
Esox	34.0	NA	21.7	Directly determined EC_{24}
Cyprinodon	27.0	0.94	28.7	Desert pupfish EO/WB (1.20) / all fish M/WB (1.27)
O. mykiss	24.5	1.92	12.8	Rainbow trout EO/M
Rudolph et al. 2008	24.7	NA	16.6	Directly calculated EC_{10}
Nautilus 2011	27.7	1.81	15.3	Cutthroat trout EO/M
O. clarkii	26.2	NA	16.0	Geometric mean of two studies
Oncorhynchus	25.3	NA	14.3	Geometric mean of two SMCVs
Micropterus	26.3	1.19	22.2	*Micropterus* EO/M
Salmo	21.0	1.14	18.5	Brown trout EO/WB (1.45) / all fish M/WB (1.27)

Taxon	EO Chronic Value	EO/M CF	Direct or Calculated Muscle Repro Chronic Value	Direct Calculation or Basis for EO/M CF (from Appendix B)
Coyle et al. 1993	26.3	1.38	19.1	Bluegill sunfish EO/M
Doroshov et al. 1992a	22.6	NA	15.7	Directly calculated EC_{10}
Hermanutz et al. 1992, 1996	14.7	NA	13.4	Directly calculated EC_{10}
Lepomis	20.6	NA	15.9	Geometric mean of three studies
Acipenser	15.6	NA	11.9	Directly calculated EC_{10}

Table 3.7. The Lowest Four Reproductive-Effect Fish Muscle GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw muscle)
4	*Salmo*	18.5
3	*Lepomis*	15.9
2	*Oncorhynchus*	14.3
1	*Acipenser*	11.9

Because the factors used to convert egg-ovary to muscle concentrations vary across species based on empirical data, the whole-body rankings differ from both from the egg-ovary rankings and the muscle rankings. With N=15 GMCVs, the 5th percentile projection yields a muscle criterion element concentration of 11.3 mg Se/kg dw muscle, lower than the muscle value for the most sensitive fish genus tested, *Acipenser*. **Figure 3.3** compares the fish muscle criterion element concentration to the distribution of fish muscle reproductive chronic values in **Table 3.6**.

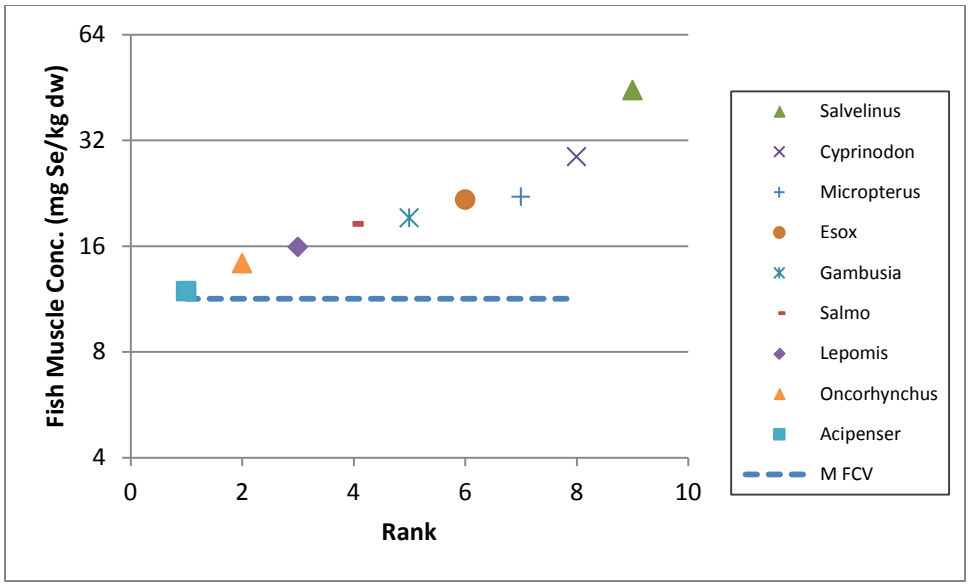


Figure 3.3. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Muscle in the Original tests, or Measured as Egg-Ovary Concentrations but Converted to Muscle Concentrations.
(As shown in **Table 3.6**). (Live-bearer *Gambusia* was converted from WB to muscle).

3.1.4 Invertebrate Chronic Effects

Below is a brief synopsis of the experimental design of the available invertebrate chronic toxicity tests, and the resulting chronic values.

Brachionus calyciflorus (rotifer)

Dobbs et al. (1996) exposed *Brachionus calyciflorus* to selenate in natural creek water for 25 days in a three-trophic level food chain test system. This is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In the Dobbs et al. (1996) study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but consumed selenium bioaccumulated in the next lower trophic level. Rotifers did not grow well at concentrations exceeding 108.1 µg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 µg Se/L in the water (40 µg Se/g dw in the algae). Regression analysis of untransformed growth data (dry weight), determined 4 day post-test initiation, resulted in a calculated EC_{10} of 37.84 µg Se/g dw tissue.

Lumbriculus variegatus (oligochaete, blackworm)

Although not intended to be a definitive toxicity study for blackworms, Besser et al. (2006) evaluated the bioaccumulation and toxicity of selenized yeast to the oligochaete, *Lumbriculus variegatus,* which was intended to be used for dietary exposure in subsequent studies with the endangered desert pupfish, *Cyprinidon macularius*. Oligochaetes fed selenized-yeast diets diluted with nutritional yeast (54 to 210 mg Se/kg) had stable or increasing biomass and accumulated Se concentrations as high as 140 mg/kg dw. The oligochaetes fed the undiluted selenized-yeast (826 µg/g Se dry wt.) showed reduced biomass. The effect level is considered >140 mg Se/kg dw.

Centroptilum triangulifer (mayfly)

Mayfly larvae (*Centroptilum triangulifer*) were exposed to dietary selenium contained in natural periphyton biofilms to eclosion (emergence) (Conley et al. 2009; Conley et al. 2011; Conley et al. 2013). In Conley et al. (2009), the periphyton fed to the mayfly larvae were exposed to dissolved selenite (radiolabeled ^{75}Se) in November 2008 (12.6 and 13.9 µg/L) and in January 2009 (2.4, 2.4, 4.9, 10.3, and 10.7 µg/L). Periphyton bioconcentrated selenium an average of 1113-fold over the different aqueous selenium concentrations (see Table E-22 in Appendix E). Twenty 4 to 6-day old mayfly larvae were exposed for 4.5 to 6 weeks to each of the periphyton diets until the larvae eclosed to subimagos (final pre-adult winged stage). The subimagos were allowed to emerge to the adult imago stage which deposited their egg masses in Petri dishes. Selenium concentrations were measured in postpartum adults along with their dry weights and clutch size. Selenium increased in concentration from periphyton to the adult mayflies (trophic transfer factor) an average of 2.2-fold. The authors observed a reduction in fecundity with diets containing more than 11 mg Se/kg dw, which is considered the dietary threshold for this study. Using the trophic transfer factor of 2.2, the periphyton selenium concentration of 11 mg/kg dw translates to an adult mayfly selenium concentration of 24.2 mg/kg dw.

Conley et al. (2011) exposed larval *C. triangulifer* larvae similar to Conley et al. (2009) to two different rations of periphyton (1x and 2x) containing low, medium and high selenium levels to evaluate the effect of feeding ration on the bioaccumulation of selenium and life cycle performance of the mayfly. Mayfly larvae were fed either a 1x or 2x ration of periphyton loaded with the three different selenium levels until the larvae eclosed to subimagos after 25-29 days.

Average periphyton Se concentrations for the three treatments in the 1x ration study were 4.2, 11.9, and 27.2 mg/kg dw, respectively. In the 2x ration study, average periphyton concentrations for the three treatments were 9.5, 19.9, and 40.9 mg/kg dw, respectively (Conley et al. 2011). Subimagos were induced to emerge to adults in petri dishes and their clutch size measured through digital imaging. Mayflies fed the 1x ration had 54% and 72% reductions in survival relative to controls in the medium and high Se treatment levels, respectively, both of which were significant ($p<0.05$). The mayflies fed the 1x ration also had significant reductions in fecundity in the low (44% reduction), medium (63% reduction) and high (77% reduction) Se treatment levels. However, for the mayflies that were fed the 2x ration, there were no significant differences between the controls and any of the three Se treatment levels for any of the endpoints measured including survival and fecundity. The 2x ration mayflies had 60% more biomass than the 1x ration mayflies. This growth difference explains why the 1x ration mayflies had higher concentrations of Se in their tissues (see Table E-23 in Appendix E). The two different rations resulted in vastly different effect levels for Se, <12.8 mg/kg dw in the 1x ration test and >37.3 mg/kg dw in the 2x ration. It is apparent from this study that if the mayflies do not obtain sufficient nutrition, they are more sensitive to selenium. Although reduced feeding levels occur in nature, it is a confounding variable in this study that cannot be used to set a chronic effect level for selenium.

Conley et al. (2013) evaluated the accumulation of selenite and selenate into periphyton with a subsequent feeding exposure to mayfly larvae. As in the previous studies, *C. triangulifer* larvae were fed periphyton previously exposed to different concentrations of selenium. In this study, periphyton plates were first exposed to low (10 µg/L) and high (30 µg/L) concentrations of either selenite or selenate and then fed to mayfly larvae to eclosion and to subimagos. The mean concentrations of selenium in the periphyton fed to the mayflies were 2.2, 12.8 and 37 mg/kg Se dw in the control, low and high treatments, respectively. Mayfly tissue (subimago) concentrations (extrapolated from Figure 4a in Conley et al. 2013) were approximately 4-7, 20-35, and 45-75 mg/kg Se dw, in the control, low and high treatments, respectively. The authors reported significant reductions in survival from the control in the high Se treatment (both pooled data and individual selenite and selenate treatments), but no significant differences were observed in the low Se treatments. Secondary production (mayfly biomass) was significantly reduced relative to the control in the high Se treatment for both selenium species. For the low Se

exposure treatments, secondary production was not significantly different than the control for the selenite treated periphyton exposure, but was for the selenate and pooled data suggesting an effect level between 20 and 35 mg/kg Se dw. These results as well as those observed in 2x ration exposures in Conley et al. (2011) where no effects were observed at 37.3 mg/kg Se dw generally support the chronic value determined for Conley et al. (2009) of 24.2 mg/kg Se dw. This information included tabulated data from these studies presented in Appendix E.

3.1.5 Summary of Relevant Invertebrate Tests

The available measured invertebrate whole-body effect concentrations are shown in **Table 3.8**. Because the intent of this assessment is to derive a concentration expressed in terms of fish tissue, **Table 3.8** also provides information on how concentrations in invertebrate tissue are translated (in **Section 3.2**) across media to predicted WB fish tissue concentrations (Trophic Level 3, TL3) in a system having invertebrates and fish. That is, consistent with the bioaccumulation modeling approach of **Section 3.2**, the second column of **Table 3.8** uses the median trophic transfer factor of 1.21 from **Table 3.11** to yield expected WB fish tissue concentrations in a system having invertebrates and fish. Whether comparing TL2 (invertebrate) whole-body GMCVs directly to **Table 3.4** TL3 (fish) whole-body GMCVs, or via the trophic transfer adjustment in the second column of **Table 3.8**, it is apparent that invertebrates are not among the most sensitive species.

The relative insensitivity of invertebrates overall when compared with the fish whole-body concentrations demonstrates that invertebrates are expected to be generally protected by selenium criterion values derived from fish. It should be noted that mayflies appear to be the most sensitive invertebrate group tested; their whole-body effects levels just below the *least* sensitive fish genus (*Salvelinus*, Dolly Varden) on whole-body basis. However these mayfly results have some uncertainty due to the indicated effect of feeding ration on selenium toxicity to mayflies that has not been fully defined. The rotifer and lumbriculus tests indicate that these organisms are less sensitive than any tested fish genus on a whole-body basis. Therefore, the invertebrates are considered implicitly in the species sensitivity distribution, and are counted toward the number of values available to calculate the fish tissue criterion elements (as egg-ovary, whole-body, and muscle), and the missing invertebrate MDRs (4 and 5) are considered satisfied by the available invertebrate data.

Table 3.8. Ranked Invertebrate Whole-Body Chronic Values with Translation to Expected Accompanying Fish Whole-Body Concentrations

SMCV & GMCV as measured (Trophic Level 2) (mg Se/kg dw WB)	Accompanying Trophic Level 3 Median Whole-Body Concentration Predicted by Bioaccumulation Model (Section 3.2) (mg Se/kg dw WB TL3)	Species
> 140	> 169.4	Oligochaete, black, *Lumbriculus variegatus*
37.84	45.8	Rotifer, *Brachionus calyciflorus*
24.2	29.3	Mayfly, *Centroptilum triangulifer*

3.1.6 Selenium Fish Tissue Toxicity Data Fulfilling Minimum Data Needs

The toxicity data currently available for genera and species fulfilling the EPA Ambient Water Quality Criteria Guidelines recommendations for calculation of the freshwater chronic criterion are described in **Sections 3.1.1, 3.1.4, 6.1.2** and Appendix C, and are summarized in **Table 3.9**.

Table 3.9. Minimum Data Requirements Summary Table Reflecting the Number of Species and Genus Level Mean Values Represented in the Chronic Toxicity Dataset for Selenium in Freshwater.

Freshwater Minimum Data Requirement	Genus Mean Chronic Value (GMCV)	Species Mean Chronic Value (SMCV)
1. Family Salmonidae in the class Osteichthyes	3	4
2. Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	2	2
3. Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	5	5
4. Planktonic Crustacean	See text	See text
5. Benthic Crustacean	See text	See text
6. Insect	1	1
7. Family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida, or Mollusca)	1	1
8. Family in any order of insect or any phylum not already represented	1	1
Total	**15**	**16**

The first three of these MDRs in **Table 3.9** are easily fulfilled by the fish species represented in **Sections 3.1.1, 6.1.2** and Appendix C. Because the field observations of contaminated sites have found effects on fish and birds in the absence of changes in invertebrate assemblages, scientific studies on the chronic toxicity of dietary selenium for invertebrates has been very limited. The few dietary chronic toxicity studies that are available for invertebrate species (arthropods **,** rotifers, and worms) indicate that they are generally less sensitive than fish, with available data indicating invertebrate whole body mean chronic values ranging from approximately 3 to 12 times higher than the fish whole body criterion element value recommended in this document (**Section 3.1.4**). The above invertebrate data address MDRs 6-8, leaving only MDRs 4 and 5, for the planktonic and benthic crustaceans, to be addressed. Because the 5th percentile calculation methods for the FCV use actual numeric values for the GMCVs of the four most sensitive (fish) genera in the selenium dataset, it is only necessary to know that the more tolerant genera have GMCVs that are greater than those of the lowest four. A recommendation in the draft white paper on Aquatic Life Criteria for Contaminants of Emerging Concern Part I (U.S. EPA 2008b), which was supported by the Science Advisory Board, states "because only the four most sensitive genus mean chronic values (GMCVs) are used in the criterion calculations, chronic testing requirements for a taxon needed to meet an MDR should be waived if there is sufficient information to conclude that this taxon is more tolerant than the four most sensitive genera."

Currently, there are no available data on the chronic toxicity to crustaceans via dietary exposure to selenium. Since there are data available for insects (*Centroptilum spp.* mayfly), EPA used the taxonomic association at the level of phylum (Arthropoda) to allow insects to be a surrogate for crustaceans. There is also associative evidence that macroinvertebrates in general are less sensitive than fish. At sites where there have been documented effects to fish and aquatic-dependent birds from selenium exposure (e.g., Kesterson Reservoir, Belews Lake, Hyco Reservoir), field observations and data indicate that there has been no evidence of effects to macroinvertebrates including crustaceans (Janz et al. 2010). In addition, Janz et al. (2010) notes that the key vector for selenium toxicity via maternal transfer is selenium loading in the egg via vitellogenesis. Crustaceans, and other arthropods are not known to deposit a significant amount of vitellogenin in the egg compared with oviparous vertebrates like fish, therefore, less selenium is likely transferred to the egg via deposition of vitellogenin. These mechanistic considerations

are thus consistent with the absence of observed field effects on aquatic macroinvertebrates, including crustaceans and other arthropods, and with the Chapman et al. (2009, 2010) expert consensus that it is the egg-laying vertebrates that are most at risk.

Applying this concept to the selenium criterion 5th percentile calculations, GMCVs for MDRs 4 and 5 (the two crustacean MDRs) should be waived and counted in the total number of GMCVs in the dataset, based on (a) the difference in the measured effect values discussed above, and (b) the lack of observed invertebrate field effects linked to selenium (for example, as concluded by Lemly 2002, pages 21-23, and Janz et al. 2010). Thus data are adequate to fulfill the data needs for developing a chronic selenium criterion.

The total number of GMCVs available to derive the chronic criterion is 15. These include ten fish genera from **Sections 3.1.1 and 6.1.2** (*Acipenser*, *Salmo*, *Lepomis*, *Micropterus*, *Oncorhynchus*, *Pimephales*, *Gambusia*, *Esox*, *Cyprinodon*, and *Salvelinus*) [Added to these are the tested invertebrate genera *Centroptilum*, *Brachionus*, and *Lumbriculus* from **Section 3.1.4**, and lastly the two waived genera for MDRs 4 and 5 (crustaceans).

3.2 Chronic Water Column-based Selenium Criterion Element

3.2.1 Translation from Fish Tissue Concentration to Water Column Concentration

EPA derived the chronic water column selenium criterion element by translating the egg-ovary concentration to an equivalent water concentration. EPA worked with USGS to derive a translation equation that utilizes a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al. 1992; Wang et. al. 1996; Luoma and Fisher 1997; Wang 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006, 2010; Presser 2013). This model quantifies bioaccumulation in animal tissues by assuming that net bioaccumulation is a balance between assimilation efficiency from diet, ingestion rate, rate of direct uptake in dissolved forms, loss rate, and growth rate. The basic model is given as:

$$C_{tissue} = \frac{\left[\left(k_u \times C_{water}\right) + \left(AE \times IR \times C_{food}\right)\right]}{\left(k_e + g\right)} \qquad \text{(Equation 1)}$$

Where:

C_{water}	=	Concentration of chemical in water (µg/L)
C_{tissue}	=	Average concentration of chemical in all tissues at steady-state (µg/g)
k_e	=	Efflux rate (/d)
g	=	Growth rate (/d)
k_u	=	Uptake rate (L/g-d)
AE	=	Assimilation efficiency (%)
IR	=	Ingestion rate (g/g-d)
C_{food}	=	Concentration in food (µg/g)

3.2.1.1 Simplifying the Bioaccumulation Model

Specific application to selenium bioaccumulation permits the simplification of Equation 1 in two ways. One simplification is removing the parameter representing growth rate (*g*), and the other simplification is removing the parameter representing direct aqueous uptake (k_u).

Growth Rate

The growth rate constant *g* is included in Equation 1 because the addition of body tissue has the potential to dilute the concentration of bioaccumulative chemicals when expressed as chemical mass per tissue mass. For very hydrophobic chemicals with low excretion rates such as polychlorinated biphenyls, growth can be an important factor in bioaccumulation estimates (Connolly and Pedersen 1988). However, Luoma and Rainbow (2005) suggest that for selenium, growth rate is a relatively inconsequential parameter under most circumstances. Food consumption is typically high during periods of high growth rate. Because food consumption is the primary route of selenium uptake in aquatic organisms (Ohlendorf et al. 1986a, b; Saiki and Lowe 1987; Presser and Ohlendorf 1987; Lemly 1985a; Luoma et al. 1992; Presser et al. 1994; Chapman et al. 2010), high consumption rates of selenium-contaminated food may counteract the selenium dilution that occurs with the addition of body tissue during periods of fast growth.

EPA evaluated the effect of removing the parameter *g* in the Equation 1 by performing a sensitivity analysis. EPA analyzed a series of hypothetical tissue concentration estimates using Equation 1 with *g* ranging between 0 (no growth) and 0.2/day (a relatively high rate of growth).

In one analysis, tissue concentrations of selenium were estimated using static values of *IR*. In a second analysis, tissue concentrations of selenium were estimated using values of *IR* that were adjusted for growth rate using a method similar to the approach used in a model of organic chemical accumulation in aquatic food webs (Thomann et al. 1992). As expected, estimates of selenium tissue concentrations were significantly reduced at progressively higher growth rates when *IR* remained constant. However, selenium concentrations remained fairly steady or slightly increased with progressively higher growth rates when *IR* was adjusted for the bioenergetics of growth. This analysis supports the hypothesis that a higher *IR* (and consequently greater rate of selenium ingestion) associated with the higher bioenergetic requirements of rapidly growing young fish tends to oppose the dilution of selenium in their tissues due to growth, whereas a lower *IR* (and consequently lower rate of selenium ingestion) associated with the lower bioenergetic requirements of slower growing older fish tends to oppose the bioconcentration of selenium in their tissues. EPA concludes from this analysis that omitting the growth rate parameter *g* is an appropriate simplification of Equation 1. A more detailed description of this sensitivity analysis is provided in Appendix J.

Uptake Rate

The uptake rate constant k_u is included in Equation 1 to account for direct absorption of bioaccumulative chemicals in the dissolved phase. However, dietary intake of selenium is the dominant source of exposure, suggesting that k_u may also be relatively inconsequential for selenium accumulation (Luoma and Rainbow 2005). Because aqueous uptake of selenium makes up a small percentage of bioaccumulated selenium (Fowler and Benayoun 1976; Luoma et. al. 1992; Roditi and Fisher 1999; Wang and Fisher 1999; Wang 2002; Schlekat et. al. 2004; Lee et. al. 2006), Presser and Luoma (2010a, b, 2013) deemed removal of k_u from Equation 1 as an acceptable simplification.

EPA evaluated the effect of removing the parameter k_u in the Equation 1 by performing a sensitivity analysis. EPA analyzed a series of tissue concentration estimates using Equation 1 and a realistic range of k_u values for trophic level 2 and trophic level 3 organisms. The analysis suggests that approximately 75% of selenium exposure in trophic level 2 organisms (invertebrates) and over 90% of selenium exposure in trophic level 3 organisms occurs through consumption of selenium-contaminated food. EPA concluded that omitting the aqueous uptake

rate constant k_u is an appropriate simplification of Equation 1. A more detailed description of this sensitivity analysis is provided in Appendix J.

Derivation of the Translation Equation

Disregarding growth (g) and uptake of selenium dissolved in water ($k_u \times C_{water}$), Equation 1 becomes Equation 2 (Reinfelder et al. 1998):

$$C_{tissue} = \frac{AE \times IR \times C_{food}}{k_e}$$

or:

$$C_{tissue} = \frac{AE \times IR}{k_e} \times C_{food} \qquad \text{(Equation 2)}$$

Because application of the bioaccumulation model applies to a single species, the combination of species-specific physiological parameters expressed as $\frac{AE \times IR}{k_e}$ remains constant for the species. Thus the EPA defines the expression $\frac{AE \times IR}{k_e}$ as a single species-specific Trophic Transfer Factor (*TTF*) given as Equation 3 (Reinfelder et al. 1998):

$$TTF = \frac{AE \times IR}{k_e} \qquad \text{(Equation 3)}$$

Substituting *TTF* for $\frac{AE \times IR}{k_e}$ in Equation 2 yields:

$$C_{tissue} = TTF \times C_{food} \qquad \text{(Equation 4)}$$

The trophic level of the organisms considered can be denoted by superscripts given as:

$$C_{tissue}^{TL2} = TTF^{TL2} \times C_{food}^{TL2} \qquad \text{(Equation 5)}$$

C_{tissue}^{TL2} as defined here represents the steady-state proportional concentration of selenium in the tissue of trophic level 2 organisms relative to the concentration of selenium in their food source.

Using the same rationale, the average concentration of selenium in the tissues of trophic level 3 organisms can be expressed as the concentration of selenium in its food multiplied by a *TTF* which is given as:

$$C_{tissue}^{TL3} = TTF^{TL3} \times C_{food}^{TL3} \qquad \text{(Equation 6)}$$

For trophic level 3 organisms that consume trophic level 2 organisms, $C_{food}^{TL3} = C_{tissue}^{TL2}$. Thus:

$$C_{tissue}^{TL3} = TTF^{TL3} \times C_{tissue}^{TL2} \qquad \text{(Equation 7)}$$

Substituting C_{tissue}^{TL2} in Equation 7 with $TTF^{TL2} \times C_{food}$ in Equation 5 yields:

$$C_{tissue}^{TL3} = TTF^{TL3} \times TTF^{TL2} \times C_{food}^{TL2} \qquad \text{(Equation 8)}$$

Defining the term C_{tissue}^{TL3} as the concentration of selenium in fish tissue, defining the term C_{tissue}^{TL2} as the concentration of selenium in living and nonliving particulate material ingested by invertebrates, and expressing the product of all *TTF* values as a single term results in the equation:

$$C_{whole-body} = TTF^{composite} \times C_{particulate} \qquad \text{(Equation 9)}$$

where:

$C_{particulate}$	=	the concentration of selenium in particulate material
$C_{whole\text{-}body}$	=	the concentration of selenium in the whole body of fish
$TTF^{composite}$	=	the product of all trophic transfer factor values

Equation 9 quantitatively expresses selenium bioaccumulation in fish ($C_{whole\text{-}body}$) as the product of the concentration of selenium at the base of the food web ($C_{particulate}$) and a parameter representing the trophic transfer of selenium through all dietary pathways ($TTF^{composite}$). This model of bioaccumulation is conceptually similar to the model of bioaccumulation utilizing a bioaccumulation factor (BAF). A BAF is the ratio of the concentration of a chemical in the tissue

of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). Similar to the term $TTF^{composite}$, a BAF quantitatively represents the relationship between the chemical concentrations in multiple environmental compartments. However, a BAF is empirically derived from site-specific measurements, whereas $TTF^{composite}$ is derived from knowledge of the ecological system. Because each *TTF* is associated with a particular taxon, $TTF^{composite}$ can be inferred for an aquatic system using existing knowledge and reasonable assumptions, without the considerable time and cost of collecting and analyzing tissue and water samples.

Equation 9 characterizes the bioaccumulation of selenium as a combination of *TTF* parameters from all steps in the dietary pathway of the predator species of interest. Thus it is possible to differentiate bioaccumulative potential for different predator species and food webs by modeling different exposure scenarios. For example, where the fish species of interest is a trophic level 4 predator that primarily consumes trophic level 3 fish, the term $TTF^{composite}$ can be represented as the product of all *TTF* parameters that includes the additional trophic level given as:

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2} \qquad \text{(Equation 10)}$$

where:

TTF^{TL2} = the trophic transfer factor of trophic level 2 species

TTF^{TL3} = the trophic transfer factor of the trophic level 3 species

TTF^{TL4} = the trophic transfer factor of the trophic level 4 species

$TTF^{composite}$ = the product of all trophic transfer factors

Similarly, the consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* at a particular trophic level as the weighted average of the *TTF*s of all species consumed given as:

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \text{(Equation 11)}$$

where:

TTF_i^{TLx} = the trophic transfer factor of the i^{th} species at a particular trophic level

w_i = the proportion of the i^{th} species consumed

These concepts can be used to formulate an expression of $TTF^{composite}$ to model selenium bioaccumulation in ecosystems with different consumer species and food webs. **Figure 3.4** describes four example food web scenarios and the formulation of $TTF^{composite}$ to model selenium bioaccumulation in each of them.

The parameter $TTF^{composite}$ quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system, w_i, the proportion of species consumed. See text for further explanation.

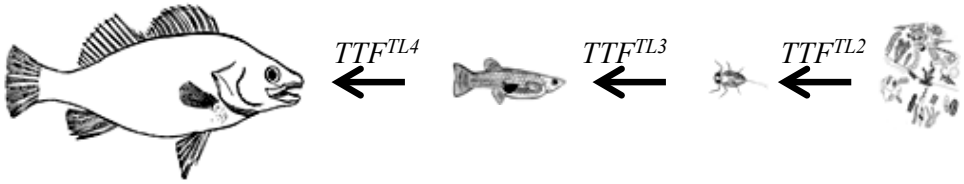
A) Three trophic levels (simple):

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$



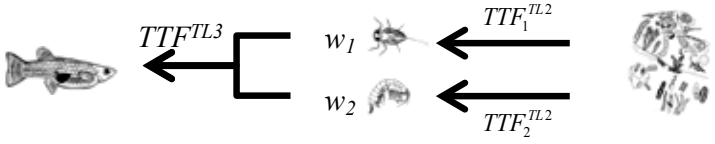
B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$



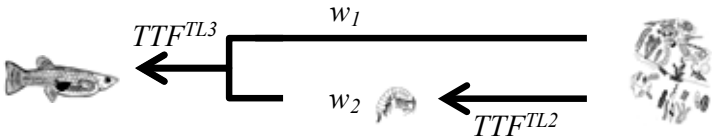
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

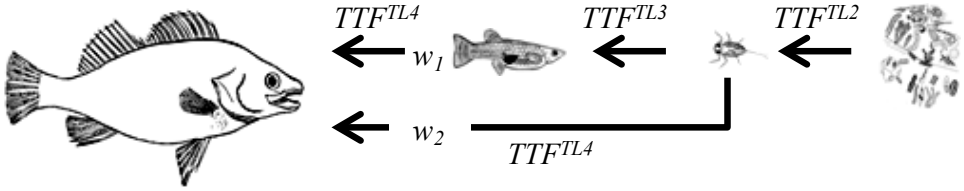


Figure 3.4. Example Aquatic System Scenarios and the Derivation of the Equation Parameter $TTF^{composite}$.

Because EPA's objective is to derive an equation that translates a fish tissue concentration of selenium to a water column concentration, the term C_{water} is reintroduced into

Equation 9 by defining the enrichment function EF representing the steady state proportional bioconcentration of dissolved selenium at the base of the aquatic food web given as:

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \text{(Equation 12)}$$

Where:

$C_{particulate}$ = Selenium concentration in particulate material (µg/g)
C_{water} = Concentration of selenium dissolved in water (µg/L)
EF = Enrichment function (L/g)

Rearranging the terms of Equation 12:

$$C_{particulate} = EF \times C_{water} \qquad \text{(Equation 13)}$$

Substituting $EF \times C_{water}$ for $C_{particulate}$ in Equation 9 results in:

$$C_{whole-body} = TTF^{composite} \times EF \times C_{water} \qquad \text{(Equation 14)}$$

Solving for the concentration of selenium in water in Equation 14 results in:

$$C_{water} = \frac{C_{whole-body}}{TTF^{composite} \times EF} \qquad \text{(Equation 15)}$$

Because Equation 15 relates a concentration of selenium in water to the concentration of selenium throughout all tissues of the body, $C_{whole\text{-}body}$ must be converted to an equivalent concentration in eggs or ovaries. EPA achieved this conversion by incorporating a species-specific conversion factor (*CF*) into Equation 15. *CF* represents the species-specific proportion of selenium in egg or ovary tissue relative to the concentration of selenium in all body tissues and is given as:

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \text{(Equation 16)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (µg/g).

Rearranging the terms of Equation 16 yields:

$$C_{whole-body} = \frac{C_{egg-ovary}}{CF} \qquad \text{(Equation 17)}$$

Substituting $C_{whole\text{-}body}$ in Equation 15 with $\frac{C_{egg-ovary}}{CF}$ yields the translation equation:

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \text{(Equation 18)}$$

where $TTF^{composite}$ equals the product of all trophic transfer factors from trophic level 2 through the target fish species.

Equation 18 describes an ecosystem-dependent relationship between the concentration of selenium in the eggs and ovaries of fish with the concentration of selenium in the water column. This approach explicitly recognizes the sequential transfer of selenium between environmental compartments (water, particulate material, invertebrate tissue, fish tissue, and eggs and/or ovary tissue) by incorporating quantitative expressions of selenium transfer from one compartment to the other. Because this approach uses food web modeling along with species-specific *TTF* and *CF* parameters to quantify most of the transfer between compartments, the only field measurements needed to relate selenium in egg-ovary and water are measurements from the water column and particulate material sufficient to calculate *EF*.

3.2.2 Equation Parameters

Empirical or laboratory data related to selenium bioaccumulation in aquatic organisms are needed to derive the equation parameters *EF*, *TTF*, and *CF*. EPA obtained data from published literature as described above The search resulted in the retrieval of 63 acceptable studies containing a total of 8,707 selenium measurements at 768 aquatic sites (2,927 from water, 373 from algae, 29 from detritus, 821 from sediment, 1,324 from various species of

invertebrates, and 3,233 from various species of fish) and 34 acceptable studies yielding 139 physiological constants (48 values of k_e, 81 values of *AE*, and 10 values of *IR*). EPA used this collection of selenium measurements to calculate site-specific *EF* values and develop species-specific *TTF* and *CF* values in an unbiased and systematic manner. A more detailed description of how EPA calculated *EF* is described below. How EPA calculated *TTF* and *CF* is described in detail in Appendix B.

3.2.2.1 Derivation of Trophic Transfer Factor (*TTF*) Values

EPA derived *TTF* values for taxonomic groups of invertebrates and fish by either using physiological coefficients found in the literature, or by evaluating the empirical relationship between matched pairs of selenium measurements in organisms and the food they consumed. When physiological coefficients were available, EPA calculated a *TTF* value using Equation 3 (**Section 3.2.1**):

$$TTF = \frac{AE \times IR}{k_e} \qquad \text{(Equation 3)}$$

Where:

k_e = Elimination rate constant (/d)

AE = Assimilation efficiency (%)

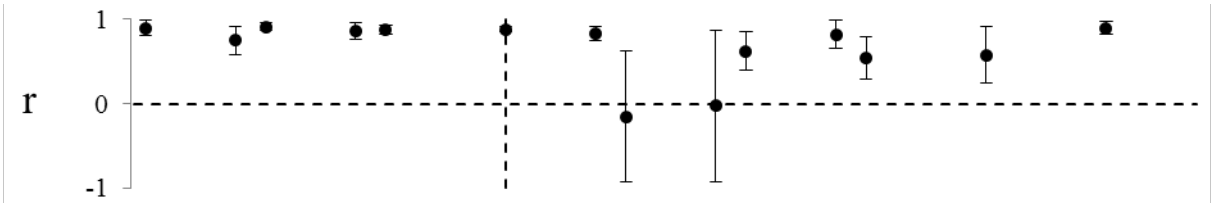
IR = Ingestion rate (g/g-d)

EPA also derived *TTF* values using empirical measurements of selenium from field studies. EPA searched its collection of available selenium measurements and identified measurements taken from aquatic organisms. For each measurement from an aquatic organism, EPA searched for additional measurements from other aquatic organisms or particulate material that was collected from the same aquatic site and of a type deemed likely to be ingested as a food source or in conjunction with feeding activity (i.e., a lower trophic level). If multiple lower trophic level measurements were matched to an aquatic organism measurement, the median of the lower trophic level measurements was calculated. Each pair of measurements, one taken from an aquatic organism and the other taken from lower trophic level organisms or particulate material, was designated as a matched pair. EPA limited particulate data used to calculate

invertebrate *TTF*s from field data to those aquatic sites with at least two particulate selenium measurements paired with invertebrate selenium measurements, and only used sediment measurements if there was at least one measurement from algae or detritus. If selenium concentrations from more than category of particulate material (algae, detritus, or sediment) were available, EPA used the median Se concentration of the available categories as the particulate concentration for that site.

Because selenium is transferred to aquatic animals primarily through aquatic food webs, the observable concentration of selenium in different environmental compartments may vary over time. To establish an appropriate time period with which to define matched pairs of selenium measurements, the effect of sample collection time on the relationship between selenium concentrations in different media was analyzed. EPA defined matched pairs of selenium measurements as described above using different relative collection time ranges and estimated the strength of the relationship between the two measurements by calculating the Pearson product-moment correlation coefficient (r). **Figure 3.5** shows the correlation coefficients for selenium measurements taken from the same aquatic sites when the measurement collection times were systematically shifted relative to one another. Each correlation coefficient was calculated from a set of data within a specified range of relative collection times with respect to the higher trophic level. For example, the correlation coefficient calculated from invertebrate and fish measurements with a relative sample collection time of 30 to 60 days were from invertebrate and fish samples collected at the same site, with the fish samples collected 30 to 60 days after the invertebrate samples. Similarly, the correlation coefficient calculated from invertebrate and fish measurements with a relative collection time of -60 to -30 days were from invertebrate and fish samples that were collected at the same site, with the fish samples collected 30 to 60 days before the invertebrate samples.

Particulate versus invertebrate



Invertebrate versus fish

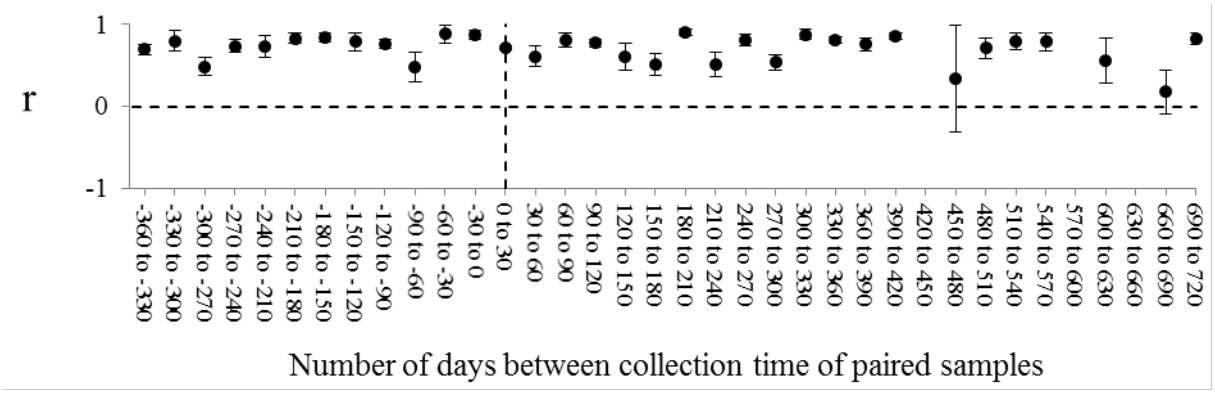


Figure 3.5. Effect of Relative Sample Collection Time on Correlation Coefficients of Selenium Measurements in Particulate Material, and Invertebrate and Fish Tissue. Error bars indicate the 95% confidence interval of r calculated using Fisher's r to z transformation. Horizontal dashed line indicates r = 0; vertical dashed line indicates relative collection time expected to have the highest correlation. The absence of a correlation coefficient indicates an insufficient quantity of data at the specified relative collection time range.

The results of this analysis suggest that the relationship between selenium concentrations in particulate material and invertebrate tissue and between invertebrate tissue and fish tissue is insensitive to relative collection time within a one year time period. These results also suggest that selenium becomes relatively persistent in the aquatic ecosystem once dissolved selenium transforms to particulate selenium and becomes bioavailable. On the basis of these analyses, EPA concludes that selenium measurements from samples collected at the same aquatic site within one year of each other are acceptable to use as matched pairs of measurements from the aquatic sites. Note that EPA chose a relative collection time period of one year on the basis of data taken from many different aquatic sites. Individual aquatic sites may have selenium loads and/or bioaccumulation characteristics that require different relative collection time criteria to accurately characterize selenium relationships.

After matched pairs of selenium measurements from samples collected in the field were identified, EPA evaluated two different analytical approaches to derive species-specific *TTF*

values from them. *TTF* was previously defined above as the steady state proportion relating the concentration of selenium in the tissue of aquatic organisms to the concentration of selenium in the food they ingest such that:

$$C_{tissue} = TTF \times C_{food} \qquad \text{(Equation 4)}$$

Rearranging the terms of Equation 4 yields Equation 19:

$$TTF = \frac{C_{tissue}}{C_{food}} \qquad \text{(Equation 19)}$$

Because *TTF* can be defined as the ratio of the concentration of selenium observed in the tissue of an aquatic organism to the concentration of selenium observed in the tissue or material the organism ingests, one approach for deriving *TTF* values from field data is to simply use the ratio of the two values. EPA evaluated this approach by calculating the ratios for all matched pairs of selenium measurements, and for each species or taxonomic group, used a statistic of central tendency of the distribution of ratios as the *TTF* value. An advantage of quantifying the relationship between selenium in two environmental compartments using ratios is that it is a simple and straightforward method that is conceptually similar to a bioaccumulation factor (BAF). A disadvantage of this approach is that it presumes that the quality and quantity of data used to derive the ratios adequately represent the relationship being characterized. Furthermore, many aquatic organisms tend to bioaccumulate more metals at low environmental concentrations (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007; U.S. EPA 2007). Thus a distribution of ratios could be biased toward larger values if the data are obtained from aquatic systems with low selenium concentrations.

Another analytical approach for deriving *TTF* values from matched pairs of selenium measurements is to model the species-specific relationships using linear regression. One possibility is to regress the concentration of selenium in the food of a particular species or taxonomic group with the concentration of selenium in the organism's tissue, and use the regression coefficient as the *TTF*. EPA evaluated this approach by applying ordinary least

squares (OLS) linear regression on the matched pairs of data. The regression coefficient (slope of the fitted line) was then taken as the *TTF* value for that species or taxonomic group. An advantage of this regression approach is that it estimates the quantitative relationship of selenium across a range of environmental concentrations in a manner that allows statistical assessment. Disadvantages of this regression approach include the assumption that the underlying data are normally distributed; one or a few very high values can have a disproportionate influence on the slope of the fitted line; and the bioaccumulation model does not account for a non-zero y-intercept. Constraining the y-intercept to zero (also known as regression through the origin or RTO) eliminates the added complexity of a non-zero y-intercept. However, RTO further increases the disproportionate influence of one or a few high values on the slope of the fitted line. Furthermore, RTO does not provide a straightforward way of evaluating goodness of fit (Gordon 1981).

After evaluating both approaches, EPA decided to use a hybrid approach by designating the median of the ratio of matched pairs of selenium measurements as the *TTF* value, but only if OLS linear regression of those data resulted in a significant ($P \leq 0.05$) fit and positive regression coefficient. Requiring a significant positive OLS linear regression coefficient confirms the relationship between selenium in organisms and the food they ingest is adequately represented by the available data. Using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias from measurements taken from aquatic systems with very high or very low selenium concentrations. Some aquatic organisms exhibit selenium bioaccumulation inversely related to water concentration (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). This inverse relationship is likely due to saturation uptake kinetics of specific transport mechanisms that regulate metals bioaccumulation within certain ranges (U.S. EPA 2007). EPA evaluated the effect of very high and very low selenium concentrations on the calculation of *TTF* values using the hybrid approach described above by calculating TTF values excluding selenium measurements above 10, 25, 50, and 100 μg/g and below 0.1, 0.5, 1.0, and 2.0 μ/g. EPA found that excluding very high or very low selenium measurements had minor effects on *TTF* values. EPA concludes that using the median ratio effectively attenuates effects of selenium concentration on the calculation of *TTF* values using the hybrid approach described above and allows the use of all available data without the need to introduce additional arbitrary exclusion criteria.

EPA calculated *TTF* values for 13 invertebrate species and 32 fish species that live in freshwater aquatic environments in North America. The data used to derive these *TTF* values are provided in Appendix B. The final *TTF* values are listed in **Table 3.10** and **Table 3.11**. The presence of physiological coefficients for a taxon in **Table 3.10** and **Table 3.11** indicates that the *TTF* values were calculated using those parameters. The absence of physiological coefficients for a taxon indicates that EPA derived the *TTF* value using field data. If a *TTF* value could be calculated from both physiological coefficients and field data, EPA used the *TTF* value calculated from the substantially larger number of field measurements to minimize statistical uncertainty.

Table 3.10. EPA-Derived Trophic Transfer Factor (*TTF*) Values for Freshwater Aquatic Invertebrates.

Common name	Scientific name	AE	IR	k_e	TTF
Crustaceans					
amphipod	*Hyalella azteca*	-	-	-	1.22
copepod	copepods	0.520	0.420	0.155	1.41
crayfish	*Astacidae*	-	-	-	1.46
water flea	*Daphnia magna*	0.406	0.210	0.116	0.74
Insects					
dragonfly	*Anisoptera*	-	-	-	1.97
damselfly	*Coenagrionidae*	-	-	-	2.88
mayfly	*Centroptilum triangulifer*	-	-	-	2.38
midge	*Chironimidae*	-	-	-	1.90
water boatman	*Corixidae*	-	-	-	1.48
Mollusks					
asian clam[a]	*Corbicula fluminea*	0.550	0.050	0.006	4.58
zebra mussel	*Dreissena polymorpha*	0.260	0.400	0.026	4.00
Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	zooplankton	-	-	-	1.89

[a] Not to be confused with *Potamocorbula amurensis*

Table 3.11. EPA-Derived Trophic Transfer Factor (*TTF*) Values for Freshwater Fish.

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

For fish species without sufficient data to directly calculate a *TTF* value, EPA estimated the *TTF* value by sequentially considering higher taxonomic classifications until one or more taxa for which a calculated *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species. For example, although data to directly calculate *TTF* for *Chrosomus eos* (northern redbelly dace) were not available, this species is in the family Cyprinidae, which also includes *Rhinichthys atratulus* (blacknose dace), *Cyprinus carpio* (common carp), *Semotilus atromaculatus* (creek chub), *Pimephales promelas* (fathead minnow), *Cyprinella lutrensis* (red shiner), *Richardsonius balteatus* (redside shiner), and *Notropis stramineus* (sand shiner). Because Cyprinidae is the lowest taxonomic classification where *Chrosomus eos* matches a species with an available *TTF* value, the median of the blacknose dace, common carp, creek chub, fathead minnow, red shiner, redside shiner, and sand shiner *TTF* values was used as the *TTF* value for northern redbelly dace. The data and analyses used to calculate all *TTF* values including those estimated by taxonomic classification are provided in Table B-8 of Appendix B.

3.2.2.2 Derivation of Whole-Body to Egg-Ovary Conversion Factor (*CF*) Values

The parameter *CF* (conversion factor) in Equation 18 (**Section 3.2.1**) represents the species-specific partitioning of selenium as measured in the whole-body and in egg-ovary tissue. EPA derived species-specific *CF* values by applying the same method used to derive species-specific *TTF* values using empirical measurements of selenium concentrations in different tissues of the same fish. To derive whole-body to egg-ovary *CF* values, EPA defined matched pairs of selenium measurements from the whole-body and from the eggs or ovaries measured from the same individual fish or from matched composite samples. Egg-ovary concentration was defined as a measurement from either the eggs or the ovaries. If multiple measurements from both eggs and ovaries of the same individual or matched composite sample were available, the average value was used. For example, both egg and ovary measurements were available for five of the 27 egg-ovary concentrations used to calculate the bluegill egg-ovary to whole body *CF* (Coyle et al. 1993), and 16 of the 69 measurements used to calculate the cutthroat trout egg-ovary to muscle *CF* (Kennedy et al. 2000).

Similar to the procedure used to derive *TTF* values, EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using OLS linear

regression of the matched pairs of measurements. If the regression resulted in a significant fit ($P \leq 0.05$) with a positive regression coefficient, EPA calculated the ratio of the egg-ovary to whole body selenium concentration of each matched pair and used the median ratio as the *CF* value for the species**.** A detailed comparison of the advantages and disadvantages of the median ratio and least squares regression approaches to calculating *CF*s, along with a comparison of *CF*s calculated from median ratios, OLS regression following log transformation, and total least squares (TLS) regression following log transformation is included in Appendix N.

EPA had sufficient egg-ovary and whole-body selenium measurements to directly derive egg-ovary to whole body *CF* values for 13 species of fish. However, matched pairs of selenium measurements in eggs and/or ovaries and muscle tissue, and matched pairs of selenium measurements in muscle and whole body were also available. To derive *CF* values for additional fish species, EPA used either the additional data or a taxonomic classification approach to estimate *CF*.

For those species of fish with neither sufficient data to directly calculate an egg-ovary to whole body *CF*, nor data to calculate a conversion factor for egg-ovary to muscle or whole body to muscle, EPA first estimated *CF* following the approach described above for the estimation of *TTF* values. In this first approach, EPA sequentially considered higher taxonomic classifications until one or more taxa for which a calculated *CF* value was available matched the taxon being considered, and if the lowest matching taxon was common to more than one species with a *CF* value available, EPA used the median *CF* from the matching species. For example, *CF* data are not available to directly calculate *CF* for *Lepomis microlophus* (redear sunfish); however, genus-level *CF*s for *Lepomis cyanellus* (green sunfish) and *Lepomis macrochirus* (bluegill) are available. Thus, EPA used the median *CF* values of *Lepomis cyanellus* and *Lepomis macrochirus* for redear sunfish.

For fish species without sufficient data to directly calculate an egg-ovary to whole body *CF*, but which had sufficient data to calculate a conversion factor for either egg-ovary to muscle or whole body to muscle, EPA followed a two stage approach based on taxonomic similarity. If a fish species had a species specific egg-ovary to muscle conversion factor, but no whole body data with which to calculate an egg to whole body *CF*, available data for other species would be used to estimate a muscle to whole body conversion factor for that species based on taxonomic relatedness. The estimated muscle to whole body factor would be multiplied by the directly

measured egg-ovary to muscle factor to estimate an egg-ovary to whole body *CF* for that species. For example, rainbow trout has a species specific egg-ovary to muscle conversion factor of 1.92, but does not have a species specific egg-ovary to whole body *CF*. Using the taxonomic approach described above, the most closely related taxa to rainbow trout with muscle to whole body conversion factors are in the class Actinopterygii. The median conversion factor for the eight species within that class is 1.27. The final egg-ovary to whole body *CF* for rainbow trout is 2.44 (**Table 3.12**), or 1.92 x 1.27.

EPA derived 13 *CF* values directly from matched pairs of egg-ovary and whole-body selenium measurements and an additional seven *CF* values by multiplying EO/M and M/WB conversion factors. Variability in the *CF* values for 19 of the 20 fish species was low (**Table 3.12**). Excluding mountain whitefish, *CF*s ranged from 1.20 to 3.11, a 2.6-fold difference. *CF* variability within each species was also low for 7 of 13 species for which egg-ovary to whole-body *CF*s were calculated. The two species with relatively high standard deviations for their *CF* values (brown trout and cutthroat trout) contained potentially anomalous hatchery data that contributed to the variability (see **Table 3.12** footnote). These species specific *CF* values are listed below in **Table 3.12** and in Table B-5 of Appendix B. All *CF* values including those estimated using the taxonomic classification approach are provided in Table B-6 in Appendix B.

Table 3.12. EPA-Derived Egg-Ovary to Whole-Body Conversion Factor (*CF*) Values.

Common name	Scientific name	CF	Std. Dev.[a]
Acipenseriformes			
white sturgeon	*Acipenser transmontanus*	1.69	
Cypriniformes			
bluehead sucker	*Catostomus discobolus*	1.82	0.19
flannelmouth sucker	*Catostomus latipinnis*	1.41	0.20
white sucker	*Catostomus commersonii*	1.38	0.36
desert pupfish	*Cyprinodon macularius*	1.20	0.10
common carp	*Cyprinus carpio*	1.92	0.49
roundtail chub	*Gila robusta*	2.07	0.29
fathead minnow	*Pimephales promelas*	1.40	0.75
creek chub	*Semotilus atromaculatus*	1.99	1.00
razorback sucker	*Xyrauchen texanus*	3.11	
Esociformes			
northern pike	*Esox lucius*	2.39	
Perciformes			
bluegill	*Lepomis macrochirus*	2.13	0.68

Common name	Scientific name	CF	Std. Dev.[a]
green sunfish	*Lepomis cyanellus*	1.45	0.23
smallmouth bass	*Micropterus dolomieu*	1.42	0.19
Salmoniformes			
brook trout	*Salvelinus fontinalis*	1.38	
Dolly Varden	*Salvelinus malma*	1.61	
brown trout	*Salmo trutta*	1.45	1.81[b]
rainbow trout	*Oncorhynchus mykiss*	2.44	
cutthroat trout	*Oncorhynchus clarkii*	1.96	2.03[b]
mountain whitefish	*Prosopium williamsoni*	7.39	

[a] Standard deviation for *CF* values for those species that had egg-ovary and whole body selenium concentrations.

[b] The brown trout and cutthroat trout standard deviations for *CF* values of 1.81 and 2.03 are considerably higher than the other standard deviations in this table. The brown trout data were taken from two studies, NewFields (2009) and Osmundson et al. (2007). *CF* values for three of the four fish samples from Osmundson et al. were four to six times greater than the median. Also, the NewFields data consisted of samples collected from natural streams and samples collected from a fish hatchery. The *CF* values for the fish hatchery samples were four to seven times lower than the median value. Although collectively, the data set meets the criteria for including the brown trout *CF*, the *CF* values for Osmundson et al. and NewFields hatchery samples may be anomalously high and low, respectively. Excluding these potentially anomalous data reduces the brown trout standard deviation to 0.47. The cutthroat trout *CF* values are from two sources (Formation 2012 and Hardy 2005). The reason for the higher variability in the cutthroat trout *CF* values is due to the relatively higher *CF* values in the hatchery fish from the Formation study. The standard deviation for cutthroat trout drops to 0.62 if the hatchery fish are excluded. See Appendix B for a presentation of the data for both of these species.

3.2.2.3 Calculation of Site-Specific Enrichment Factor (EF) Values

The most influential step in selenium bioaccumulation occurs at the base of aquatic food webs (Chapman et al. 2010). The parameter *EF* characterizes this step by quantifying the partitioning of selenium between the dissolved and particulate state. *EF* can vary by at least two orders of magnitude across aquatic systems (Presser and Luoma 2010). The greatest reduction in uncertainty when translating a fish tissue concentration of selenium to a water column concentration using Equation 18 is achieved when spatially and temporally coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity are used to accurately characterize *EF*. Thus, EPA only used aquatic sites with sufficient data to calculate a reasonably reliable *EF* value.

To calculate the *EF* of aquatic systems, EPA searched its collection of selenium concentration measurements from field studies (see **Section 2.7.8** for a description of data

sources and acceptability criteria) and identified aquatic sites with measurements from both particulate material and the water column. EPA first identified all selenium measurements from algae, detritus, or sediment, and then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water measurement was available for any given particulate measurement, the median was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median ratio. The geometric mean of the algae, detritus, and sediment ratios was used as the site *EF*. Because there were at most only three possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric mean in order to reduce the potential for one of the values to have excessive influence on the final site *EF* value.

The availability of selenium measurements from particulate material was limited. In addition, the majority of particulate measurements were from sediment samples with a significantly lower correlation to selenium in water ($r = 0.34$) compared to algae ($r = 0.68$; Fisher r-to-z transformation, $P < 0.001$) and detritus ($r = 0.94$; Fisher r-to-z transformation, $P < 0.001$). Therefore, to reduce uncertainty in estimating site-specific *EF* values, EPA limited its analysis to those aquatic sites with at least two particulate selenium measurements with corresponding water column measurements, and only used sediment measurements if there was at least one other measurement from either algae or detritus. On the basis of these requirements, *EF* values were calculated for 96 individual aquatic sites.

3.2.3 Food-Web Models

For the aquatic sites with a calculated *EF* value, EPA modeled the food webs for the fish species the studies indicated were present. Some of those studies provided information about the species and proportions of organisms ingested by fish, either through direct analysis of stomach contents, or examination of the presence and prevalence of invertebrate species. For those studies, that site-specific information in the food web models was used. Most studies, however, did not provide site-specific food web information. In those cases, the food webs of fish species present were modeled using information about their typical diet and/or eating habits obtained from the NatureServe database (http://www.natureserve.org).

After EPA developed food web models, EPA identified the appropriate species-specific *TTF* values for each model and calculated $TTF^{composite}$. Although individual *TTF* values were derived for several different taxa of invertebrates and fish (**Table 3.10** and **Table 3.11**), some of the food web models included one or more taxa for which no *TTF* value was available. EPA estimated *TTF* values for these taxa using the same taxonomic approach used to estimate egg-ovary to whole body, egg-ovary to muscle, and muscle to whole body conversion factors for taxa without sufficient data. In brief, for taxa with insufficient data to calculate a *TTF* value, EPA sequentially considered higher taxonomic classifications until one or more taxa for which a *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species. EPA used site-specific food-web models to translate the egg-ovary criterion element to a set of water column concentrations in order to derive the water column concentration element of the selenium criterion. Details of these food web models are shown in Table B-8 of Appendix B.

3.2.4 Classifying Categories of Aquatic Systems

Transformation reactions that convert dissolved selenium to particulate forms are the primary route of entry into aquatic food webs, and are critical steps in selenium bioaccumulation and toxicity (Chapman et al. 2010). Site-specific characteristics can result in substantial bioaccumulation variability and consequently different risks of selenium toxicity for a given dissolved selenium concentration. Freshwater systems fall into two distinct categories: lotic systems such as rivers and streams, characterized by flowing water, and lentic systems, such as lakes and ponds, characterized by largely standing water (e.g., Jones 1997). Water residence time is generally shorter in lotic systems than in lentic systems, and subsequently, aquatic organisms living in lentic systems tend to bioaccumulate more selenium than organisms living in lotic systems for a given dissolved selenium concentration (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005).

Although the distinction between lotic and lentic aquatic systems is often straightforward, some aquatic systems possess both lotic and lentic characteristics. For example, flow rate can vary greatly among lotic systems, with slow flowing low gradient systems (such as sloughs) having longer residence times relative to fast flowing high gradient systems. Lotic systems can also become more lentic during dry periods as hydrologic connectivity between deeper pools

decrease or cease with decreasing flow (Buffagni et al. 2009). Downstream reaches of some low gradient coastal rivers can also be influenced by tides (Riedel and Sanders 1998). Some lentic systems can exhibit some degree of flow, such as lakes fed and drained by one or more streams; however, the magnitude of flow is generally small compared to a lotic system. Even after accounting for flow, the majority of water movement in a lentic system is driven typically by wind or convection rather than gravity (e.g., Jones 1997). Finally, human-made reservoirs have some features that are intermediate between typical lotic and lentic systems. For example, reservoirs tend to be longer and narrower than natural lakes, and they have somewhat shorter water retention time than a natural lake of comparable volume (Thornton et al. 1990). Overall, however, reservoirs as a general class are considered more lentic than lotic, and have historically been classified as a type of lake (Thornton et al. 1990).

To verify the suitability of lentic and lotic aquatic system categories as the basis for independent water column criterion element values, EPA evaluated the aquatic systems that provided data for the 96 *EF* values. EPA utilized the description provided by the study authors to categorize each aquatic system as either lotic or lentic. Of the 39 lentic sites, the authors identified them as ponds (n = 18), lakes (n = 13), reservoirs (n = 4), or marshes (n = 4). Of the 57 lotic sites, the authors identified them as creeks (n = 31), rivers (n = 16), artificial channels (drains and wasteways, n = 3), springs (n = 2), sloughs (n = 2), or ephemeral systems (draws and washes, n = 3). The three ephemeral aquatic sites (two washes and one draw) were categorized as lotic because there was flowing water at the time they were sampled (Butler et al. 1995; Presser and Luoma 2009). *EF* values for these aquatic systems are shown in **Figure 3.6**.

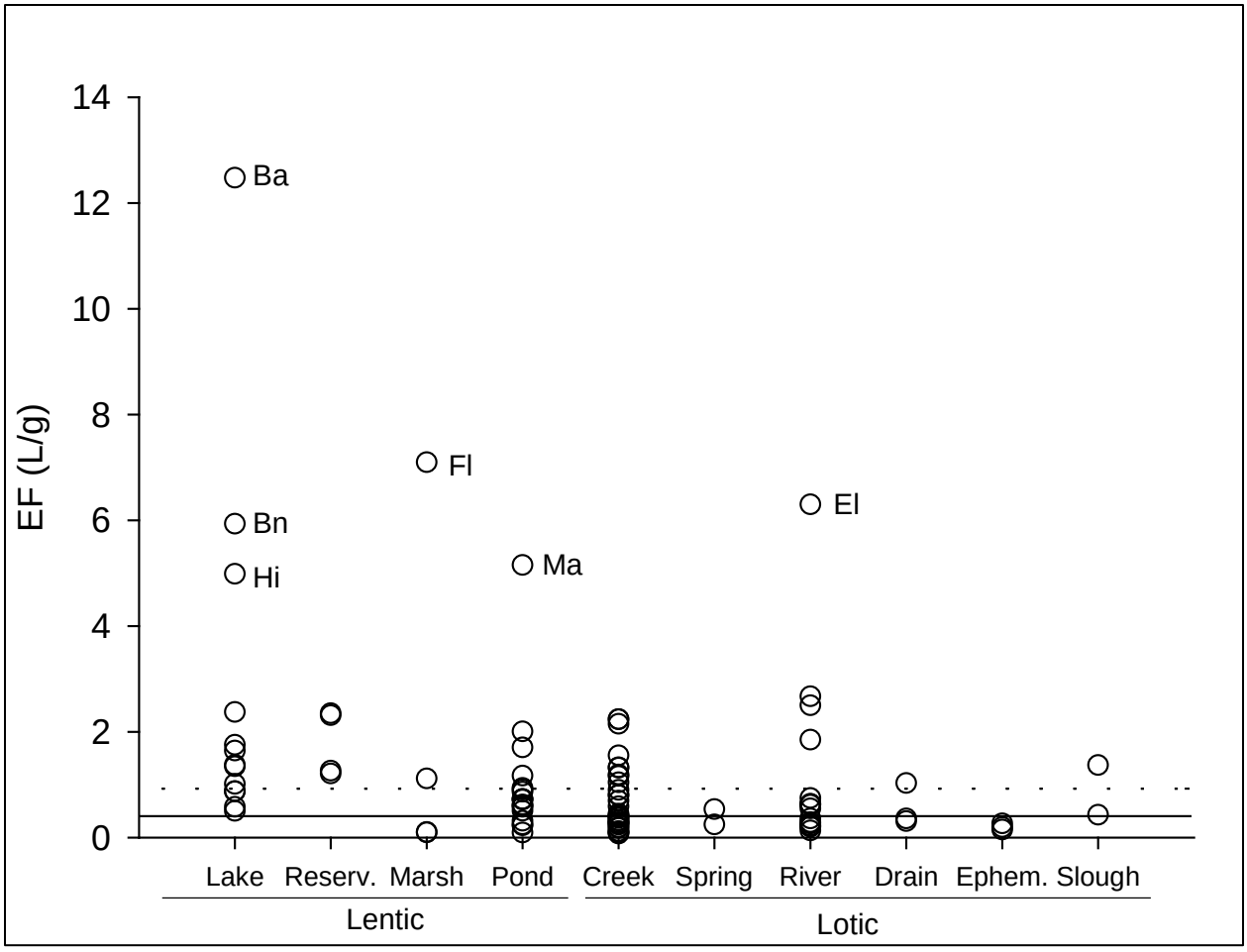


Figure 3.6. Enrichment Factors (*EF*) for 96 Aquatic Sites Derived from Published Studies and Organized by Waterbody Type.
The dashed line represents the median *EF* for the 39 lentic sites (0.9 L/g), and the solid line represents the median *EF* for the 57 lotic sites (0.4 L/g). See text for information on labeled data points.

Because the six labeled aquatic sites in **Figure 3.6** (Ma, Ba, Bn, Hi, El and Fl) appear as outliers, EPA selected them for further scrutiny. Data from site "Ma" result in an *EF* value of 5.2 L/g. Site "Ma" was a small irrigation pond within the Mancos River Valley watershed in southwestern Colorado (Butler et al. 1997). This watershed drains the Mancos Shale, a region that is naturally high in selenium. Data from sites "Hi," "Bn," and "Ba" resulted in *EF* values of 5.0, 5.9, and 12.5 L/g, respectively. Data from site "Hi" were from High Rock Lake, NC, data from site "Bn" were from Barnes Lake, British Columbia (Orr et al. 2006), and data from site "Ba" was from Badin Lake, NC (Lemly 1985). The high *EF* values at these three lakes were the result of a relatively high selenium concentration in particulate matter coupled with low aqueous

selenium concentrations. Data from site "El" result in an *EF* value of 6.3 L/g. Site "El" is an upstream site in the Elk River watershed in southeastern British Columbia, and the relatively large *EF* is the primarily the result of a low aqueous selenium concentration (McDonald and Strosher 1998). Data from "Fl" result in an *EF* value of 7.1 L/g. Site "Fl" is within Flathead wetland in southeastern British Columbia, and the relatively large *EF* is primarily the result of a low aqueous selenium concentration (Orr et al. 2012).

Figure 3.7 illustrates the variability in *EF* values across aquatic systems and substantial overlap between lotic and lentic categories. Some of this variability can be attributed to differences in the ambient concentration of selenium in the water column at these aquatic sites. *EF* is the ratio of selenium in particulate material ($C_{particulate}$) to selenium in the water column (C_{water}). As expected, the selenium concentrations in particulate material are positively correlated with the selenium concentrations in the water column (**Figure 3.7A**). The plot of $C_{particulate}$ versus C_{water} shows a significant ($P<0.001$) positive relationship for both lentic (slope = 0.65, 95% confidence interval = [0.50, 0.80]) and lotic (slope = 0.55, 95% confidence interval = [0.43, 0.68]) aquatic systems. However, selenium accrual in particulate matter is lower at aquatic sites with a higher water concentration of selenium compared to aquatic sites with a lower water concentration of selenium (**Figure 3.7B**). The plot of C_{water} versus *EF* shows a significant ($P<0.001$) negative relationship for both lentic (slope = -0.36, 95% confidence interval = [-0.51, -0.22]) and lotic (slope = -0.42, 95% confidence interval = [-0.55, -0.30]) aquatic systems. Consistent with other studies (e.g., Hamilton and Palace 2001; Brix et al. 2005; Orr et al. 2006), these results illustrate that the overall longer residence times of lentic systems result in increased bioaccumulation of selenium compared to lotic systems.

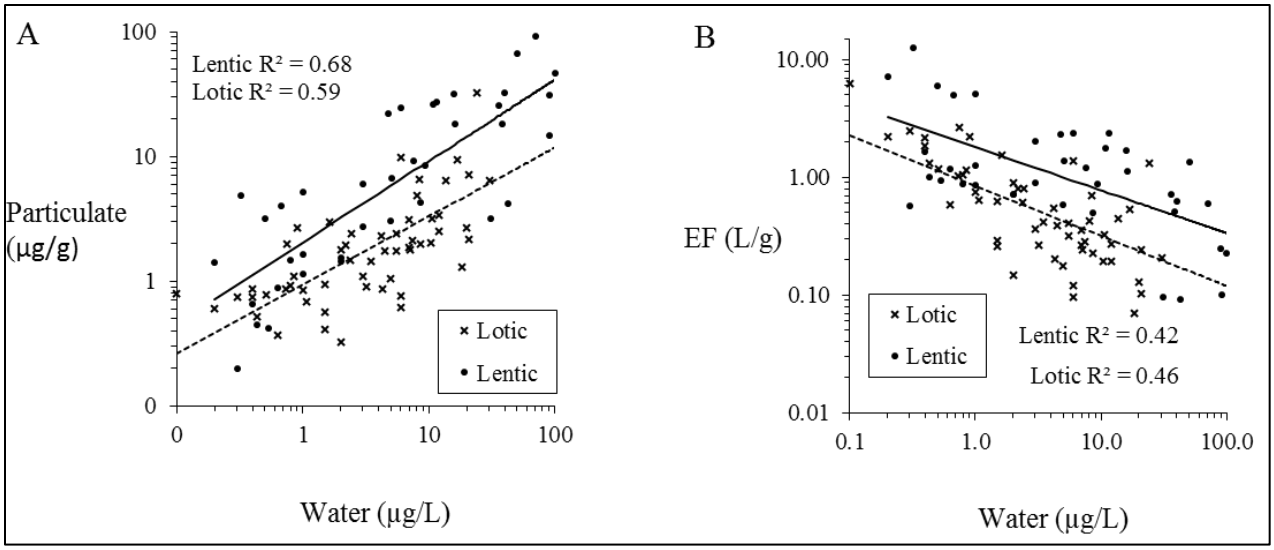


Figure 3.7. The Relationship between C_{water} and $C_{particulate}$, and C_{water} and *EF* for the 39 Lentic and 57 Lotic Aquatic Systems.
A: Relationship between C_{water} and $C_{particulate}$ by site category.
B: Relationship between C_{water} and *EF* by site category.
Solid line, ordinary least squares linear regression of logged data from lentic aquatic systems.
Dashed line, ordinary least squares linear regression of logged data from lotic aquatic systems.

Figure 3.8 shows the distribution of *EF* values grouped by lotic and lentic aquatic system categories. Although EPA derived the lentic and lotic *EF* values from aquatic sites with a similar range of water concentrations, the relative proportion of *EF* values collected at sites with higher water concentrations is larger for lentic sites than lotic sites. In particular, 6 of the 39 lentic *EF* values were from ponds in the Kesterson National Wildlife Refuge where C_{water} ranged from 38.6-196 µg/L (Saiki and Lowe 1987; Schuler et al. 1990). Despite the influence of selenium water concentration on *EF*, the median of *EF* values from lentic and lotic aquatic systems are significantly different from each other (Mann-Whitney U, P = 0.002). EPA concludes from these analyses that lentic and lotic aquatic system categories are appropriate categories for differentiating Se bioaccumulation. A listing of all aquatic-sites from which *EF*s were calculated is provided in Appendix H.

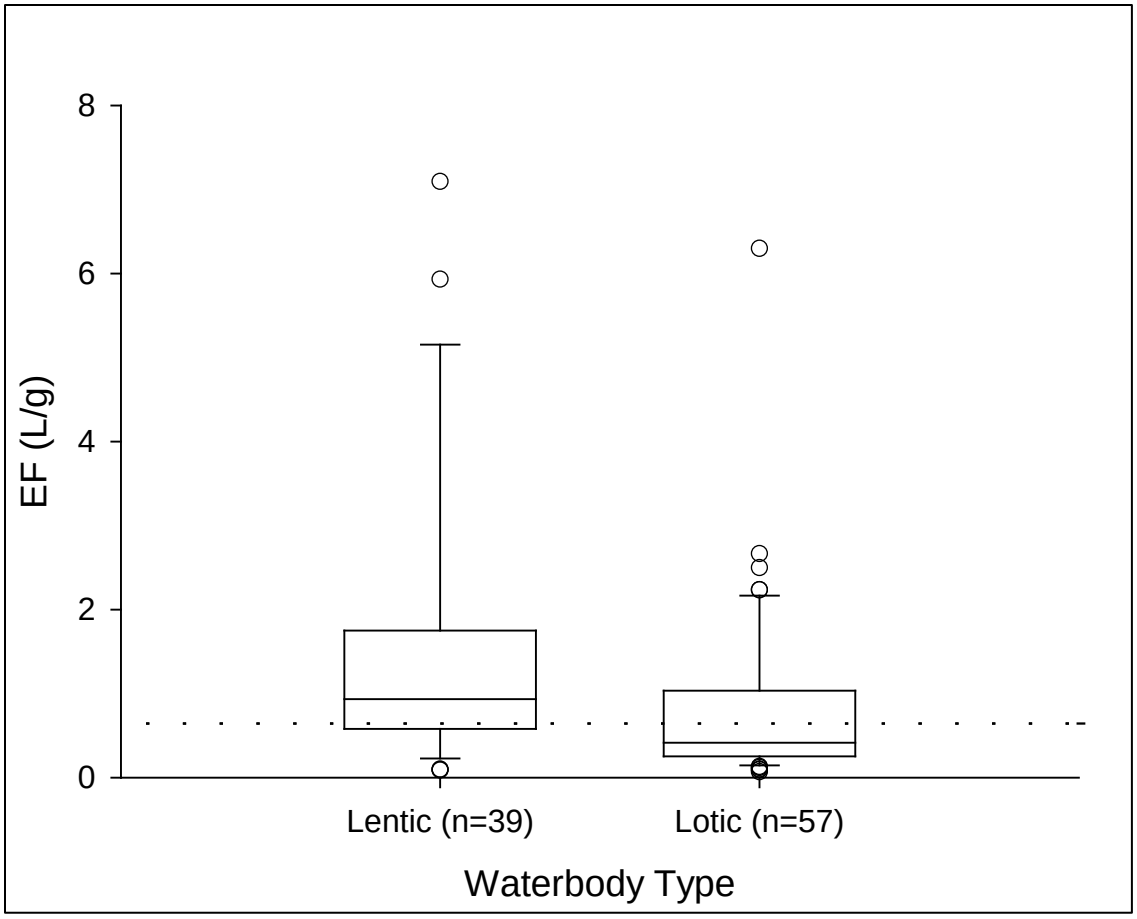


Figure 3.8. Distribution of *EF* Values for the Same 96 Aquatic Systems.
(As shown in **Figure 3.6** and **Figure 3.7** grouped by lentic and lotic aquatic system categories). Boxes show the 25th centile, median, and 75th centile *EF* values; whiskers show the 10th and 90th centiles. Circles represent *EF* values greater than 1.5 times the interquartile (25th-50th – lower circles; 50th-75th –upper circles) range. Dashed line represents the median *EF* of all 96 sites (0.63 L/g). The *EF* value of 12.48 L/g from Badin Lake (Lemly 1985) is off scale.

3.2.5 Deriving Protective Water Column Concentrations for Lentic and Lotic System Categories

To derive the water column element of the selenium criterion, EPA translated the egg-ovary criterion element to a distribution of water column concentration values for lentic and lotic aquatic systems. EPA uses the *EF* values calculated for 96 aquatic sites, available information about the fish species present at those sites, and food web models of those fish species. Because translation of the egg-ovary criterion element is site- and species-specific, several studies identifying more than one species of fish could potentially provide more than one translated water column concentration (one translated water value for each species). EPA considered using

all water column values for all species present to generate distributions of translated water column values from lentic and lotic aquatic sites. However, the number of reported fish species at aquatic sites with an *EF* value varied from one to six fish species. Furthermore, the studies providing data for 31 of the 96 sites with *EF* values do not provide information on the species of fish that may have been present at the aquatic site. Because the number of fish species at an aquatic site was not consistently reported, and because the number of reported fish species does not necessarily indicate the number of species present at a site, EPA calculated one translated egg-ovary criterion element to water column value for each aquatic site with both an *EF* value and at least one reported fish species. When more than one species was reported at a site, the EPA used the lowest translated water value for that site. Using this methodology, EPA translated the egg-ovary FCV into water column concentrations at 26 lentic and 39 lotic aquatic sites. EPA used these distributions of water concentration values translated from the egg-ovary criterion element to derive chronic water column criterion element values for lentic and lotic aquatic systems. **Table 3.13** shows the model parameter values used to translate the egg-ovary criterion element to site-specific water concentrations, and **Figure 3.9** shows the distribution of the translated values.

Table 3.13. Data for the 65 Site Minimum Translations of the Egg-Ovary Criterion Concentration Element to a Water Column Concentration.

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Birkner 1978	East Allen Reservoir, Medicine Bow WY	Iowa darter	Lentic	2.31	1.45	2.87	1.57
Birkner 1978	Galett Lake, Laramie WY	Iowa darter	Lentic	0.88	1.45	2.87	4.15
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	northern plains killifish	Lentic	1.70	1.20	2.44	3.04
Birkner 1978	Meeboer Lake, Laramie WY	northern plains killifish	Lentic	0.58	1.20	2.44	8.96
Birkner 1978	Miller's Lake, Wellington CO	Iowa darter	Lentic	2.37	1.45	2.87	1.53
Birkner 1978	Sweitzer Lake, Delta CO	fathead minnow	Lentic	0.87	1.40	2.78	4.45
Birkner 1978	Twin Buttes Reservoir, Laramie WY	Iowa darter	Lentic	1.21	1.45	2.87	3.01
Bowie et al. 1996	Hyco Reservoir	bluegill	Lentic	2.35	2.13	2.00	1.51
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	brown trout	Lentic	1.26	1.45	2.78	2.98
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	fathead minnow	Lentic	2.00	1.40	2.78	1.94
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	smallmouth bass	Lentic	5.15	1.42	1.93	1.07
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	Lentic	0.90	1.40	2.78	4.29
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	Lentic	0.86	1.40	2.78	4.49
Lemly 1985	Badin Lake	red shiner	Lentic	12.48	1.95	2.27	0.27
Lemly 1985	Belews Lake	red shiner	Lentic	1.75	1.95	2.27	1.94
Lemly 1985	High Rock Lake	red shiner	Lentic	4.99	1.95	2.27	0.68
Muscatello and Janz 2009	Vulture Lake	northern pike	Lentic	1.01	2.39	4.02	1.56
Orr et al. 2012	Clode Pond 11	cutthroat trout	Lentic	0.71	1.96	2.29	4.70
Orr et al. 2012	Elk Lakes 14	cutthroat trout	Lentic	1.64	1.96	2.29	2.05
Orr et al. 2012	Fording River Oxbow 10	cutthroat trout	Lentic	1.34	1.96	2.29	2.50
Orr et al. 2012	Henretta Lake 27	cutthroat trout	Lentic	0.50	1.96	2.29	6.72
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	Lentic	0.51	1.20	2.37	10.52
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	Lentic	0.32	1.20	2.37	16.83
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	Lentic	0.60	1.20	2.37	8.84
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	Lentic	0.93	1.20	2.37	5.69

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Stephens et al. 1988	Marsh 4720	common carp	Lentic	0.10	1.92	1.58	52.02
Butler et al. 1991	Uncompahgre River at Colona	rainbow trout	Lotic	0.63	2.44	2.33	4.21
Butler et al. 1993	Spring Cr. at La Boca	brown trout	Lotic	0.18	1.45	2.78	20.97
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	Lotic	0.15	1.40	2.78	26.04
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	fathead minnow	Lotic	0.90	1.40	2.78	4.32
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	fathead minnow	Lotic	0.37	1.40	2.78	10.57
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	red shiner	Lotic	0.12	1.95	2.27	28.34
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	Lotic	0.10	1.95	2.27	35.60
Butler et al. 1995	Navajo Wash near Towaoc	speckled dace	Lotic	0.20	1.95	1.36	29.07
Butler et al. 1995	San Juan River at Four Comers	red shiner	Lotic	0.26	1.95	2.27	12.97
Butler et al. 1995	San Juan River at Mexican Hat Utah	common carp	Lotic	0.29	1.92	1.58	17.24
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	Lotic	0.40	1.40	2.78	9.60
Butler et al. 1997	Cahone Canyon at Highway 666	green sunfish	Lotic	0.20	1.45	2.29	23.22
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	Lotic	0.07	1.40	2.78	55.27
Casey 2005	Deerlick Creek	rainbow trout	Lotic	2.24	2.44	2.33	1.18
Casey 2005	Luscar Creek	rainbow trout	Lotic	0.33	2.44	2.33	8.14
Formation 2012	Crow Creek - 1A	brown trout	Lotic	0.80	1.45	2.96	4.42
Formation 2012	Crow Creek - 3A	brown trout	Lotic	0.81	1.45	2.97	4.37
Formation 2012	Crow Creek - CC150	brown trout	Lotic	1.04	1.45	2.91	3.44
Formation 2012	Crow Creek - CC350	brown trout	Lotic	1.16	1.45	2.97	3.02
Formation 2012	Crow Creek - CC75	brown trout	Lotic	1.19	1.45	2.87	3.07
Formation 2012	Deer Creek	brown trout	Lotic	1.55	1.45	3.00	2.25
Formation 2012	Hoopes Spring - HS	brown trout	Lotic	0.24	1.45	3.86	11.06
Formation 2012	Hoopes Spring - HS3	brown trout	Lotic	0.54	1.45	2.63	7.40
Formation 2012	Sage Creek - LSV2C	brown trout	Lotic	0.45	1.45	3.01	7.76
Formation 2012	Sage Creek - LSV4	brown trout	Lotic	0.69	1.45	2.88	5.22
Formation 2012	South Fork Tincup Cr.	brown trout	Lotic	1.32	1.45	3.05	2.58
Hamilton and Buhl 2004	Lower East Mill Creek	cutthroat trout	Lotic	1.32	1.96	2.29	2.55
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	mountain whitefish	Lotic	6.30	7.39	2.97	0.11
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	cutthroat trout	Lotic	0.23	1.96	2.29	14.91
Orr et al. 2012	Elk River 1	cutthroat trout	Lotic	0.55	1.96	2.29	6.14

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Orr et al. 2012	Elk River 12	cutthroat trout	Lotic	2.67	1.96	2.29	1.26
Orr et al. 2012	Fording River 23	cutthroat trout	Lotic	0.21	1.96	2.29	16.20
Orr et al. 2012	Michel Creek 2	cutthroat trout	Lotic	0.28	1.96	2.29	11.85
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	Lotic	0.36	1.20	2.37	14.81
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	Lotic	1.03	1.20	2.37	5.17
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	Lotic	1.37	2.13	1.47	3.53
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	Lotic	0.43	2.13	1.47	11.29
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	Lotic	0.36	2.13	1.47	13.50
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	Lotic	0.75	2.13	1.47	6.46

a - Geometric mean of the median enrichments functions (*EF*) for all available food types (algae, detritus, and sediment). *EF* (L/g) = C_{food}/C_{water}.
b - Taxa-specific conversion whole-body to egg ovary conversion factor (*CF*; dimensionless ratio).
c - Composite trophic transfer factor ($TTF^{composite}$). Product of *TTF* values for all trophic levels.
d - Translated water concentration corresponding to an egg-ovary criterion element of 15.1 mg Se/kg dw, calculated by Equation 18.

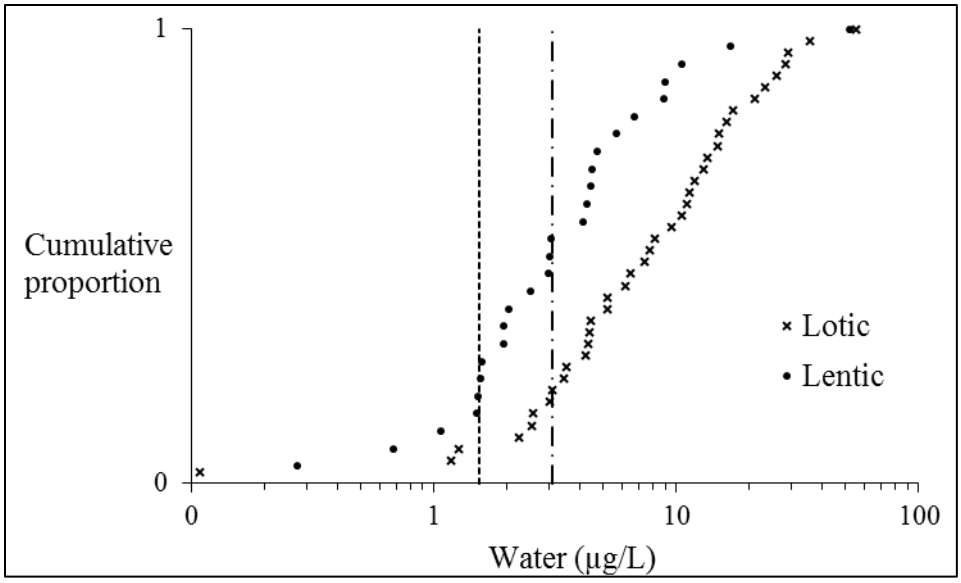


Figure 3.9. Probability Distribution of the Water Column Concentrations Translated from the Egg-Ovary Criterion Element at 26 Lentic and 39 Lotic Aquatic Sites.
Dashed and dash-dot lines show the 20th percentiles of the lentic and lotic distributions, respectively.

EPA selected the 20th percentile from the distribution of translated water column values of each category as the final national water column criterion element concentrations (3.1 µg/L for lotic waters and 1.5 µg/L for lentic waters) because the 20th percentile is consistent with past practice as it provides a high probability of protection for most aquatic systems in both lentic and lotic categories. **Table 3.14** provides the 20th percentile of the water concentration values translated from the egg-ovary criterion element value.

Table 3.14. Water Column Criterion Element Concentration Values Translated from the Egg-Ovary Criterion Element.

	Lentic	Lotic
20th percentile (final EPA-recommended water column criterion element)	**1.5 µg/L**	**3.1 µg/L**

As discussed in **Section 2.2.2**, selenium bioaccumulation potential depends on several biogeochemical factors that characterize a particular aquatic system. Uncertainty in the translation of the egg-ovary criterion element to the water column element can be reduced by deriving a site-specific criterion that uses site-specific selenium data and information on food-web dynamics from a biological assessment of the aquatic system. The general considerations are provided in Appendix K. The derivation of water column criterion element values described

above is constrained by the need to apply a national criterion value to a large number of aquatic systems for lentic and lotic systems.

3.2.6 Derivation of Averaging Period for Chronic Water Criterion Element

In the context of selenium bioaccumulation in a single trophic level, k would be the first-order depuration coefficient, and 1/k would equal the time needed to depurate to a concentration of 1/e times the initial concentration (where e=2.718). For depuration of two trophic levels sequentially, invertebrates and fish, the characteristic time is likewise the time needed for c/c_o to reach a value of 1/e. This differs from typical criteria averaging periods based on U.S. EPA (1995), where the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action due to direct waterborne toxicity of metals (i.e., where characteristic time = 1/k, where k is the first-order kinetic coefficient in a toxicokinetic model fitted to the relationship between LC_{50} and exposure duration). For the first trophic level, the kinetics for algal bioaccumulation and depuration were assumed to be rapid compared to the kinetics for larger organisms at higher trophic levels; that is, the characteristic time for algae was assumed to be negligible.

For the second trophic level, invertebrates, values for k_{TL2} are tabulated elsewhere in the document. A value of 0.1/day appears to be environmentally conservative, considerably higher than those for *Lumbriculus*, Asian clam, and zebra mussel, but slightly lower than copepods, which are very small in size. This corresponds to a characteristic time of 10 days.

For fish, the median depuration coefficient measured by Bertram and Brooks (1986) for 6-9 month-old (early adult) fathead minnows is applied, providing a k_{TL3} value of 0.02/day. This corresponds to a characteristic time of 50 days. Because of the small size of adults of this species, this represents faster kinetics than would likely be applicable to the salmonids and centrarchids of greatest concern for selenium toxicity, consonant with the Newman and Mitz (1988) inverse relationship between depuration rate and organism size. The striped bass k value of Baines et al. (2002) is inapplicable here because it was measured in the early juvenile life stage, a size that is too small to be relevant to reproductive impairment stemming from exposure of adult females.

As shown in Appendix J, the characteristic time for the combined second and third trophic levels (invertebrates and fish) is the approximate sum of the above two characteristic times, or 60 days. The analysis of the protectiveness of a 30-day averaging period, shorter than

the characteristic time, was performed and is shown in Appendix J. That analysis demonstrated that a 30-day averaging period for the chronic water criterion element affords protection under all conditions, and is therefore the duration recommended for the chronic water column criterion element.

3.3 Intermittent-Exposure Water Criterion Element: Derivation from the Chronic Water Criterion Element

Chapman et al. (2009) noted that selenium acute toxicity has been reported rarely in the aquatic environment and that traditional methods for predicting effects based on direct exposure to dissolved concentrations do not work well for selenium. As demonstrated in Appendix J, the kinetics of selenium accumulation and depuration are sufficiently slow that attainment of the water criterion element concentration by ambient 30-day averages will protect sensitive aquatic life species even where concentrations exhibit a high degree of variability.

To address situations where pulsed exposures of selenium could result in bioaccumulation in the ecosystem and potential chronic effects in fish, EPA is providing an intermittent exposure water criterion element concentration intended to limit cumulative exposure to selenium, derived from the chronic 30-day water criterion element magnitude and from its duration, which was obtained from the kinetic analysis of Appendix J. That is, the intermittent criterion element is based on the same kinetic analysis used to derive the water chronic averaging period (30 days).

To illustrate the concept of the intermittent criterion element and its dependence on the 30-day criterion element magnitude and duration, **Figure 3.10** shows a possible sequence of exposures over a 30-day period.

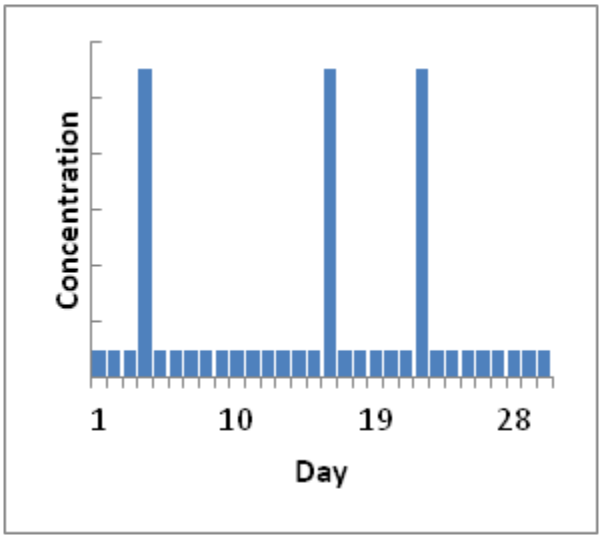


Figure 3.10. Illustration of Intermittent Spike Exposure Occurring for a Certain Percentage of Time (e.g., 10%) Over a 30-Day Period, and Background Exposure Occurring for the Remaining Percentage of Time (e.g., 90%).

The 30-day average concentration, $C_{30\,day}$, is given by Equation 20:

$$C_{30-day} = C_{int} f_{int} + C_{bkgrnd}(1 - f_{int}) \qquad \text{(Equation 20)}$$

Where:

C_{int} = the intermittent spike concentration (µg/L)

f_{int} = the fraction of any 30-day period during which elevated selenium concentrations occur

C_{bkgrnd} = the average daily background concentration occurring during the remaining time, integrated over 30 days.

$C_{30\text{-}day}$ is not to exceed the chronic criterion element, $WQC_{30\text{-}day}$. If the intent is to apply a criterion element, WQC_{int}, to the intermittent spike concentrations, then replacing C_{int} with WQC_{int} and $C_{30\text{-}day}$ with $WQC_{30\text{-}day}$ in the above equation, and then solving for WQC_{int} yields Equation 21:

$$WQC_{int} = \frac{WQC_{30\,day} - C_{bkgrnd}(1-f_{int})}{f_{int}} \qquad \text{(Equation 21)}$$

The equation expresses the intermittent exposure water criterion element in terms of the 30-day average chronic water criterion element, for a lentic or lotic system, as appropriate, while accounting for the fraction in days of any 30-day period the intermittent spikes occur and for the concentration background occurring during the remaining time. The reasonable worst-case assumption inherent in this approach is that selenium bioaccumulation is linear over a very wide range of concentrations, that is, *EF* and *TTF* values do not decrease significantly as concentrations increase.

If the heights of three spikes in **Figure 3.10** were to differ somewhat among each other, the intermittent element would apply to the arithmetic mean of the three. If the background concentrations were to vary somewhat, then the arithmetic mean background would be entered into the equation. Where concentrations vary smoothly over time, it does not matter where the line is drawn defining elevated versus background concentrations. The intermittent element will yield the same level of protection as the 30-day average element, provided that the equation uses (a) the average of the concentrations occurring for the fraction of time defined as being intermittently elevated, and (b) the average of the concentrations occurring for the remaining time, defined as being background. The intermittent element will only be exceeded under conditions that would have caused the 30-day element to be exceeded, had it been applied.

Table 3.15 illustrates example values for the intermittent element. The bottom row of the lotic and lentic values and the right column are to emphasize that WQC_{int} is not an independent element but a re-expression of the 30-day average water criterion element concentration. WQC_{int} converges to $WQC_{30\text{-}day}$ when the background concentration is already at $WQC_{30\text{-}day}$ or when the intermittent exposure is said to occur throughout the 30-day period.

Table 3.15. Representative Values of the Intermittent Water Criterion Element Concentration.

Bkgrnd Conc, C_{bkgrnd} (µg/L)	Fraction of Time, f_{int} in a 30-day period					
	0.03333 (1 day)	0.05 (1.5 days)	0.1 (3 days)	0.2 (6 days)	0.5 (15 days)	1 (30 days)
	Lotic Intermittent Criterion Element, WQC_{int} (µg/L)					
0	93	62	31	15.5	6.2	3.1
1	64	43	22	11.5	5.2	3.1
2	35	24	13	7.5	4.2	3.1
2.5	20.5	14.5	8.5	5.5	3.7	3.1
3.1	3.1	3.1	3.1	3.1	3.1	3.1
	Lentic Intermittent Criterion Element, WQC_{int} (µg/L)					
0	45	30	15	7.5	3	1.5
0.5	30.5	20.5	10.5	5.5	2.5	1.5
1	16	11	6	3.5	2	1.5
1.25	8.8	6.3	3.8	2.5	1.8	1.5
1.5	1.5	1.5	1.5	1.5	1.5	1.5

If the value of f_{int}, the intermittent exposure fraction of the month, is assigned a value less than one day, the intermittent criterion element value could exceed water concentrations that have been shown to be acutely toxic to sensitive species in 2- or 4-day toxicity tests (compiled in U.S. EPA 2004). Because the concentrations that would be acutely toxic in exposures of less than one day might not be much greater than those observed to be toxic in 2-4 day exposures, the intermittent fraction of the month must *not* be assigned a value less than 0.033, corresponding to one day.

4 NATIONAL CRITERION FOR SELENIUM IN FRESH WATERS

The available data indicate that freshwater aquatic life would be protected from the toxic effects of selenium by applying the following four-part criterion, recognizing that fish tissue elements supersede the water elements (except in special situations, see footnotes 3 and 4, Table 4.1) and that the egg-ovary tissue element supersedes all other tissue elements:

1. The concentration of selenium in the eggs or ovaries of fish does not exceed 15.1 mg/kg, dry weight; [1]
2. The concentration of selenium (a) in whole-body of fish does not exceed 8.5 mg/kg dry weight, or (b) in muscle tissue of fish (skinless, boneless fillet) does not exceed 11.3 mg/kg dry weight; [2]
3. The 30-day average concentration of selenium in water does not exceed 3.1 µg/L in lotic (flowing) waters and 1.5 µg/L in lentic (standing) waters more than once in three years on average;
4. The intermittent concentration of selenium in either a lentic or lotic water, as appropriate, does not exceed $WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1-f_{int})}{f_{int}}$ more than once in three years on average.[3]

Table 4.1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	**Fish Tissue**[1]		**Water Column**[4]	
Criterion Element	**Egg/Ovary** [2]	**Fish Whole Body or Muscle** [3]	**Monthly Average Exposure**	**Intermittent Exposure**[5]
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. Water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with fint assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

EPA recommends that states and tribes adopt into their water quality standards a selenium criterion that includes all four elements, and express the four elements as a single criterion composed of multiple parts, in a manner that explicitly affirms that the whole-body or muscle elements supersede the water column element, and the egg-ovary element supersedes any other element. The magnitude of the fish egg-ovary element is derived from analysis of the

available toxicity data. The magnitudes of the fish whole-body element and fish muscle elements are derived from the egg-ovary element coupled with data on concentration ratios among tissues. The magnitudes of the water column elements are derived from the egg-ovary element coupled with bioaccumulation considerations. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements into the selenium criterion ensures protection when neither fish egg-ovary nor fish whole-body nor muscle tissue measurements are available. To ensure that the contribution of short-term exposures to the bioaccumulation risks is accounted for in all situations, EPA is also recommending that the intermittent exposure element be included in the selenium criterion, as noted above. EPA is not recommending a separate acute criterion derived from the results of toxicity tests having water-only exposure because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. Application of the intermittent exposure criterion element values to single day, high exposure events will provide protection from the most important selenium toxicity effect, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high exposure events. It is unnecessary to have an additional acute water column criterion element because the intermittent exposure criterion element will be more stringent than an acute criterion element. Further, as noted in this document, there have been few if any acute exposure, water column-only selenium aquatic toxicity events documented in the literature.

In implementing the water quality criterion for selenium under the NPDES permits program, states may need to establish additional procedures due to the unique components of the selenium criterion. Where states use the selenium water column concentration criterion element values only (as opposed to using both the water column and fish tissue elements) for conducting reasonable potential (RP) determinations and establishing water quality-based effluent limitations (WQBELS) per 40 CFR 122.44(d), existing implementation procedures used for other acute and chronic aquatic life protection criteria may be appropriate. However, if states also decide to use the selenium fish tissue criterion element values for NPDES permitting purposes, additional state WQS implementation procedures (IPs) will be needed to determine the need for and development of WQBELs necessary to ensure that the fish tissue criterion element(s) are met.

EPA recommends that states use the default monthly average exposure water column elements of the criterion, adopted as part of the state's water quality criterion. Alternatively, states may want to develop and adopt, and submit for EPA approval, either a site-specific water column criterion (see Appendix K for details), or a procedure to facilitate the translation of a fish tissue criterion element concentration into site-specific water concentration values. A site-specific water column criterion element or set of lentic/lotic criterion element values may be developed using a mechanistic modeling approach (Presser and Luoma 2010) or using the empirical bioaccumulation factor approach, both described in Appendix K, for the specific waterbody or waterbodies, or a on a state-wide basis. A translation procedure must be scientifically defensible and able to produce repeatable and predictable outcomes, and must be consistent with either the mechanistic modeling approach or the empirical bioaccumulation factor approach described in Appendix K. The chronic selenium criterion is derived to be protective of the entire aquatic community, including fish, amphibians, and invertebrates. Fish are the most sensitive to selenium effects. Selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) sample data override the criterion elements based on water column selenium data due to the fact, noted above, that fish tissue concentrations provide the most robust and direct information on potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) in “fishless” waters, and 2) in areas with new selenium inputs.

Fishless waters are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (i.e., extirpation) due to temporary or permanent changes in water quality (e.g., due to selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas.

New inputs are defined as new activities resulting in selenium being released into a lentic or lotic waterbody. New inputs will likely result in increased selenium in the food web, likely resulting in increased bioaccumulation of selenium in fish over a period of time until the new or

increased selenium release achieves a quasi-"steady state" balance within the food web. EPA estimates that concentrations of selenium fish tissue will not represent a "steady state" for several months in lotic systems, and longer time periods (e.g., two to three years) in lentic systems, depending upon the hydrodynamics of a given system such as the location of the selenium input related to the shape and internal circulation of the waterbody, particularly in reservoirs with multiple riverine inputs, hydraulic residence time, and the particular food web. Estimates of steady state under new or increased selenium input situations are expected to be site dependent, so local information should be used to better refine these estimates for a particular waterbody. Thus, EPA recommends that fish tissue concentration not override water column concentration in these situations until these periods of time have passed in lotic and lentic systems, respectively, or steady state conditions can be estimated.

4.1 Protection of Downstream Waters

EPA regulations at 40 CFR 131.10(b) provide that "[i]n designating uses of a waterbody and the appropriate criteria for those uses, the state shall take into consideration the water quality standards of downstream waters and ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." Especially in cases where downstream waters are lentic waterbody types (e.g., lakes, impoundments), or harbor more sensitive species, a selenium criterion more stringent than that required to protect in-stream uses may be necessary to ensure that water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

5 SITE-SPECIFIC CRITERIA

All four elements of the freshwater selenium criterion may be modified to reflect site-specific conditions where the scientific evidence indicates that different values will be protective of aquatic life and provide for the attainment of designated uses.

Since the fish egg-ovary criterion element is based on toxicity data, a state may modify that element by applying the Recalculation Procedure (U.S. EPA 2013b) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while including tested surrogates for untested resident species.

It is important to note that species in the national data set that are not present at a site should not be deleted from the data set because those species serve as surrogate(s) for other species known or expected to be present at a site. Confidence in the applied tissue criterion element can be improved by further testing of fish species resident at the site. The most relevant testing would measure the survival and occurrence of deformities in offspring of wild-caught female fish to determine an EC_{10} for selenium in the eggs or ovaries (e.g., following Janz and Muscatello 2008).

Using either the EPA national recommended egg-ovary, whole-body, or muscle criterion concentration element or a site-specific egg-ovary, whole-body, or muscle criterion element, translation of the fish tissue criterion to a protective water concentration can be performed in a manner that accounts for site-specific conditions. Appendix K provides a step-wise process for deriving each parameter used in Equation 18 to perform a site-specific translation. These steps include:

1. selecting a target fish species for the waterbody,
2. determining the primary food source for the target species
3. determining the appropriate *TTF* values,
4. determining the appropriate *EF* value, and
5. determining the appropriate *CF* value.

Appendix K also provides information on the data necessary to derive a site-specific criterion, as well as scientifically defensible methods, including the use of traditional Bioaccumulation Factors (BAFs), in addition to the more comprehensive mechanistic modeling.

6 EFFECTS CHARACTERIZATION

6.1 FISH AND AMPHIBIANS

6.1.1 Principles for Using Studies for which EC_{10}s Cannot Be Calculated

When the data from an acceptable chronic test met the conditions for logistic regression analysis, the EC_{10} was used. When data did not allow calculation of ECs but did allow calculation of closely spaced NOECs and LOECs, the NOEC was used to approximate the EC_{10}. No NOEC values were used in calculating the tissue criterion element values.

When significant effects were observed at all treatment concentrations, such that no treatment concentration was classified as a NOEC, then the chronic value was assigned as "less than" (<) the lowest tested concentration. When no significant effects were observed at any concentration, such that no treatment concentration was defined as an LOEC, then the chronic value was assigned as "greater than" (>) the highest tested concentration.

A number of the chronic values in **Sections 3.1.1** and **6.1.2** (reproductive effects) and in **Section 6.1.9** (nonreproductive effects) include a "greater than" (>) or "less than" (<) sign because of an inability to resolve an exact value when all exposure concentrations of a study yielded either too little or too much effect to provide a point estimate of a chronic value. The decision to use chronic values with a "greater than" or "less than" sign in calculating an SMCV followed a rule based on whether these values add relevant information to the mean, as described below. None of these values were used in this assessment to derive the tissue criterion element values.

6.1.1.1 Evaluation Approach

- Neither a low "greater than" value nor a high "less than" value were used to calculate the SMCV;
- Both a low "less than" value and a high "greater than" value were included in the SMCV calculation. However, none of these values were used in this assessment to calculate the numeric criterion values for fish tissue.

For example, a chronic value reported here as ">15 mg Se/kg" is ignored if the tentative SMCV is 20 mg Se/kg. The ">15 mg Se/kg" value indicates that no significant effects were

observed at the study's highest tested concentration of 15 mg Se/kg. As this is consistent with what would be expected if the SMCV were 20 mg Se/kg, it provides no information to support modifying the SMCV. However, a different study showing no effects at its highest tested concentration and yielding the value ">25 mg Se/kg" is not consistent with an SMCV of 20 mg Se/kg, and indicates that the ">25 mg Se/kg" value provides information for modifying the mean upwards. Conversely, a chronic value reported here as "<15 mg Se/kg" indicates that significant effects were observed even at the study's lowest tested concentration of 15 mg Se/kg. As this is not consistent with a 20 mg Se/kg SMCV, it indicates the utility of the "<15 mg Se/kg" information for modifying the SMCV downwards. On the other hand, a value reported here as "<25 mg Se/kg" would not be used to recalculate a 20 mg Se/kg SMCV. The intent of the approach is to use all quality information that is relevant and appropriate for calculating the SMCVs.

6.1.2 Acceptable Studies of Fish Reproductive Effects of Genera that were not among the Four Most Sensitive Genera

The following is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the genera that were not the four most sensitive but were included in the number of GMCVs in the dataset (see **Section 3.1.3**). The studies in this section involve effects on the offspring of exposed female fish. Data are summarized in **Table 3.1**. Details of these studies are contained in Appendix C.

6.1.2.1 Cyprinidae

Pimephales promelas (fathead minnow)

Schultz and Hermanutz (1990) examined the effects of selenium transferred from parental fish (females) on fathead minnow larvae. The parental fathead minnows were first exposed to selenite (10 µg/L) that was added directly to the water in artificial streams in a mesocosm study. The selenite entered the food web and contributed to exposure via diet. Spawning platforms were submerged into treated and control streams. Embryos were collected from the spawning platforms and transferred to a proportional diluter where they were reared in incubation cups for observation. Treated embryos in the egg cups were exposed to 10 µg/L selenium. Edema and lordosis were observed in approximately 25 percent of the larvae spawned and reared in natural water spiked with 10 µg Se/L and in ≤ 6 percent of control larvae. Although

a case can be made that the selenium treatment had a higher rate of edema and lordosis, there are some issues that add uncertainty to the estimation of an effect concentration (R. Erickson, personal communication). Heavy mortality/loss of embryo/larvae during monitoring and the erratic occurrence of the abnormalities (e.g., significant incidence of edema in only 3 of 10 replicates for the Se treatment) led to the conclusion that results should not be used for criterion derivation. The data from this study support the range of reproductive effect levels determined in other fish studies. The Se concentration in embryos from the 10 µg/L treatment stream of 3.91 mg/kg ww converts to 25.6 mg/kg dw using 15.3% dw (N=3 range 14.7 – 15.6%) for fathead minnow eggs (R. Erickson, personal communication).

Two other studies suggest fathead minnows are less sensitive to selenium than other fish. Young et al. (2010) observed that fathead minnow populations remained after selenium contamination of Belews Lake had eliminated most other fish species, including bluegill and largemouth bass. In a maternal transfer laboratory study with fathead minnows, GEI (2008) estimated EC_{10}s for larval survival and deformities that ranged from 35 – 65 mg Se/kg dw expressed as maternal whole body, as noted in Appendix E, Figures E-2 and E-3.

6.1.2.2 Esocidae

Esox lucius (northern pike)

Muscatello et al. (2006) collected spawning northern pike from four sites near a uranium milling operation in north-central Saskatoon, Canada, with egg concentrations ranging from 2.7 to 48 mg Se/kg dw. The four sites included a reference site and three sites 2, 10 and 15 km downstream of the effluent discharge, representing a gradient of selenium exposure. Milt and ova were stripped from gravid fish. Eggs were then fertilized in the field and incubated in the laboratory for observations and measurements. The test was terminated when the majority of the fry exhibited swim-up and had absorbed the yolk.

Mean egg diameter, fertilization success and cumulative embryo mortality were not significantly different among the sites. Significant increases in percent total deformities including edema, skeletal deformities, craniofacial deformities and fin deformities were observed in fry originating from pike collected at the medium exposure site. The concentrations of selenium in the northern pike eggs collected at the reference and low exposure site were very similar, as were the percent total deformities in embryos/fry. The geometric mean of selenium in

the eggs of the adult females at the reference and low exposure sites was 3.462 mg Se/kg dw and the corresponding arithmetic mean of the percent total deformities was 13.20%. There were only 4 adult females from exposed sites, and all had relatively similar concentrations in their eggs, all close to the geometric mean concentration of 34.00 mg Se/kg dw. Likewise, all four exposed females had relatively similar percent total deformities, not far from their arithmetic mean of 33.40%. This is not a sufficient level of effect for applying TRAP to determine an EC_{10}. Furthermore, the relatively large spread between the two clusters of exposure concentrations (3.462 and 34.00 mg Se/kg dw) would render a NOEC and LOEC unreliable and unsuitable for defining a threshold. That is, the NOEC and LOEC would be "greater than" and "less than" values, >3.462 and <34.00 mg Se/kg dw respectively, providing little information on the sensitivity of northern pike compared to other species.

Instead, making use of the clustering of data at low exposure and effects and at elevated exposure and effects, the effect level for the elevated exposure eggs was normalized to the low exposure condition and rescaled to a 0-100% range. The rescaled (i.e., Abbott-adjusted) percent of total deformities for the elevated exposure eggs was 24% (relative to the low exposure eggs). Thus the concentration of selenium in the elevated exposure eggs (34 mg Se/kg dw) was equivalent to an EC_{24}, and is *the only effects concentration that can be calculated for this test*, given the limitations in the range of concentrations tested and effects observed. Although the EC_{24} is not directly translatable to an EC_{10} for use in determining the criterion, it is useful for comparison with the EC_{24} in other species in order to determine species sensitivity rank. The EC_{24} for skeletal deformities from the Holm et al. (2005) study of rainbow trout, calculated to be 30.9 mg Se/kg dw in eggs, is slightly lower than the northern pike value, indicating these two species may be similar in tolerance, with the northern pike being slightly more tolerant (see Appendix C for more details.)

6.1.2.3 Salmonidae

Seven publications provide quantitative data on the effects of selenium on salmonid embryo/larval survival and deformity that were used in calculating criterion values. All involve wild-caught adults taken from selenium contaminated streams and spawned for effects determination. Exposure for all studies was therefore through the parents. Data are available for rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*), Dolly Varden

(*Salvelinus malma*) and brown trout (*Salmo trutta*). The studies with *Salvelinus* are discussed below; *Oncorhynchus* and *Salmo* were previously discussed in **Section 3.1.2**.

Salvelinus fontinalis (brook trout)

These data were not used directly in the criterion calculations. See **Section 6.1.5** for discussion of the available data.

Salvelinus malma (Dolly Varden)

Golder (2009) collected adult Dolly Varden from a reference site and two sites downstream from the Kemess Mine in northern British Columbia, one with a high and one with a moderate selenium exposure in the fall of 2008. Fertilized eggs were taken to the laboratory where they were monitored for survival and deformities until 90% of the larvae reached swim-up, approximately five months after fertilization. Alevin mortality was <1% in the treatments collected from the exposed sites and not considered an effect. The prevalence of deformities increased sharply after the selenium egg concentration exceeded 50 mg/kg dw (Appendix C). The proportion of Dolly Varden larvae without any type of deformity (skeletal, craniofacial, and finfold as well as edema), as a function of the log of the selenium concentration in the eggs using TRAP, produced an EC_{10} value of 56.22 mg Se/kg dw and an EC_{20} value of 60.12 mg Se/kg dw.

6.1.2.4 Salmonidae SMCV and GMCV Summary

As given in **Section 3.1.2**, the SMCV for cutthroat trout, *Oncorhynchus clarkii*, is 26.2 mg Se/kg dw in eggs derived from Rudolph et al. (2008), and Nautilus Environmental (2011), (24.7, and 27.7 mg Se/kg dw respectively). The GMCV for the genus *Oncorhynchus* is 25.3 mg Se/kg dw in eggs, derived from the 24.5 mg Se/kg dw EC_{10} from the combined Holm (2002) and Holm et al. (2005) rainbow trout data, the above mean of the Rudolph et al. (2008) and Nautilus Environmental (2011) Westslope cutthroat trout studies. The GMCV for the genus *Salvelinus* is the EC_{10} value of 56.22 mg Se/kg dw for Dolly Varden (*S. malma*) from the Golder (2009) study.

6.1.2.5 Poeciliidae

Data are available for two species in this family. These studies are not represented in **Table 3.1** because they are live-bearing rather than egg-laying, but the relative tolerance of these species is accounted for in derivation of the criterion.

Gambusia holbrooki (eastern mosquitofish)

Staub et al. (2004) collected male and gravid female eastern mosquitofish from a contaminated ash basin and a reference pond in July 1999. Male fish were used for measuring standard metabolic rate and the reproductive endpoints. Brood size and percent viability of live offspring at parturition were measured using the live-bearing females. Standard metabolic rates of males, brood size of females, and offspring viability were not significantly different between sites. Average concentrations of selenium in females were 11.85 and 0.61 mg/kg dw in the contaminated ash basin and reference sites, respectively. The chronic value in whole body tissue is >11.85 mg Se/kg dw whole-body (Appendix C). In a community of equally exposed fish taxa (fish taxa having whole body tissue concentrations >11.85 mg Se/kg dw), the median egg-ovary concentration among egg-laying fish would be expected to be 1.71 higher, or >20.26 mg Se/kg dw.

Gambusia affinis (western mosquitofish)

Western mosquitofish were collected in June and July 2001 from sites in the grassland water district in Merced County, California. Mosquitofish were collected from two sites that were contaminated with selenium and from two reference sites in the same area with relatively low selenium water concentrations (Saiki et al. 2004). Seventeen to 20 gravid females (mosquitofish are live-bearers) from each site were held in the laboratory for two weeks to quantify live and dead births and to make other measurements. Live and dead fry were visually examined under low magnification with a binocular microscope for evidence of external abnormalities (teratogenic symptoms such as spinal curvature, missing or deformed fins, eyes and mouths and edema). The percentage of live births was high at both selenium-contaminated sites (96.6 to 99.9%) and reference sites (98.8 to 99.2%). There were no obvious anomalies (e.g., deformities, edema) observed during the study. The concentration of selenium in four postpartum females from the site with the highest selenium concentration ranged from 13.0 to 17.5 mg Se/kg dw (geometric mean of the high and low is 15.1 mg Se/kg dw). The chronic value in whole body tissue is >15.1 mg Se/kg dw (Appendix C). Similar to Staub et al. (2004), this value can be converted to egg-ovary concentrations that would be expected in accompanying egg-laying fish, by multiplying by the median fish egg-ovary to whole-body concentration ratio, 1.71. This yields a >25.82 mg Se/kg dw equivalent egg-ovary concentration.

Gambusia, which have been reported to be tolerant to selenium contamination, are often one of the few remaining species at sites with high levels of selenium contamination (Cherry et al. 1976; Lemly 1985a; Saiki et al. 2004; Young et al. 2010; Janz et al. 2010). The two studies discussed above support this observation with a GMCV of >13.4 mg Se/kg dw in whole body tissue, combining these "greater than" values as described in **Section 6.1.1**. It may be concluded that this genus is not among the most sensitive to selenium.

6.1.2.6 Cyprinodontidae

Cyprinodon macularius (desert pupfish)

Besser et al. (2012), using a diet of oligochaete *Lumbriculus* that had fed on selenized yeast, exposed desert pupfish to six levels of dietary and waterborne selenium. Five-week old juveniles (F_0) were exposed for 85 days, during which time survival and growth were measured. Upon reaching maturity at the end of this exposure period, the 60-day reproductive study was begun, during which F_1 eggs were collected, counted, and further tested for percent hatch, survival, growth, and deformities. The authors observed no significant differences in pupfish survival, growth, total egg production, hatch, or deformities among treatments. Although the authors noted a potential interaction between the timing of egg production and treatment, a comprehensive re-analysis of this data, described in Appendix C, indicated that the phenomenon was neither statistically nor biologically significant. It is concluded that the egg concentration, 27 mg Se/kg (dw), for the test's highest treatment was not sufficiently high to define a concentration-response curve. Although desert pupfish is thus not among the most sensitive species, the slightly reduced survival observed at 27 mg Se/kg egg dw egg suggests that the EC_{10} may be close to that concentration, as also noted by the authors.

6.1.2.7 Centrarchidae

Micropterus salmoides (largemouth bass)

A laboratory study was conducted by Carolina Power & Light (1997) in which adult largemouth bass obtained from a commercial supplier were fed an artificial diet spiked with a gradient of selenomethionine concentrations for several months. Approximately 100 eggs from each spawn were monitored for mortality and deformities up to the larval swim-up stage. The authors combined survival and deformities into a single metric (i.e., survival as normal

offspring). The average concentration of selenium in the ovaries ranged from 3.1 mg/kg dw in the control to 77.6 mg/kg dw in the high dietary treatment (53.1 mg/kg dw). A plot of the percent survival of larval largemouth bass as a function of the selenium concentration in the parental female ovary shows two groups of data; one at background survival with considerable variability (mean 90.3%, standard deviation 10.9%) and one with <10% survival, with most of the data being at 0% survival (see *Micropterus* summary in Appendix C, Figure 1). Due to inadequate partial effects, a TRAP interpolation was used to estimate an EC_{10} value. Based on a risk management decision that the LOEC cannot be any higher than the lowest concentration with 0% survival (32.9 mg/kg) and that any ECx should be below this, this establishes the higher concentration point for the interpolation (an EC_{100} of 32.9 mg/kg) and requires that the highest 4 NOECs not be considered in setting the EC_0. The lower concentration point for the interpolation is therefore set here to 24.6, the next highest NOEC with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg (and a steep slope of 16). Please see Appendix C for more detailed information.

6.1.3 Reproductive Effects in Catfish (Ictaluridae)

Some important families of fish are not represented in the effects assessment, such as the catfish family (Ictaluridae). In their compilation of egg-ovary versus whole-body ratios, Osmundson et al. (2007) found comparatively high concentrations of selenium in egg-ovary compared to whole body in black bullhead, *Ameiurus melas*, which are related to the Ictaluridae. This raises a question about the potential risks of reproductive effects in this species and possibly in related Ictaluridae. In addition to this concern about how much selenium such species may accumulate in their eggs, U.S. Fish and Wildlife Service (2005) has suggested that offspring of channel catfish (*Ictalurus punctatus*) and related species might be affected at unusually low egg concentrations. This is based on results of a study in which adult female catfish were injected with seleno-L-methionine (Doroshov et al. 1992b). Effects were found in the offspring at egg concentrations between 3.2 mg/kg (NOEC) and 6.3 mg/kg (LOEC), below levels observed in the studies summarized in **Section 3.1.2** and documented in Appendix C. These data were not included in derivation of the criterion because the injection route of exposure is not an acceptable experimental protocol for studies used in criterion derivation due to its difference from exposure routes in the environment (water column and diet).

In the absence of valid tests yielding an Ictaluridae EC_{10} or chronic value, EPA evaluated the potential vulnerability of the taxonomic group that includes catfish by examining comparative fisheries observations of Ictaluridae and Centrarchidae sharing the same selenium-contaminated water body. Crutchfield (2000) reports results of annual cove rotenone sampling performed from 1982 to 1997 in Hyco Reservoir, North Carolina. The sampling was begun after centrarchid populations in this reservoir had collapsed due to the release of ash pond selenium from a coal-fired power plant. The plant began operating a dry fly ash handling system in January 1990, thereby eliminating the aquatic discharge of selenium; the sampling continued through the recovery period.

Crutchfield (2000) reports abundance data (kg/ha) for 20 fish taxa, including four Ictaluridae and three Centrarchidae. These data were examined to determine the relationship between the Ictaluridae and the selenium-affected Centrarchidae populations. The correlation matrix between annual measured abundance of the seven taxa is shown below in **Table 6.1**. Correlation with the reciprocal of measured average concentrations of selenium in invertebrates is also shown. Because the reciprocal of the selenium concentration is used, a positive correlation means that abundance decreases as selenium concentration increases. Conversely, a negative correlation means abundance decreases as selenium concentration decreases.

Table 6.1. Correlation Matrix (Values of r) for Ictaluridae and Centrarchidae Abundance and for Selenium Food Chain Contamination for the Hyco Reservoir.
(Data Reported by Crutchfield 2000).

	Ictaluridae				**Centrarchidae**			
	Channel catfish	White catfish	Flat bullhead	*Ameiurus* spp.	Bluegill	Large-mouth bass	*Pomoxis* spp. (crappie)	1 ÷ Inverteb. Se Conc
Channel catfish	1.00	-0.36	0.18	0.68	0.08	-0.33	-0.08	-0.44
White catfish	-0.36	1.00	0.02	-0.32	-0.31	-0.24	-0.15	-0.06
Black bullhead	0.18	0.02	1.00	0.40	0.32	-0.08	0.08	-0.03
Ameiurus spp.	0.68	-0.32	0.40	1.00	0.22	-0.24	-0.05	-0.31
Bluegill	0.08	-0.31	0.32	0.22	1.00	0.78	0.76	0.80
Largemouth bass	-0.33	-0.24	-0.08	-0.24	0.78	1.00	0.78	0.92
Pomoxis spp. (crappie)	-0.08	-0.15	0.08	-0.05	0.76	0.78	1.00	0.69
1 ÷ Inverteb. Se Conc.	-0.44	-0.06	-0.03	-0.31	0.80	0.92	0.69	1.00

The centrarchid abundances are well correlated with each other and are closely related to selenium concentrations in the food chain, with fish abundance decreasing as selenium concentrations increase. Ictaluridae abundances, however, are unrelated either to the selenium-sensitive centrarchid abundances or to the selenium concentrations in the food chain.

Figure 6.1 shows abundance as both mass and numbers of individuals of channel catfish (CCF) and largemouth bass (LMB) observed by Crutchfield (2000) during the period 1982-1997. Both species are long lived. Crutchfield (2000) notes that the decline of reproductive success and abundance of Hyco's largemouth bass (and bluegill) was first documented in the mid-1970s. Because this study was initiated after the largemouth bass recreational fishery had collapsed, **Figure 6.1** does not show the largemouth bass decline, only the period of its depression and subsequent recovery.

Numbers of largemouth bass were very low at the beginning of the study period; their numbers and mass do not begin to recover until invertebrate selenium drops below 30 mg Se/kg dw. In the later portion of the study period, 1989-1997, largemouth bass numbers and mass increase 100-fold. These observations are fully consistent with the premise that the earlier observations of elevated selenium concentrations had been impairing reproduction of largemouth bass.

In contrast, the ups and downs of channel catfish numbers, mass, and size seem to vary randomly throughout the period of study. In 1984 catfish numbers reached their third highest value while their average size was at its minimum: that is, there were many young individuals. Simultaneously, largemouth bass was near its minimum for both numbers and mass. The next year (1985) catfish numbers jumped to their maximum for the study period, and mass reached near maximum. Such observations are easily explained if reproduction is taking place. But they seem inexplicable under a premise that channel catfish reproduction was even more impaired than largemouth bass reproduction, and its population merely a senescent non-reproducing remnant of the pre-contamination population. Rather the observations indicate that *if* selenium was having *any* effect on catfish reproduction, it was far less than on largemouth bass reproduction and was no hindrance to rapid population increases.

Observations of selenium-contaminated Belews Lake accord with the above observations of Hyco Reservoir. Young et al. (2010) indicate that as many as 29 resident fish species were documented prior to contamination, but only common carp, catfish, and fathead minnows

remained after contamination. The Doroshov et al. (1992b) injection study results suggesting that channel catfish is sensitive at egg concentrations of 5 mg Se/kg dw, four-fold below the largemouth bass Chronic Value, thus conflict with field observations. As demonstrated in the Appendix C discussion of the Cleveland et al. (1993) toxicity tests with juvenile bluegill, the exposure route by which selenium was accumulated can have a dramatic influence on the potency of a given tissue concentration. That is, to accord with the Cleveland et al. (1993) data, the whole-body EC_{50} would be expected to be at least four-fold higher when accumulated via diet than when accumulated via water. For this reason, the criterion is derived only from tests using the environmentally relevant exposure route of diet.

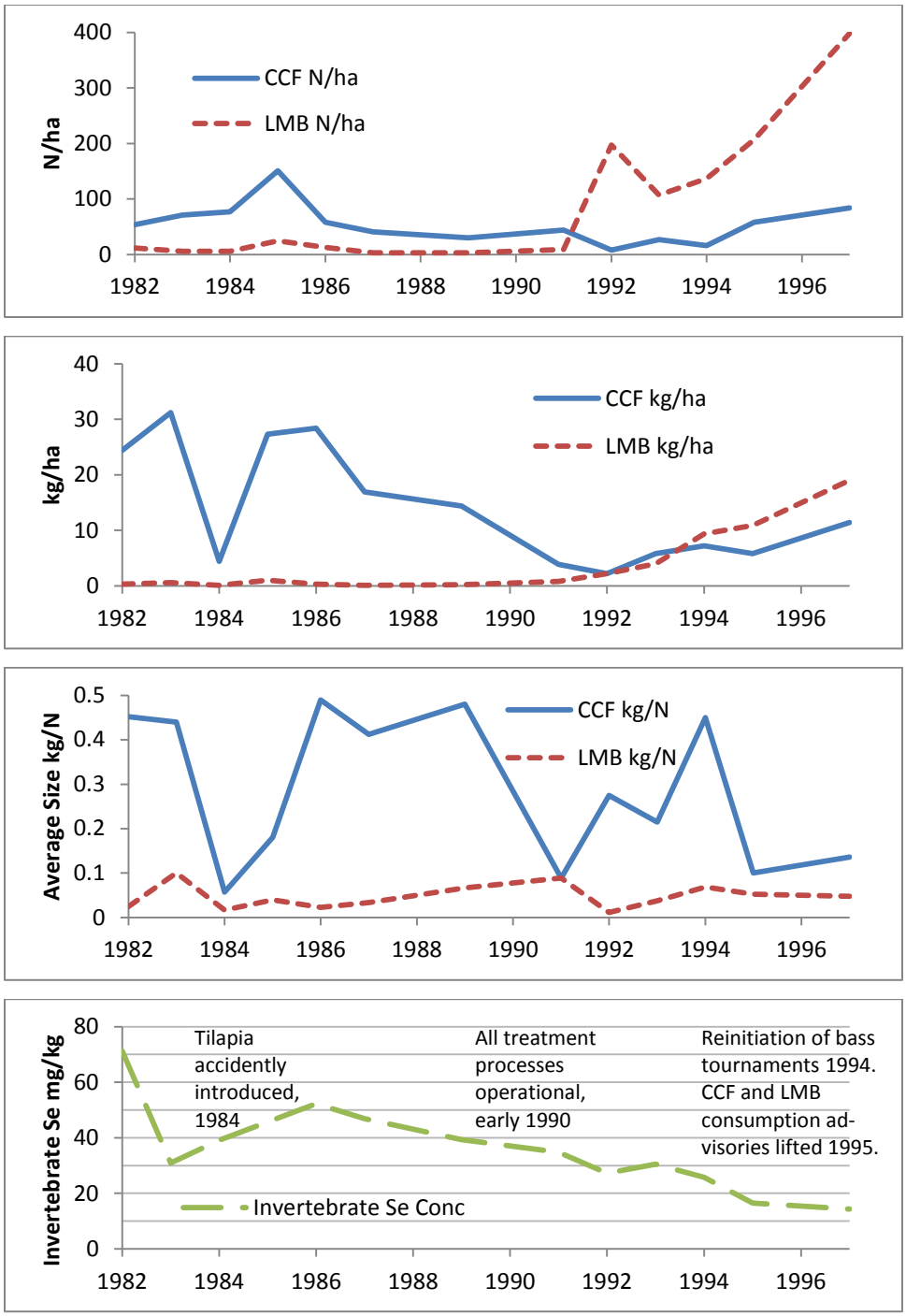


Figure 6.1. Crutchfield (2000) Observations of Channel Catfish (CCF) and Largemouth Bass (LMB) in Hyco Reservoir Beginning a Few Years after Populations of Largemouth Bass had been Reduced by Se Contamination.
(A) number of individuals/ha, (B) mass/ha, (C) mass/ha divided by number/ha, yielding average weight per individual, and (D) invertebrate Se concentration (mg Se/kg dw), and noting other events relevant to management of the fishery.

6.1.4 Reproductive Effects in Amphibians (*Xenopus laevis*)

Massé et al. (2015) has conducted the only maternal transfer study conducted with an amphibian under controlled laboratory conditions. The African clawed frog (*Xenopus laevis*) was fed a control diet (0.73 mg/kg Se dw) and three spiked diets containing selenium concentrations of 10.92, 30.4 and 94.2 mg/kg dw. Trophic transfer to the frog's eggs was approximately 1:1 with measured selenium concentrations in the control and three spiked diets of 1.6, 10.82, 28.13, and 81.66 mg/kg egg dw, respectively. Deformities were assessed in 200 tadpoles per female (1800 – 2000 tadpoles per treatment group). EC_{10} values determined by the authors for abnormal spinal curvature, abnormal craniofacial structure and abnormal lens structure were 57.3, 38.4, and 34.5 mg/kg Se egg dw, respectively. The EC_{10} value for total deformities of 44.9 mg/kg Se egg dw is in the upper-range of EC_{10} values for fish (see **Table 3.2**). Although *X. laevis* is a non-native amphibian with a different reproductive strategy, their upper-range sensitivity suggests amphibians are protected by the fish chronic criterion elements.

6.1.5 Reproductive Studies Not Used in the Numeric Criterion Derivation

Danio rerio (zebrafish)

Two studies (Penglase et al. 2014; and Thomas and Janz 2014) have shown the zebrafish, *Danio rerio* (family Cyprinidae), to be sensitive to selenium. Penglase et al. (2014) assessed the interaction of selenium with mercury through a maternal transfer study but did have two treatments with selenium exposures resulting in 1.17 mg/kg egg dw (control) and 6.24 mg/kg egg dw. The higher Se egg concentration had significantly reduced embryo survival and fecundity relative to the control, however embryo survival in the controls was low at 54%. With only one selenium treatment exposure, the data were not amenable to TRAP analysis. Thomas and Janz (2014) conducted a maternal transfer study using adult zebrafish that were fed a control diet and three levels of selenomethionine, 3.7, 9.6, and 26.6 mg/kg Se dw for 90 days before breeding the exposed fish and collecting the fertilized embryos for assessment. TRAP analysis of larval survival and larval deformities of 2-6 days post fertilization fish produced very low EC_{10} values. The lowest EC_{10} was for deformities at 7.0 mg/kg egg dw. This value is markedly lower than any of the EC_{10}'s in the current data set. The slope of the concentration-response curve for both deformities and larval survival was very shallow, which was different than the selenium responses for all other fish species for which data were available (see Figure E-6 in Appendix E). Further, the control mortality in the experiment continuing over 160 days was high, over 40%.

This zebrafish EC_{10} for deformities contrasts with the absence of deformities in the related species, fathead minnow, observed by GEI (2008) at concentrations up 40 mg/kg in adult whole body (dw) as presented in Figure E-3 in Appendix E. The GEI (2008) fathead minnow study was not directly used for criterion derivation because the offspring survival data for Sand Creek appeared to be confounded by multiple stressors in this industrial waterway. However, its deformity data appear unequivocal, indicating that the fathead minnow deformity endpoint is relatively insensitive to selenium.

Since the zebrafish is a non-native cyprinid species, EPA assessed the information available on zebrafish sensitivity to selenium compared to the sensitivity of native cyprinid (minnow) species across the U.S. (Appendix E in the 2016 criterion document), including several studies where native cyprinids were investigated in selenium-impacted waters (NAMC 2008). Data from these studies suggest that native cyprinids are likely less sensitive to selenium than the non-native zebrafish.

The anomalous nature of the concentration-response curve, with the very low value coupled with field and other laboratory data suggesting that cyprinids are not particularly sensitive to selenium was the basis for not including the zebrafish EC_{10} in the data for deriving the criterion. A detailed write up of this study and a summary of field and laboratory studies indicating native cyprinids are not one of the more sensitive families are provided in Appendix E.

Oncorhynchus clarkii (cutthroat trout)

Kennedy et al. (2000) reported no significant differences in mortality and deformity in eggs, larvae, and fry from wild-caught cutthroat trout between a reference and an exposed site (Fording River, British Columbia, Canada). The observations were made on eggs reared in well water from spawning age females collected from the two locations (N = 17 and 20, respectively) and fertilized by one male collected at each site. The mean selenium content in eggs from fish collected from the reference site was 4.6 mg/kg dw and from fish collected from the Fording River was 21.2 mg/kg dw. The chronic value for eggs is >21.2 mg Se/kg dw. These values were not used in the criterion derivation because they represent high "greater than" values, as discussed above, and provide no additional important quantitative data for the analyses.

Hardy (2005) fed cutthroat trout experimental diets containing a range of selenomethionine (0-10 mg/kg dw) for 124 weeks. No significant growth or survival effects were

observed in the adult fish over the 124 weeks. The whole body concentration reached 12.5 mg/kg dw selenium after 44 weeks. Embryo-larval observations (percent hatch and percent deformed) were not related to whole body selenium concentrations in the spawning females (9.37 mg/kg dw) fed the selenium-laden diet for 124 weeks. The concentration of selenium in eggs from these females was 16.04 mg/kg dw. For this study the chronic value, an unbounded NOEC, is thus >16.04 mg Se/kg dw in eggs. This value was not used in the criterion derivation.

Salvelinus fontinalis (brook trout)

Holm et al. (2005) collected spawning brook trout from streams with elevated selenium contaminated by coal mining activity and from reference streams in 2000, 2001 and 2002. Similar to procedures described by these authors for rainbow trout, above, fertilized eggs were monitored in the laboratory for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. Embryos from the contaminated stream had on average a higher frequency of craniofacial deformities than fry from the reference stream (7.9% for the contaminated stream compared to 2.1% in the reference stream). Although this increased rate of craniofacial deformities was calculated to be statistically significant when compared across sites, the Abbott-adjusted effect is only 6% and is thus below the 10% effect represented by an EC_{10}. But more important, when comparing across adult females (the more reliable analysis for selenium reproductive toxicity studies of this type, and the one used to obtain the related rainbow trout EC_{10} for these authors' studies), there is no apparent relationship between brook trout craniofacial deformities and exposure across a broad range of concentrations, as illustrated in Appendix C. An environmentally conservative estimate of the NOEC might be considered to be the average concentration of selenium in eggs from the high exposure site (Luscar Creek), >7.78 mg Se/kg ww or >20.5 mg Se/kg dw using the 61.2% moisture content for rainbow trout eggs cited above. However, the effect threshold appears to be substantially higher based on the absence of any consistent concentration-response relationship up to the maximum observed egg concentration of 18.9 mg Se/kg ww or 48.7 mg Se/kg dw, as shown in the Appendix C graphs. Given the point estimate EC_{10} available for the related species, *Salvelinus malma* (Dolly Varden, **Section 6.1.2.3**), the "greater than" chronic value for brook trout is not used to obtain the GMCV, in accordance with the principles listed in **Section 6.1.1**.

Lepomis macrochirus (bluegill)

Applicable chronic reproductive data for bluegill can be grouped by exposure type: field and laboratory. In some field studies, chronic value estimates were "less than" fairly high selenium concentrations (Bryson et al. 1984, 1985a; Gillespie and Baumann 1986). This low resolution is due to the observed effect occurring at a single observed high exposure concentration relative to a reference condition. In the Bryson et al. (1984, 1985a) and Gillespie and Baumann (1986) studies, the artificially crossed progeny of females collected from a selenium contaminated reservoir (Hyco Reservoir, Person County, NC) did not survive to swim-up stage, irrespective of the origin of milt used for fertilization. Measured waterborne selenium concentrations prior to the experiments ranged from 35 to 80 µg/L. The ovary tissue selenium concentration associated with this high occurrence of mortality of hatched larvae was <30 mg/kg dw tissue, as reported by Bryson et al. (1985a), and <46.30 mg/kg dw tissue, as reported by Gillespie and Baumann (1986). In the case of the latter, nearly all swim-up larvae from the Hyco Reservoir females were edematous, none of which survived to swim-up.

Bryson et al. (1985b) examined percent hatch and percent swim-up larvae from spawns using bluegills collected from Hyco Reservoir and a control site. There were no differences in the Hyco measurements relative to the control. The concentration of selenium in the liver of the parental Hyco bluegill was 18.6 mg/kg dw. The chronic values for this embryo-larval development test was >18.6 mg Se/kg dw liver. The high "less than" and low "greater than" chronic values obtained from Bryson et al. (1984, 1985a, b) and Gillespie and Baumann (1986) were not used in the SMCV calculation because these values are consistent with and yet provide no numeric basis for modifying the SMCV obtained from the EC_{10}s.

6.1.6 *Salmo* GMCV: EPA Re-analysis of a Key Study Used in Criterion Derivation

Previously, in the draft selenium criterion document submitted for external peer review in May 2014, the lowest GMCV in the reproductive effects dataset was for *Salmo* (15.91 mg/kg dw) based on larval deformities. Subsequently, in 2015, EPA conducted a careful re-analysis in response to stakeholder comments to confirm the validity of the approach used in 2014, resulting in the calculation of an EC_{10} at 18.09 mg/kg dw based on larval mortality from hatch through swim up, prior to a lab overflow accident during the post swim up feeding portion of the test. The dataset was constrained to hatch through swim up information due to uncertainty introduced by the loss of larvae from an overflow event caused by clogged drains during the post swim-up

portion of the test (Formation 2011). The hatch through swim up deformity endpoint was not considered because of the preferential selection of visibly non-deformed fish for the post swim up portion of the test (Response letter to EPA, J.R. Simplot Company 2014). This is important because the primary endpoint of interest during the post-swim up phase is deformity rate. Random selection of living fish would have been more appropriate since visibly healthy fish may be less likely to express deformities in this later stage of the test.

Following the release of the 2015 draft selenium criterion document, the larval survival from hatch through swim up dataset was reanalyzed, and it was determined that the TRAP model resulting in an EC_{10} of 18.09 mg/kg was not appropriate, because the EC_{10} was lower than one of the treatment levels within the background no-effect range. In order to calculate an EC_{10} that would not fall below the highest background concentration, a weighted least squares nonlinear regression was calculated in TRAP, resulting in an EC_{10} of 21.0 mg/kg. Additional details describing this weighted nonlinear regression approach are described in Appendix C.

6.1.7 Impact of Number of Tested Species on Criterion Derivation

Many of the species used for testing the toxicity of selenium are those observed to be affected at contaminated sites or otherwise suspected to be particularly sensitive. Six of the eight minimum data requirements were met, and the other two (for planktonic and benthic crustaceans) were waived (see **Section 2.6**). Of the N=15 genera used for the calculation of the criterion, ten are fish, which are more sensitive than invertebrates, based on the available data. Of the ten fish genera, five are either salmonids or centrarchids. Had a broader array of expected insensitive taxa been included, the four most sensitive genera would not likely change, but N would increase. The criterion calculation for selenium is relatively insensitive to the effect of increasing the value of N by adding more tests with different genera than those already represented. Setting N=20 (leaving the four most sensitive the same) would only raise the egg-ovary criterion element from 15.1 mg Se/kg to 16.0 mg Se/kg. This insensitivity occurs because the four lowest GMCVs are closely spaced, such that the calculated egg-ovary criterion element is never distant from the lowest GMCV.

6.1.8 Comparisons between Concentrations in Different Tissues

Researchers often report concentrations of selenium in fish eggs or ovaries (e.g., Formation Environmental 2011, 2012; Holm et al. 2005; Osmundson et al. 2007). Osmundson et

al. (2007) found reduced levels of selenium in ovaries after spawning, presumably due to the loss of selenium through spawning and release of eggs with relatively high concentrations of selenium. Of the 14 chronic values determined from the maternal transfer reproductive studies, 12 values represent selenium measured in eggs. Two values represent selenium measured in the ovaries: Hermanutz et al. (1992, 1996) and Carolina Power & Light (1997). Hermanutz et al. (1992, 1996) sampled adult female bluegill just prior to spawning and at the end of the test (post spawning) and found no decreases in the concentration of selenium in the post-spawned fish. In the Carolina Power & Light (1997) study, selenium in ovaries of largemouth bass was measured from fish sampled just after spawning. No comparison to prespawning fish or selenium in eggs can be made for the largemouth bass study, however, the EC_{10} of 26.3 mg Se/kg ovary dw was mid-range in the SSD indicating this test was not overly conservative due to lower selenium measurements in post spawning ovaries. Based on the observations stated above, egg selenium and ovary selenium were considered equal for the toxicity data set. Any potential error resulting from this assumption would be conservative since the effect of spawning only lowers the selenium concentration in the ovary. EPA recognizes selenium ovary concentrations may vary in field collected samples due to fish reproductive cycles and will address such concerns in the implementation information.

6.1.9 Studies of Non-Reproductive Effects

Non-reproductive effect studies do *not* involve effects on the offspring of exposed female adults, and their results are *not* expressed as selenium concentrations in egg or ovary tissue. Because selenium concentrations in whole body and muscle are generally lower than in egg and ovary, with observed egg-ovary to whole-body ratios ranging from 1.3 to 7.4, and egg-ovary to muscle ratios ranging from 1.0 to 5.8, *whole-body, muscle, and egg-ovary effect concentrations can only be compared after accounting for the inherent differences in the selenium concentrations in these different media*. Non-reproductive effects were determined to provide a less reliable basis for a criterion, in part because comparatively few of such studies provided sigmoidal concentration-response curves. Non-reproductive SMCVs and GMCVs are shown in **Table 6.2** below and summaries of the acceptable non-reproductive studies are included in Appendix D.

Table 6.2. Freshwater Chronic Values from Acceptable Tests - Non-Reproductive Endpoints.
(Parental Females Not Exposed).

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Acipenser transmontanus white sturgeon	Tashjian et al. 2006	dietary (lab) 8 weeks	seleno-L-methionine in artificial diet	EC_{10} juvenile growth	15.08 WB 27.76 M	EC_{10} 15.1 WB 27.8 M	15.1 WB 27.8 M
			seleno-L-methionine in artificial diet	EC_{20} juvenile growth	17.82 WB 32.53 M	EC_{20} 17.8 WB 32.5 M	
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	dietary (lab) 9 months	selenized-yeast	NOEC	10.1 M	10.1 M 15.1 M 12.3 M	10.1 M 15.1 M 12.3 M
				LOEC	15.1 M		
				MATC juvenile deformities (juvenile exposure only)	12.34 M		
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab) 9 to 19 days	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40 WB	51.40 WB 69.83 M	51.40 WB 69.83 M
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab) 8 days	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOEC for larval fish dry weight after 8 d	<73 WBb		
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>12.9 WBb	see text	see text
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001b	dietary and waterborne (lab) 28 days	water: site waters; diet: algae exposed to site water then fed to rotifers which were fed to fish	NOEC for survival and growth	>42 WBb		
Catostomus latipinnis flannelmouth sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>10.2 WB	>10.2 WB	>10.2 WB
Oncorhynchus tshawytscha	Hamilton et al. 1990	dietary (lab) 60 days	mosquitofish spiked with seleno-DL-methionine	EC_{10} for juvenile growth	7.355 WB	EC_{10} 9.052 WB	EC_{10} 9.052 WB

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
chinook salmon				EC_{20} for juvenile growth	10.47 WB	EC_{20} 12.83 WB	
			mosquitofish spiked with SLD diet	EC_{10} for juvenile growth	11.14 WB		
				EC_{20} for juvenile growth	15.73 WB		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab) 16 weeks	sodium selenite in food preparation	juvenile growth NOEC	21 Liver	NOAEC 28.98 L	
				LOEC	71.7 Liver		
				MATC	38.80 Liver	LOAEC 84.68 L	
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juvenile survival and growth NOEC	40 Liver		
				LOEC	100 Liver	MATC 49.52 L	
				MATC	63.25 Liver		
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab) 80 days	Se-laden shiners from Belews Lake, NC	LOEC for survival of yearling bass	<16.2 M[c]	<16.2 M	<16.2 M
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab) 180 days 20 to 4°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOEC for juvenile mortality at 4°C	<7.91 WB	4°C EC_{10}-NOAEC 8.15 WB	4°C & 9°C 9.33 WB
				Threshold prior to "winter stress"	5.85 WB		
		dietary and waterborne (lab) 180 days 20°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC for juvenile mortality at 20°C	>6.0 WB		
Lepomis macrochirus bluegill	McIntyre et al. 2008	dietary and waterborne (lab) 182 days 20 to 4°C (ES1)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES1	9.27 WB	4°C EC_{20}-LOAEC 8.80 WB	
				EC_{20} juv. survival ES1	9.78 WB		
		dietary and waterborne (lab) 182 days 20 to 9°C (ES3)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES3	14.00 WB	9°C EC_{10} 14.0 WB	
				EC_{20} juv. survival ES3	14.64 WB	9°C EC_{20} 14.6 WB	
		dietary and waterborne (lab) 182 days 20 to 4°C (ES2)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC juv. surv. ES2	>9.992 WB		

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary (lab) 60 days	seleno-DL-cysteine	NOEC for juvenile growth	>3.74 WB[b]		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary (lab) 90 days	seleno-L-methionine	NOEC for juvenile survival	>13.4 WB[b]		

[a] All chronic values reported in this table are based on the measured concentration of selenium in whole body (WB), muscle (M) or liver (L) tissues.

[b] Chronic value not used in SMCV calculation (see text).

[c] Tissue value converted from ww to dw. See Appendix C for conversion.

6.1.9.1 Comparison of Fish Chronic Reproductive Effects and Chronic Non-Reproductive Effects

A chronic criterion element concentration of 15.1 mg/kg dw in the egg/ovary addresses the toxic effect identified by the Chapman et al. (2009, 2010) expert workshop to be of greatest concern, reproductive effects, and is expected to be protective of non-reproductive endpoints such as juvenile survival and growth.

If the information in the reproductive-effect GMCV **Table 3.2** (expressed as whole-body) were combined with the information in the nonreproductive-effect **Table 6.2**, and the lower of the reproductive or nonreproductive GMCVs for each taxon were used to construct a combined distribution of whole-body chronic values, the resulting criterion element (corresponding to N=18, accounting for three additional fish genera only having nonreproductive-effect GMCVs), the FCV would be calculated to be 9.1 mg Se/kg WB dw, similar to the 8.5 mg Se/kg WB dw FCV for reproductive effects expressed as whole-body **Figure 6.2**.

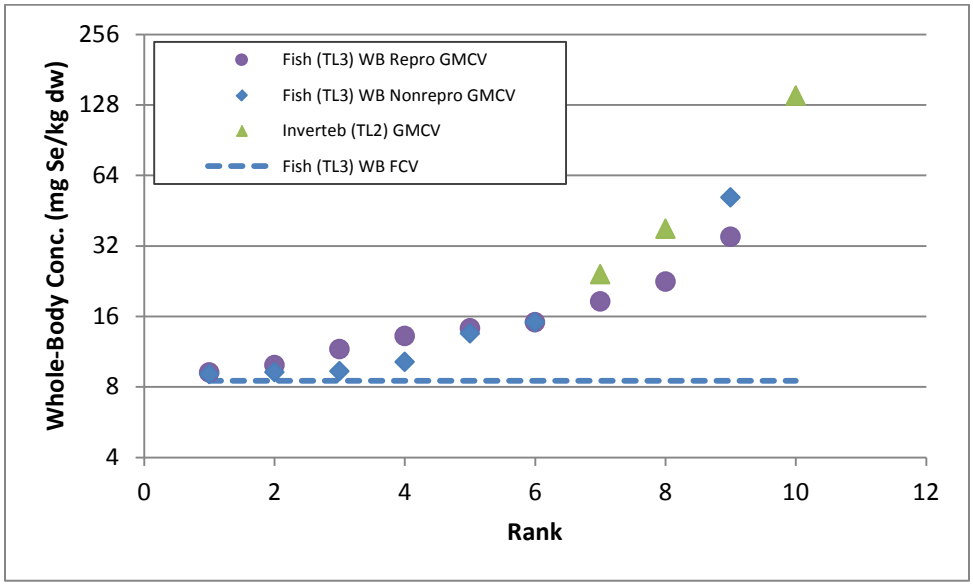


Figure 6.2. Distribution of Fish Reproductive Effect GMCVs from Figure 3.2 and Distribution of Fish Nonreproductive Effect GMCVs and Invertebrate GMCVs.

For establishing a reliable criterion, the sufficiency of and consistency among the data underlying the reproductive-endpoint GMCVs favor their use over any non-reproductive endpoint data (see **Section 3.1.1** and Appendix C). Most of the reproductive studies involved

examining the offspring of wild-caught females, exposed under real-world conditions. Most had concentration-response curves that supported EC_{10} estimates.

In contrast, the non-reproductive endpoint studies provide fewer data for supporting a criterion, and fewer of these studies yielded the type of concentration-response data that could support EC_{10} estimates. Furthermore, the non-reproductive data are not as consistent, as noted by Janz et al. (2010). The reproductive effect data also show more clear-cut concentration-response relationships than the non-reproductive effect data (11 of the reproductive chronic values are specific ECs, compared to only five of the non-reproductive chronic values), are more readily reproducible, and are better corroborated by field observations. Reproductive effects represent the endpoint of greatest concern (Chapman et al. 2009, 2010); all non-reproductive GMCVs are protected by a criterion derived from the reproductive GMCVs. The reproductive endpoint data, expressed relative to selenium concentrations in fish eggs and ovaries, thus provide a more reliable and protective basis for the criterion. Because the data set used to derive the criterion is comprised primarily of the aquatic species considered most sensitive to selenium (salmonids and centrarchids) and because the criterion is designed to protect 95% of the genera, the egg-ovary criterion element concentration of 15.1 mg/kg dw ovary/egg should be protective of aquatic populations of fish and invertebrates.

6.1.10 Special conditions for consideration of primacy of water column criterion elements over fish tissue criterion elements

The chronic selenium criterion is derived to be protective of the entire aquatic community, including fish, amphibians, and invertebrates. Fish are the most sensitive taxa to selenium effects. Selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) sample data supersede the criterion elements based on water column selenium data, when measured in the same approximate time frame (approximately one year) and site. This is due to the fact, noted above, that fish tissue concentrations provide the most robust and direct information on potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) In "fishless" waters, and 2) new selenium inputs. *Fishless waters* are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported

populations of one or more fish species but no longer support fish (i.e., extirpation) due to temporary or permanent changes in water quality (e.g., due to selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish within such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas. *New inputs* are defined as new activities resulting in selenium being released into a lentic or lotic waterbody. New inputs will likely result in increased selenium in the food web, resulting in increased bioaccumulation of selenium in fish over a period of time until the new selenium release achieves a quasi-"steady state" balance within the food web. EPA estimates that concentrations of selenium fish tissue will not represent a "steady state" for several months in lotic systems, and longer time periods (e.g., 2 to 3 years) in lentic systems, dependent upon the hydrodynamics of a given system; the location of the selenium input related to the shape and internal circulation of the waterbody, particularly in reservoirs with multiple riverine inputs; and the particular food web. Estimates of time to achieve steady state under new selenium input situations are expected to be site dependent, so local information should be used to better refine these estimates for a particular waterbody. Thus, EPA recommends that fish tissue concentration not supersede water column concentration until these periods of time have passed in lotic and lentic systems, respectively, or until steady state concentrations can be determined.

6.2 WATER

6.2.1 Validation of Translation Equation for Developing Water Column Concentrations

EPA evaluated the efficacy of the equation used to translate the egg-ovary criterion element to a water column concentration. EPA's translation equation is given as:

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \text{(Equation 18)}$$

Because selenium levels in fish are relatively stable over a long time period if the ecosystem is at steady state with respect to selenium concentration, single measurements of selenium in fish tissue are likely to be less variable and a better representation of selenium loads to the aquatic system than single measurements of selenium in the water column. Thus, EPA used a validation approach based on fish tissue measurements rather than single water measurements.

Rearranging Equation 18 to solve for egg-ovary concentration yields:

$$C_{egg-ovary} = C_{water} \times TTF^{composite} \times EF \times CF \qquad \text{(Equation 22)}$$

EPA used Equation 22 to calculate the predicted concentration of selenium in the eggs and ovaries of fish from all spatially and temporally relevant measurements in the water column. EPA then compared those predicted values to the measured concentration in the fish.

EPA searched its collection of selenium measurements in fish tissue taken from aquatic sites with a previously calculated *EF* value. Identified tissue measurements from other than eggs or ovaries were converted to equivalent egg-ovary concentrations using species-specific conversion factors as described previously. For each tissue measurement, EPA searched its collection of selenium measurements again for water column measurements that were taken from the same aquatic site and within one year of the tissue measurement. If more than one water column measurement was matched to a tissue measurement, the median water column measurement was used. For each matched pair of tissue and water measurements, appropriate species-specific *TTF* and *CF* values were identified as described previously, and the *EF* value from the site samples were taken. EPA then used Equation 22 to calculate the predicted egg-ovary concentration from the observed water column concentration. Finally, EPA compared the predicted egg-ovary concentrations with the observed egg-ovary concentrations.

EPA identified 317 tissue measurements associated with one or more water column measurements. A predicted egg-ovary concentration was calculated for each water column concentration as described above. **Figure 6.3** shows all 317 predicted egg-ovary concentrations plotted against the measured egg-ovary concentrations. Because both the predicted and observed selenium concentrations exhibited substantial heteroscedasticity (the variability of one variable is unequal across the range of values of a second variable that predicts it), they are plotted and analyzed on a log scale. The predicted and measured concentrations are highly correlated ($r=0.82$, $t_{(315)}=25.30$, $P<0.001$). Data used to generate **Figure 6.3** can be found in Appendix I.

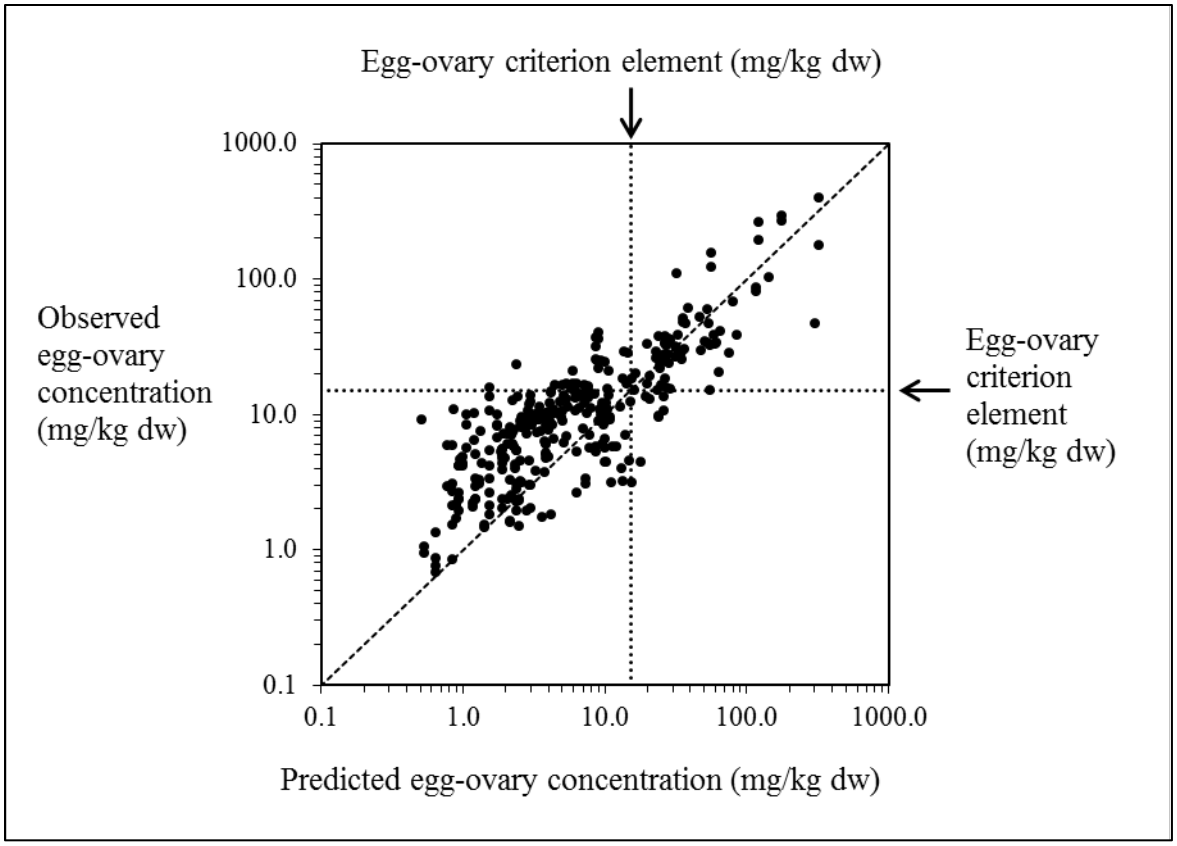


Figure 6.3. Scatter Plot of Predicted Versus Measured Concentrations of Selenium in Fish. Solid line shows unity y = x line; dashed lines show the egg-ovary criterion element value.

Although there is a strong correlation between predicted and observed egg-ovary concentration values, **Figure 6.3** shows more data points above the y = x line at low selenium concentrations. This result suggests the model underestimates bioaccumulation at low selenium concentrations. Such behavior is likely the result of the inherent model assumption of constant bioaccumulation rates regardless of selenium concentration, whereas selenium bioaccumulation has been shown to be inversely related to water concentration (see **Sections 3.2.2** and **3.2.4** for further discussion). Within the range of concentrations near the egg-ovary criterion element value, however, the relationship between predicted and observed selenium concentrations are evenly dispersed around the y = x line. Thus the model is unlikely to result in biased estimates near egg-ovary concentrations that may require regulatory action.

Dispersion around the unity line is likely attributable to several sources of uncertainty including small sample sizes, temporal or spatial variability in selenium exposure, and local variability in aquatic food webs. EPA limited this analysis to only those aquatic sites with at least

two particulate measurements available to calculate an *EF* value and with at least one of them from algae or detritus. The requirement of at least two particulate measurements was made because a single measurement was considered insufficient. The requirement that at least one of the measurements be for algae and/or detritus was made because selenium within these particulate types was more highly correlated to water (**Section 3.2.2.3**). Nevertheless, only one or two measurements of algae and/or detritus were available for 62 of the 96 aquatic sites evaluated. Although the minimum data requirements described above reduce uncertainty when applying Equation 22 to available data, EPA believes that two particulate measurements are only marginally sufficient. Another potential source of uncertainty is the frequent absence of site-specific information about the types and proportions of organisms ingested by fish. In most cases, EPA estimated the type and proportion of prey organisms using general knowledge of the fish species and aquatic system location. Notwithstanding the limitations in available data, the EPA concludes from this analysis that Equation 18 provides a reasonable translation of the egg-ovary criterion element to a site-specific water concentration.

6.2.2 Sulfate-Selenium Interactions

Several investigators (Brix et al. 2001; Ogle and Knight 1996; Williams et al. 1994) have previously evaluated the role of sulfate on the bioavailability and toxicity of selenium in freshwater organisms. A report from DeForest et al. (2014) notes that a sulfate-dependent selenium criteria would apply only to selenate-dominated, well-oxygenated streams, a subset of freshwater systems in the U.S. The DeForest publication discussed experiments to assess influence of sulfate on selenate uptake on one species of macrophyte (*Lemna minor*) and one algal species (*Pseudokirchnella subcapitata*), a limited data set of primary producers. The authors note that, "It does need to be emphasized here, however, the analysis currently does not include Se data for periphyton and benthic diatoms, as these data are not available." The authors also note that, "due to methodological challenges and high costs, it is difficult to comprehensively evaluate the influence of sulphate on bioconcentration and transfer up the food chain."

Including any type of sulfate relationship in the national criterion derivation would necessitate having sulfate measurements to accompany all observed selenium water concentrations included in the derivation database. That is, the absence of an accompanying sulfate observation would necessitate excluding the water observation. The resulting reduction in

the number of sites included in the database would reduce the confidence in its ability to represent the nation's waters. For the above reasons, EPA has not included a sulfate relationship in the 2016 selenium criterion.

6.3 UNCERTAINTY

This section examines several areas where EPA addressed uncertainty in the development of the selenium water quality criterion. This section represents a qualitative treatment of specific parts of the derivation process for the selenium freshwater chronic criterion where EPA has identified the potential for uncertainty, and also describes the approaches that the Agency used to reduce uncertainty.

EPA developed a tissue-based water quality criterion designed to be protective of aquatic life from the chronic effects of selenium. In general, EPA followed the procedure detailed in the document, Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the "Guidelines") (Stephan et al. 1985). The Guidelines sets forth a methodology for deriving ambient water quality criteria for the protection of aquatic life that includes a rigorous list of data quality requirements. Because selenium is a bioaccumulative chemical with maternal diet and transfer as the primary route of exposure for chronic toxicity, EPA included additional data quality requirements such as the requirement of a dietary exposure. See **Section 2.7.5** Analysis Plan for Derivation of the Chronic Fish Tissue-Based Criterion Elements for how chronic effect levels were determined for selenium. The Guidelines provide several recommended approaches that reduce uncertainty in the derivation of criterion. It provides a strict set of guidelines for the acceptance of data to be used in criteria derivation. It provides a minimum set of data requirements (MDRs) that define an assemblage of aquatic organisms that can be used in a genus sensitivity distribution to derive a criterion that is protective of 95% of aquatic species. The requirements in the Guidelines reduce the uncertainty in the ability of a criterion to be protective of aquatic life.

6.3.1 Tissue Criterion Element

The tissue criterion element is based on reproductive effects caused by selenium and is expressed in three different tissue types, egg/ovary, muscle and whole body. Non-reproductive effects were also determined but not used in the derivation of the criterion because of less certainty in the endpoints and effect levels (**Section 6.1.9** and **Table 6.2**). A comparison of fish

reproductive and non-reproductive effects and the conclusion that the reproductive criterion is protective of the non-reproductive effects is given in **Section 6.1.9.1**.

The dataset used to derive the tissue-based criterion consists primarily of fish species: 12 fish species representing 10 genera and 7 families. Although this might be viewed as a small number of the nearly 800 native freshwater fish species (36 families) of fish in the United States, it is a large number of species relative to chronic criteria derivations for other pollutants. Furthermore, the fish species that have been the focus of some of the research have been the species observed to be those first affected (most sensitive) at selenium-contaminated sites such as Belews Lake and Hyco Reservoir, i.e., bluegill sunfish and largemouth bass. The data set contains three acceptable chronic toxicity studies with bluegill sunfish, the second most sensitive genus in the dataset and one with largemouth bass, the fifth most sensitive genus. The three replicate chronic values for bluegill are 14.7, 22.6, and 26.3 mg Se/kg egg-ovary dw. Of these three values, 14.7 mg Se/kg ovary dw is likely the least certain because the study (Hermanutz et al. 1992, 1996) was not designed to minimize uncertainty in characterizing the tissue concentrations associated with its observed levels of effect.

Three genera representing five species from Salmonidae, a family considered to be generally sensitive to contaminants, are in the data set. Two salmonid genera, *Salmo* and *Oncorhynchus* are the third and fourth most sensitive taxa in the data set. *Salmo* is represented by a single study, but because the study included a large number of individuals across a broad spectrum of exposure, the uncertainty associated with its 21 mg Se/kg egg dw might not be viewed as particularly large. The chronic values for the three studies with *Oncorhynchus* are confined to the narrow range 24.5 – 27.7 mg Se/kg egg dw, and on that basis may be considered to have low uncertainty. Although the numbers of species and families of fish in the data set are a fraction of what are native to the United States, the fish species contained in the data set are known to be those most sensitive to selenium based on field observations or known to be sensitive in general to contaminants. With the lowest six chronic values falling in the relatively narrow range 15.6 – 27 mg Se/kg egg-ovary dw, the selenium tissue criterion element should probably be considered to have the smallest amount of uncertainty among the existing aquatic life criteria.

As stated in the previous paragraph, the data set primarily consists of fish species and contains only three invertebrate species. The cases in the field where adverse effects have been

observed to fish and water birds (e.g., Belews Lake, Hyco Reservoir, Kesterson Reservoir) have not documented any adverse effects on macroinvertebrates either on a species or community level (Janz et al. 2010). The effect levels determined for the three invertebrate species contained in the data set are consistent with the field observations that macroinvertebrates are in general less sensitive to selenium than fish species. EPA recognizes that there may be more sensitive oviparous taxa (fish and amphibians), as well as macroinvertebrate taxa than those in the current data set and supports the testing of different species.

6.3.1.1 Reproductive Endpoints

Reproductive endpoints were determined from studies in which adults were exposed to selenium either in the laboratory or field. Effects were measured in the offspring which received selenium exposure via maternal transfer. Larval mortality and teratogenic deformities such as skeletal, craniofacial, and fin deformities, and various forms of edema that result in mortality are the most sensitive indicators of selenium toxicity in fish larvae. Recent research suggests the mode of action of selenium-induced toxicity in fish larvae is due to oxidative stress and appears to be related to glutathione homeostasis (See **Section 2.3** for more detail on this subject.). Linking the mode of action directly to the assessment endpoint used in the derivation of the tissue-based criterion provides a consistent concentration-response relationship among the studies used in the data set. Using the most sensitive assessment endpoint (based on the state of the science) reduces uncertainty in the ability of the criterion to protect aquatic life.

6.3.1.2 Egg Ovary Chronic Values

Chronic Values (CV) were based on the most direct representation of exposure to the effect in the offspring, that is, the concentration of selenium in the egg/ovary. One way to assess the precision of the chronic values used in the derivation of the criterion is to look at the reproducibility of tests used to calculate the CV for a taxon. This precision assessment can be done with two tests conducted with cutthroat trout and three tests conducted with bluegill sunfish. The two cutthroat trout studies (Rudolph et al. 2008 and Nautilus Environmental 2011) had very similar EC_{10} values (24.7 and 27.7 mg Se/kg egg dw, respectively) for the same endpoint (larval survival) using fish collected at the same site. Two of the three bluegill tests (Coyle et al. 1993 and Doroshov et al. 1992a) also had very similar EC_{10} values (26.3 and 22.6 mg Se/kg egg dw, respectively) for larval endpoints determined in laboratory exposures. An

EC_{10} of 14.7 mg Se/kg ovary dw was determined in the third bluegill test, a mesocosm exposure study reported by Hermanutz et al. (1992, 1996). Although the mesocosm study had a lower EC_{10} value when compared with Coyle et al. (1993) and Doroshov et al. (1992a), it was within a factor of 1.8 and 1.5, respectively. Delos (2001) found such differences to be typical when equivalent toxicity tests of the same species are compared. The relatively low variability between chronic toxicity tests conducted with the same species indicates precision in the CV estimates, which reduces the uncertainty the tissue-based criterion.

Most of the CVs were determined using an EC_{10} value and a few were estimated using the NOEC. EC_{10} values were considered more appropriate than EC_{20}, because selenium is a tissue-based criterion due to its nature of exposure and effects for this bioaccumulative chemical. See **Section 2.7.1** for a discussion of why EC_{10}s were favored over EC_{20}s. The use of EC_{10}s and NOECs increases the certainty that the criterion will be protective of aquatic life.

6.3.1.3 Whole Body and Muscle Chronic Values

Effect levels (EC_{10} or NOECs) were determined directly for whole body or muscle tissues when the selenium concentrations for these tissues were measured and reported in the tests. Effect levels were calculated directly using muscle tissue for five of the chronic toxicity tests: northern pike, cutthroat trout (Rudolph), bluegill (Doroshov and Hermanutz) and white sturgeon, while effect levels for three tests were calculated directly using whole body selenium concentrations: bluegill (Coyle and Hermanutz) and brown trout. For the other tests that did not have muscle or whole body selenium measurements, conversion factors (*CF*s) were used to convert the egg/ovary CV to a muscle or whole body CV. The direct calculation of the muscle and whole body CVs (when data were available) reduced uncertainty in these effect level estimates.

6.3.1.4 Conversion Factors

When muscle or whole body chronic values could not be determined directly using selenium concentrations measured and reported for the respective tissue, conversion factors (*CF*) were used to convert the egg/ovary chronic value to either a muscle or whole body chronic value.

To derive egg-ovary to whole-body *CF* values, EPA defined matched pairs of selenium measurements from the eggs or ovaries and from the whole-body measured from the same individual fish or from matched composite samples. If multiple measurements from both eggs

and ovaries of the same individual or matched composite sample were available, the average value was used. Similar pairings were done for egg-ovary to muscle *CF* values.

After the data sets of the pairings were compiled, EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using ordinary least squares (OLS) linear regression of the matched pairs of measurements. If the regression resulted in a significant fit ($P \leq 0.05$) with a positive slope, EPA calculated the ratio of the egg-ovary to whole body (or muscle) selenium concentration of each matched pair and used the median ratio as the *CF* value for the species. A detailed comparison of the advantages and disadvantages of the median ratio and least squares regression approaches to calculating *CF*s, along with a comparison of *CF*s calculated from median ratios, OLS regression following log transformation, and total least squares (TLS) regression following log transformation is in Appendix N. Table N-3 provides a comparison of the median-based and regression-based *CF*s when they are used to convert an egg-ovary selenium concentration to muscle or whole body. Generally, the median-based and TLS-based *CF*s were similar for both tissue types and this similarity resulted in similar criterion element values (bottom row of Table N-3). The muscle criterion element value for the data set that contained directly calculated CVs and converted CVs was similar whether median or TLS *CF*s were used, 11.3 and 10.2, respectively. The whole body criterion element value was also similar using these two approaches, 8.5 and 9.4, respectively. The median-based *CF* approach was considered to be better than the regression-based *CF* approaches at reducing uncertainty. A detailed comparison and rationale for the median approach is discussed in appendix N.

EPA had sufficient egg-ovary and whole-body selenium measurements to directly calculate egg-ovary to whole body *CF* values for 13 species of fish. Similarly, there were sufficient egg-ovary and muscle selenium measurements to directly calculate egg-ovary to whole body *CF* values for 16 species of fish. To derive *CF* values for additional fish species, EPA used a taxonomic-relatedness approach (most similar taxon) approach to estimate *CF*. This approach is consistent to that done for TTF estimates, and is described in **Section 3.2.2**, and in greater detail in Appendix B.

The variability of *CF*s between fish species and within fish species was fairly low. EPA derived 13 *CF* values directly from matched pairs of egg-ovary and whole-body selenium measurements and an additional seven *CF* values by multiplying EO/M and M/WB conversion

factors (**Table 3.12**). Excluding mountain whitefish (*CF* = 7.4), *CF*s for 19 of the 20 species ranged from 1.20 to 3.11, a 2.6-fold difference. CF variability within each species was also low for 11 of the 13 species for which egg-ovary to whole-body CFs were determined directly and a standard deviation calculated (**Table 3.12**). The two species with relatively high standard deviations contained data that were potentially anomalous. When the potentially anomalous data were removed the standard deviations for these two species were reduced considerably (see footnote to **Table 3.12**).

6.3.2 Trophic Transfer Factors

A Trophic Transfer Factor (*TTF*) represents the transfer of selenium from one trophic level to the next higher trophic level. *TTF*s are used in the translation of the tissue criterion element concentration to a water element value. For a description of how *TTF*s are used in translation, see **Section 3.2.1**, Translation from Fish Tissue Concentration to Water Column Concentration.

Similar to CFs, EPA calculated *TTF*s from field data using the median-ratio approach after first performing OLS regression of matched pairs of selenium measurements for the two taxa representing successive trophic levels to determine if the relationship is significant ($P \leq 0.05$) and has a positive slope. EPA also evaluated using only OLS regression results to calculate *TTF* values. OLS regression was performed using matched concentrations of selenium in the food of a particular species or taxonomic group with the concentration of selenium in the organism's tissue, and then the slope of the regression was used as the *TTF* for that species or taxonomic group. An advantage of the regression approach is that it estimates the quantitative relationship of selenium across a range of environmental concentrations in a manner that allows statistical assessment. Disadvantages of this regression approach include the assumption that the underlying data are normally distributed; the possibility that one or a few very high or low values can have a disproportionate influence on the slope of the fitted line; and the fact that the bioaccumulation model does not account for a non-zero y-intercept. Constraining the y-intercept to zero (also known as regression through the origin or RTO) eliminates the added complexity of a non-zero y-intercept. However, RTO further increases the disproportionate influence of one or a few high values on the slope of the fitted line. Furthermore, RTO does not provide a straightforward way of evaluating goodness of fit (Gordon 1981).

The median-ratio approach, following confirmation of a significant ($P \leq 0.05$) relationship and positive slope, was considered to be more appropriate for deriving *TTF*s from field data that the OLS regression approach. Requiring a significant positive OLS linear regression coefficient confirms the relationship between selenium in organisms and the food they ingest is adequately represented by the available data. Using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias from measurements taken from aquatic systems with very high or very low selenium concentrations. Some aquatic organisms exhibit selenium bioaccumulation inversely related to water concentration (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). This inverse relationship is likely due to saturation uptake kinetics of specific transport mechanisms that regulate metals bioaccumulation within certain ranges (U.S. EPA 2007). EPA evaluated the effect of very high and very low selenium concentrations on the calculation of *TTF* values using the hybrid approach (use of median ratios for matched data with significant relationship and positive slope) described above by excluding selenium measurements above various minimum and/or below various maximum selenium concentrations. EPA found that using the median ratio effectively attenuates any effects of selenium concentration on the calculation of *TTF* values using the hybrid approach described above without the need to introduce additional arbitrary exclusion criteria.

*TTF*s were also determined using physiological coefficients (see **Section 3.2.2.1**, Derivation of Trophic Transfer Factors (TFF) Values. However, if a *TTF* value could be calculated from both physiological coefficients and field data, EPA used the *TTF* value calculated from the substantially larger number of field measurements to minimize statistical uncertainty.

*TTF*s were calculated for 32 fish species, and ranged from 0.68 to 2.67 (**Table 3.11**). The majority of fish *TTF*s fell within a relatively narrow range, with an interquartile range (25^{th} – 75^{th} centile) of 1.03 to 1.42. Variability of *TTF*s among the 13 invertebrate taxa was higher, ranging from 0.74 to 4.58 (**Table 3.10**). Much of the variability among invertebrate *TTF*s was related to taxonomic groups. The two bivalve *TTF*s ranged from 4.00 to 4.58. The five insect *TTF*s ranged from 1.48 to 2.88, the five crustacean *TTF*s ranged from 0.74 to 1.89, and the *TTF* for blackworms was 1.29.

EPA translated the tissue criterion element concentration to water element values at field sites that had selenium measurements in the required water, particulates, invertebrates, and fish.

For species without sufficient data to directly calculate a *TTF* value at these sites, EPA estimated the *TTF* value by sequentially considering higher taxonomic classifications until one or more taxa for which a calculated *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species.

6.3.3 Enrichment Factors

Enrichment factors are discussed in **Section 3.2.2.3** and Appendix H. This factor, describing how the bottom of the food chain takes up selenium, is the most variable between sites. Variability among *EF*s is the main reason that fish BAFs vary so much between sites, and this variability is the reason the national criterion for selenium needed to be tiered, with tissue having priority over water, to increase certainty that the criterion is protective as intended. The range of site *EF* values shown in Appendix H spans more than a 100-fold range.

The *EF* value measured at a particular site is also likely to be the site's most uncertain parameter, being a ratio of measurements of algae, detritus, and sediment, which may vary within a site in uncertain ways, and measurements of water, which vary over time. The approach for setting site *EF*s was designed to reduce uncertainty. As described in Appendix H, EPA calculated *EF* values by searching its database of selenium measurements and identifying all the selenium measurements from algae, detritus, or sediment. EPA then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water concentration was available for any given particulate measurement, the median water concentration was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median of those ratios. Selenium concentrations between particulate and water concentrations were higher for algae and detritus than for sediment. To reduce uncertainty in *EF* values associated with sediments, at least two particulate selenium measurements with corresponding water column measurements were required, and sediment measurements were used only if there was at least one other measurement from either algae or detritus. The geometric mean of the algae, detritus, and sediment ratios was then calculated and used as the site *EF*. Because there were at most only 3 possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric

mean in order to reduce the potential for one of the values to have excessive influence on the final site EF value. Sites with insufficient data to fulfill these data requirements were not used.

Had EPA increased the data requirements for setting a site *EF*, then the database would be restricted to a smaller number of sites. Because the variability between sites is high, reducing the number of sites in the database would decrease the confidence in the representativeness of the few sites retained (that is, it would increase the potential for sampling error in attempting to characterize the nation's waters). The *EF* determination process thus involved a balance between having enough information to reasonably characterize each site, and having enough sites to represent the range of the nation's waters.

Inclusion of selenium speciation information (such as selenate and selenite concentrations) was infeasible. Very few sites would have the requisite information, thereby increasing the uncertainty in the representativeness of any possible derived national criterion. Likewise, inclusion of a sulfate relationship was not feasible on a national basis at this time, for lack of sulfate data at many sites in the database.

Because *EF* is the BAF component that varies the most between sites, it is the most important in determining what the water concentration would be for a site if its fish tissue concentrations were hypothesized to be at the level of the fish tissue criterion element value. That is, sites with higher *EF*s tabulated in Appendix H have lower translated water concentrations in **Table 3.13**. Despite uncertainties in this parameter, model-predicted versus observed fish-tissue concentrations in the vicinity of the water element criterion concentrations are relatively unbiased, as shown in the figures of Appendix I.

For particular sites, the appropriateness of the national criterion can be resolved by site specific criteria when necessary (e.g., when a permit limit for water is required), as recommended in Appendix K. When taking measurements of a site, uncertainty in particulate measurements (the numerator of the *EF*) can be bypassed by using site-specific fish BAFs, since they only consider water and fish tissue selenium measurements. On the other hand, uncertainty in characterizing time-variable water concentrations is a problem shared by *EF*s and BAFs. However, this uncertainty can be reduced by sampling in a spatially and temporally robust manner, appropriate for the site in question, and then using the mathematical modeling approach to derive a site specific criterion.

6.3.4 Water Values

Derivation of the water criterion element from the egg-ovary criterion element is described in **Sections 2.7.8** and **3.2**, and involves *EF*s, *TTF*s, and *CF*s. Uncertainties in predicted tissue-to-water ratios combine the uncertainties in the parameters from which they are predicted. The prediction model is linear in all respects. Potential nonlinearities are therefore an uncertainty. **Section 6.2.1** and Appendix I assesses the accuracy of the predictions. As shown in the figures of Appendix I, the predicted values perform reasonably well in the vicinity of the water criterion element concentrations.

Although an earlier published draft document weighted sites by the number of fish species sampled (between 1 to 6 species per site), that overweighting of sites with several measured species was removed from this draft by using only the most bioaccumulative fish species per site, thereby reducing uncertainty that the fish tissue criterion element will be exceeded when using only water column concentration data. Lentic and lotic sites were assessed separately, per **Section 3.2.4**. This increases the likelihood that the water criterion element concentration will be appropriate for the site of application.

To reduce the likelihood that the water criterion element concentration will be under-protective for any particular site of application, the 20th percentiles of translated water concentrations for all lentic and lotic sites, respectively, were used as the water criterion element concentrations. As described previously, these distributions represented the translated water concentrations for the most bioaccumulative fish species at each site, which further reduces the likelihood that the fish tissue criterion element would be exceeded if the water criterion element was being met. These water criterion elements should not be interpreted to be potentially under-protective in 20 percent of sites, because when applied to a site's 30-day once-in-three-year maximum concentration (which is higher than its median), the 20th percentile site would not attain. The actual percentage of sites protected would thus be greater than 80 percent, but the exact percentage is uncertain.

6.4 Protection of Threatened or Endangered Species

The chronic toxicity dataset for selenium contains toxicity data for two Federally-listed endangered species, *Cyprinodon macularius* (desert pupfish) and *Oncorhynchus mykiss* (listed as steelhead, indicating anadromous individuals, but herein called rainbow trout, implying non-anadromous individuals). The dataset also contains toxicity data for *Acipenser transmontanus*

(white sturgeon), which is listed as endangered in specific locations, such as the Kootenai River white sturgeon in Idaho and Montana. The white sturgeon also serves as a surrogate for other sturgeon listed as threatened or endangered (e.g., pallid and shovelnose sturgeon). The *Acipenser* GMCV of 15.6 mg/kg dw egg is the lowest value in the dataset and therefore provides protection for other potentially sensitive sturgeon. The white sturgeon chronic value is greater than the chronic egg-ovary criterion element value.

Desert pupfish, *Cyprinodon macularius*, with a chronic value estimated to be ≥27 mg Se/kg dw egg, is not among the most sensitive species. Its chronic value of ≥27 mg Se/kg dw egg is substantially above the chronic egg-ovary criterion element value of 15.1 mg Se/kg dw.

Oncorhynchus mykiss has an SMCV of 24.5 mg Se/kg dw egg, whose genus is the fourth most sensitive species in the dataset. The dataset contains multiple studies with cutthroat trout (*Oncorhynchus clarkii*) some subspecies of which are Federally listed as threatened. The SMCV for cutthroat trout is 26.2 mg Se/kg dw egg. Both of these chronic values for *Oncorhynchus* species are greater than the chronic egg-ovary criterion element.

The dataset also contains toxicity information for *Salvelinus malma* (Dolly Varden) which is not threatened or endangered, but is so closely related to the threatened *Salvelinus confluentus* (bull trout) that it can hybridize with that species, producing fertile offspring (Baxter et al. 1997). Dolly Varden is the least sensitive fish species for which information is available, with an SMCV of 56 mg Se/kg dw egg. *Salvelinus fontinalis*, brook trout, can also hybridize with bull trout, but the offspring are sterile, suggesting that it is less closely related. With the available study of brook trout, although in **Section 6.1.5** the NOEC is conservatively set to >20.5 mg Se/kg dw egg, which was the average concentration at the Holm et al. (2005) high-exposure site. The concentration-response information for the offspring of individual females, presented in Appendix C, suggests that its EC_{10} could be substantially higher, possibly as high as that for Dolly Varden.

The egg-ovary criterion element value of 15.1 mg Se/kg (dw) is below all of the above mentioned chronic egg-ovary values for threatened and endangered (or closely related) species. However, because other threatened or endangered species could be more sensitive, if relevant new information becomes available in the future, it should be considered in state- or site-specific criterion calculations.

The protectiveness of the whole body criterion element concertation of 8.5 mg/kg dw to threatened and endangered species is also supported by a recent non-reproductive study with two sturgeon species. De Riu et al. (2014) fed juvenile green and white sturgeon (~30 g body weight) diets containing a range of selenium concentrations (selenomethionine added to diet formulation; 2.2 mg/kg Se in control diet (no added Se) and 19.7, 40.1 and 77.7 mg/kg Se in the three treatment diets). Several endpoints were monitored over the 8-week exposure period including survival and percent body weight increase (% BWI). White sturgeon had no mortalities through the highest dietary treatment. Green sturgeon juveniles had 0%, 7.7% and 23.1% mortality with the three dietary treatments. TRAP analysis (threshold sigmoid nonlinear regression) of the green sturgeon survival data resulted in a whole body EC_{10} value of 28.93 mg/kg dw. EC_{10} values were lower for % BWI using TRAP. For % BWI, the whole body EC_{10} value for green sturgeon was 16.36 mg/kg dw, and 23.94 mg/kg dw for white sturgeon.

Also notable, the background concentrations of selenium in the juvenile green and white sturgeon were also elevated at 7.2, 6.5 and 7.1 mg/kg dw (green sturgeon whole body), and 4.8 7.3 and 5.6 mg/kg dw (white sturgeon whole body) at test initiation, and after four and eight weeks of exposure, respectively.

The De Riu et al. (2014) study suggests that green sturgeon may be more sensitive to selenium than white sturgeon and also that the EPA whole body concentration of 8.5 mg/kg dw will be protective, based on the survival and growth data and the observation in De Riu 2014 that the control whole body tissue concentrations (up to 7.2 mg/kg dw) are approaching the proposed criterion. This is important because white sturgeon, as well as juvenile green sturgeon (up to three to four years), spend most of their time in the coastal rivers and estuaries. All species in the Acipenseriformes (sturgeon and paddlefish) spawn in freshwaters (Bemis and Kynard 1997) or spend their entire life in freshwater. The white sturgeon's EC_{10} in the dataset provides surrogacy for the threatened and endangered species from this group. For more information on the De Riu et al. (2014) study, see Appendix E.

6.4.1 Special Consideration for Pacific Salmonid Juveniles

The current criterion is based on reproductive effects (larval mortality and/or deformities) for offspring of selenium-exposed adults, and the whole-body criterion element is derived from the egg-ovary element, with an implicit assumption of adult exposure to selenium. One peer-reviewer of the 2014 EPA External Peer Review Draft criterion document raised concerns

regarding the protection of anadromous salmonids, since there is at least some evidence (e.g., Hamilton et al. 1990) that juvenile growth may be comparable in sensitivity to reproductive effects endpoints used by EPA. Anadromous salmon species (e.g., Chinook salmon) in the Pacific Northwest are unique in that reproductively mature adults are not exposed to selenium in the freshwater environment due to their life history; young juvenile salmon leave freshwater streams and rivers as smolts and mature to adulthood in the marine environment until migration for spawning begins. Furthermore, they are semelparous, breeding only once in their lifetime and subsequently dying, so there is no potential selenium exposure following spawning in freshwater.

Juvenile salmon have evolved different strategies for growth and maturation to the smolt stage, and may spend from three months to two years in freshwater (depending on timing of egg hatching and other factors) before migrating to estuarine areas as smolts and into the ocean to feed and mature. Salmon remain in the ocean for one to six years (more commonly two to four years), with the exception of a small proportion of yearling males (called jack salmon), which mature in freshwater or return after two or three months in salt water (NOAA 2011).

The physiological and morphological changes that allow these species to adapt to marine conditions as juveniles are reversed in returning adults preparing to migrate up natal streams to spawn. One key change is the cessation of feeding prior to re-entry into freshwater. Since mature females are not feeding after returning to freshwater, it is not representative to predict reproductive effects for anadromous salmonid species based on egg-ovary selenium concentrations, because the exposure is wholly from selenium sources in the marine environment (Groot and Margolis 1991).

6.4.1.1 Selenium Toxicity to Juvenile Salmonids

Hamilton et al. (1990) assessed the toxicity of two organoselenium diets in 90-day partial life cycle tests in freshwater with two life stages of Chinook salmon (*Oncorhynchus tshawytscha*). The first diet consisted of fish meal made from low-selenium mosquitofish (collected from a reference site) fortified with selenomethionine (here termed the SeMet diet). The second diet contained fish meal made from high-selenium mosquitofish (*Gambusia affinis*) collected from the San Luis Drain (SLD), California (here termed the SLD diet). This waterbody is known to have high concentrations of selenium. A 90-day partial life cycle study was conducted with swim-up stage salmon larvae in a standardized fresh water that simulated dilution of San Luis Drain water.

Survival and growth (length and weight) were measured at 30, 60, and 90 days. Unexplained control mortality (33%) between day 60 and day 90 introduced an unacceptable level of uncertainty into the overall health of the fish. The 1985 Aquatic Life Guidelines (Stephan et al. 1985) and the Manual of Instructions for Preparing Aquatic Life Water Quality Criteria Documents (Stephan 1987) require that excessive control mortality be treated as an exclusionary threshold in data quality assessments for regulatory purposes such as deriving water quality criteria. Therefore the 90-day survival data from this study was not used quantitatively. At 60 days, larval control mortality was acceptable (1%), and 60-day larval survival was > 90% in all SLD and SeMet treatments (3.2 ppm – 18.2 ppm) except for the high Se treatment (35.4 ppm). Whole body selenium concentrations were measured at 60 days, were 10.4 and 13.3 mg/kg dw, respectively, for larvae fed the SeMet and SLD diets of 18.2 mg/kg dw (Hamilton et al. 1990).

Although survival was similar in response to the two diets, larval growth responses differed between the SLD and SeMet diets. The salmon fed the SeMet mosquitofish diet had significant reductions in both length and weight at 30, 60, and 90 days; but only at the two highest concentrations (18.2 and 35.4 ppm). The average length and weight of the larvae fed the SLD mosquitofish diet were significantly lower at all concentrations at 30, 60, and 90 days. The greater effect on growth parameters fed the SLD mosquitofish meal diet could have been caused by one or more of several factors: 1) additional forms of organic selenium (e.g., selenocysteine) present in the SLD mosquitofish, 2) additional toxic elements (e.g., heavy metals) that were accumulated by the SLD mosquitofish, and not present in the reference site mosquitofish, and 3) differential metabolic processing of the organoselenium contained in the proteins of the SLD mosquitofish and fed to the larval salmon, versus the larvae fed the diet containing the free amino acid selenomethionine (Hamilton et.al. 1990).

EPA performed a regression on the 60-day weight and whole body concentrations, and derived a whole body EC_{10} value of 7.355 mg/kg dw for the SeMet diet for reduced growth, and a whole body EC_{10} value of 11.14 µg/g dw for the SLD diet for reduced growth. These values are the only two available EC_{10} Species Mean Chronic Values (SMCVs) for non-reproductive endpoints for the genus *Oncorhynchus*, and the Genus Mean Chronic Value (GMCV) is 9.052 mg/kg dw. This is greater than the national whole body criterion element concentration of 8.5 mg/kg dw, which will thus be protective of this genus.

EPA recommends that states and tribes consider use of the whole-body criterion element for juvenile (smolt) anadromous Pacific salmon species as the primary criterion element over the other elements due to the unique life history of these species, specifically, the lack of exposure to adult salmonids from selenium in freshwater prior to reproduction. The hierarchal structure of the egg-ovary tissue over the other tissue criterion elements applies to all other species in the family Salmonidae. The egg-ovary criterion element, as well as the other fish tissue criterion elements and the water column criterion elements still apply, as applicable, to protect the remainder of the aquatic community in these waters.

6.5 Aquatic-Dependent Wildlife is Beyond the Scope of this Aquatic Criterion Derivation

AWQC that are developed by EPA typically focus directly on aquatic life, not aquatic-dependent wildlife such as birds. As presented by Campbell (2011), EPA recognizes that selenium effects on aquatic-dependent wildlife are also of concern but considers them beyond the scope of this national criterion update. In the interest of providing updated guidance to protect against the known risks of selenium exposure to fish, EPA decided to focus its analyses on updating the existing selenium criterion for freshwater aquatic life based on the latest scientific evidence.

In the future, EPA plans to consider the effects of selenium on aquatic-dependent wildlife, potentially in the form of criteria expanded to address aquatic-dependent wildlife. When translated to a water concentration, a criterion protective of aquatic-dependent wildlife may be more stringent or less stringent than the values provided for aquatic life in this criterion document. This is because data indicate that for most ecosystems, selenium concentrations are generally conserved or increase incrementally at each trophic level in a food web (after a substantial increase from water to trophic level 1 (e.g., algae). Certain specific ecosystems (e.g., estuarine and marine systems more commonly) with mollusk-based food-webs may create a pathway for more selenium to bioaccumulate, particularly in molluscivorous predators (certain fish and aquatic bird species), since the available data indicate that mollusks generally have a higher trophic transfer factor than other invertebrate taxa. This level of bioaccumulation is typically lower, and in contrast to other bioaccumulative chemicals such as mercury, which have much greater biomagnification.

As stated previously, the single largest step in tissue selenium accumulation in aquatic environments occurs at the base of the food web where algae and other microorganisms accumulate selenium from water (Orr et al. 2012; Stewart et al. 2010). Mollusks such as mussels and clams accumulate selenium to a much greater extent than planktonic crustaceans and insects due to higher ingestion rates of both particulate-bound (algae) and dissolved selenium from the water column through filter feeding, and these organisms have a lower selenium elimination rate (Luoma and Rainbow 2005). Thus, aquatic-dependent wildlife criteria for species that are primarily molluscivores may have concentrations of concern that are not protected by the 2016 selenium criterion elements found in this document. The criteria values for aquatic-dependent wildlife would be expected to depend on the aquatic systems, species, and food webs considered, as well as spatial and temporal considerations related to selenium exposure and breeding and nesting seasons. Where sensitive aquatic-dependent (e.g., bird) species are known to exist, states should consider developing site-specific criteria based on data for such species.

6.6 SUMMARY

EPA developed the 2016 national 304(a) Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater to be protective of most aquatic life genera in most waters of the United States, with an intended goal of protecting approximately 95% of aquatic genera in an ecosystem. This freshwater chronic selenium criterion applies only to aquatic life, and is not intended to address selenium toxicity to aquatic-dependent wildlife such as aquatic-dependent birds. This document provides guidance to States and Tribes authorized to adopt water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of selenium.

The 2016 selenium criterion is a chronic criterion that is composed of four elements. All elements are protective against chronic selenium effects. Two elements are based on the concentration of selenium in fish tissue and two elements are based on the concentration of selenium in the water-column. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column - element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent element to account for potential chronic effects from short-term exposures (one value for lentic and one value for lotic aquatic systems). The assessment of the available data for fish, invertebrates, and amphibians

indicates that a criterion value derived from fish is expected to be protective of the aquatic community, based on available data.

EPA recommends that states and tribes adopt into their water quality standards a selenium criterion that includes all four elements, and express the four elements as a single criterion composed of multiple parts, in a manner that explicitly affirms that the whole-body or muscle elements supersede the water column element, and the egg-ovary element supersedes any other element. The magnitude of the fish egg-ovary element is derived from analysis of the available toxicity data. The magnitudes of the fish whole-body element and fish muscle elements are derived from the egg-ovary element coupled with data on concentration ratios among tissues. The magnitudes of the water column elements are derived from the egg-ovary element coupled with bioaccumulation considerations. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements into the selenium criterion ensures protection when neither fish egg-ovary nor fish whole-body nor muscle tissue measurements are available. There are two specific circumstances where the fish tissue concentrations do not fully represent potential adverse effects on fish and the aquatic ecosystem: 1) "fishless" waters, because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, and 2) areas with new selenium inputs, because the fish tissue concentrations in such systems would not yet represent steady state conditions upon which the criterion is based.

To ensure that the contribution of short-term exposures to the bioaccumulation risks is accounted for in all situations, EPA is recommending that the intermittent exposure element be included in the selenium criterion, as noted above. EPA is not recommending a separate acute criterion element derived from the results of toxicity tests having water-only exposure because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. Application of the intermittent exposure criterion element values to single day, high exposure events will provide protection from the most important selenium toxicity effect, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high exposure events.

The egg/ovary-based tissue criterion element of 15.1 mg Se/kg dw is based on a genus sensitivity distribution that used the most sensitive assessment endpoint observed in toxicity

tests, reproductive effects, and included fish species known to be sensitive to selenium (i.e., species from Salmonidae and Centrarchidae), as well as three endangered species (desert pupfish, rainbow trout and white sturgeon).

With respect to the chronic water column criterion elements, EPA intends the lentic and lotic values of 1.5 and 3.1 µg/L, respectively, to be protective of most surface waters in the U.S. These water concentrations represent the 20^{th} percentile of the distribution of translated water column values from sites across the U.S. The intermittent exposure water column criterion element is derived from the chronic water column criterion element, which was derived from the tissue-based criterion.

EPA recognizes selenium bioaccumulation potential depends on the structure of the food web and several biogeochemical factors that characterize a particular aquatic system. Uncertainty in the translation of the egg-ovary criterion element to the water column element can be reduced by deriving a site-specific criterion that uses site-specific selenium data and information on food-web dynamics from a biological assessment of the aquatic system. Appendix K provides recommendations and examples for developing site-specific selenium criteria.

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Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016 (Appendices A-N)

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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APPENDIX A: SELENIUM CHEMISTRY

Selenium in aquatic ecosystems exists in a broad range of oxidation states: (+ VI) in selenates ($HSeO_4^-$, SeO_4^{2-}) and selenic acid (H_2SeO_4), (+ IV) in selenites ($HSeO_3^-$, SeO_3^{2-}) and selenous acid (H_2SeO_3), 0 in elemental selenium, and (-II) in selenides (Se^{2-}, HSe^-), hydrogen selenide (H_2Se), and organic selenides (R_2Se). Selenium also shows some tendency to form catenated species like organic diselenides (RseSeR). Within the normal physiological pH range and the reduction potential range permitted by water, only Se, SeO_3^{2-}, $HSeO_3^-$, and SeO_4^{2-} can exist at thermodynamic equilibrium (Milne 1998). While ionic reactions are expected to be rapid in water, oxidation-reduction reactions may be slow, and the possibility exists for the formation of HSe^- in living systems and some environments where anoxic conditions arise. The parallel behavior of comparable species of sulfur and selenium in living systems has often been observed, but it is important to recognize that their chemical characteristics are different in many ways. For instance, selenate is comparable to chromate in oxidizing strength and far stronger than sulfate [$E^0(SeO_4^{2-}/H_2SeO_3)$ = 1.15 V; $E^0(Cr_2O_7^{2-}/Cr^{3+})$ = 1.33V; $E^0(SO_4^{2-}/H_2SO_3)$ = 0.200V (standard potentials in acid solution: Weast 1969)], whereas selenide is a much stronger reducing agent than sulfide [$E^0(Se/H_2Se)$ = -0.36 V; $E^0[S/H_2S$]= 0.14V)].

1.0 INORGANIC SELENIUM

Selenate usually predominates in well-aerated surface waters, especially those with alkaline conditions. In spite of its oxidizing strength, selenate (SeO_4^{2-}) exhibits considerable kinetic stability in the presence of reducing agents (Cotton and Wilkinson 1988). The radius of SeO_4^{2-} is comparable to that of SO_4^{2-} (Frausto da Silva and Williams 1991), and uptake by cells is expected to take place via the same ion channels or permeases for both anions. Competition between sulfate and selenate uptake has been observed in many species: algae (Riedel and Sanders 1996), aquatic plants (Bailey et al. 1995), crustaceans (Ogle and Knight 1996), fungi (Gharieb et al. 1995), HeLa cells (Yan and Frenkel 1994), and wheat (Richter and Bergmann 1993). Reduced selenate bioconcentration with increasing sulfate concentration has been demonstrated in *Daphnia magna* (Hansen et al. 1993). A significant inverse relationship was shown to exist between acute selenate toxicity to aquatic organisms and ambient sulfate concentrations (Brix et al. 2001a). Competition with selenate has also been observed for phosphate in green algae (Riedel and Sanders 1996), and with chromate and tungstate in anaerobic bacteria (Oremland et al. 1989).

Selenous acid species ($HSeO_3^-$ and SeO_3^{2-}) can predominate in solution under the moderately oxidizing conditions encountered in oxygenated waters. Between pH 3.5 and 9.0 biselenite ion is the predominant ion in water, and at pH values below 7.0, selenites are rapidly reduced to elemental selenium under mildly reducing conditions (Faust 1981), situations that are common in bottom sediments.

Most selenite salts are less soluble than the corresponding selenates. The extremely low solubility of ferric selenite $Fe_2(SeO_3)_3$ ($K_s = 2.0 \pm 1.7 \times 10^{-31}$), and of the basic ferric selenite $Fe_2(OH)_4SeO_3$ ($K_s = 10^{-61.7}$), is important to the environmental cycling of selenium. Selenites also form stable adsorption complexes with ferric oxides, forming complexes of even lower solubility than the ferric selenites. Under certain conditions, selenite (in contrast to selenate) seems to be completely adsorbed in high amounts by ferric hydroxide and, to a lesser extent, by aluminum hydroxide (Faust 1981). Coprecipitation techniques have been applied for preconcentration of selenium in natural waters, using iron (III) hydroxides, which coprecipitates selectively the selenite, but not the selenate, species in river and sea waters (Yoshii et al. 1977). Alum and iron coagulation precipitation can be used in water treatment processes to remove selenite (Clifford et al. 1986). The low levels of selenium in ocean waters have been attributed to the adsorption of selenite by the oxides of metals, such as iron and manganese (National Academy of Sciences 1976).

Relative to selenate, selenite is more reactive because of its polar character, resulting from the asymmetric electron density of the ion, its basicity (attraction to bond with proton), and its nucleophilicity (attraction to bond to a nucleus using the lone pair electrons of the ion). No evidence has yet been presented to show that $HSeO_3^-$ or SeO_3^{2-} is taken up intact into the cell interior. Evidence indicates that selenite is reduced rapidly, even before uptake in some cases, making it difficult to distinguish between uptake and metabolic processes (Milne 1998). Freshwater phytoplankton process selenate and selenite by different mechanisms, leading to different concentrations within the cell, and the concentrations attained are affected by various chemical and biological factors in the environment (Riedel et al. 1991). These authors suggested that selenate is transported into the cell by a biological process with low affinity, whereas selenite appears to be largely physically adsorbed. Contradictory evidence suggesting that selenite uptake is enzymatically mediated was found with marine phytoplankton (Baines and Fisher 2001). Experimental results supporting the hypothesis that separate accumulation mechanisms for selenate and selenite are present in *D. magna* have been published (Maier et al. 1993). However, while some organisms appear to absorb selenite nonspecifically, specific transport systems exist in other species. Sulfate competition is insignificant in the aquatic plant *Ruppia maritima* (Bailey et al. 1995), and specific uptake systems have been demonstrated in some soft line microorganisms (Heider and Boeck 1993). Selenite uptake in green algae, unlike selenate, is increased substantially at lower pH values, a property that represents another difference between these two anions (Riedel and Sanders 1996). The uptake of inorganic selenium species, selenate and selenite, by the green alga *Chlamydomonas reinhardtii* (Dang) was examined as a function of pH over the range 5 to 9, and in media with varying concentrations of major ions and nutrients using ^{75}Se as a radiotracer. Little difference was noted in the uptake of selenate as a function of pH, with the maximum uptake found at pH 8; however, selenite uptake increased

substantially at the lower pH values. Differences in speciation are suggested to be the cause of these differences. Selenate exists as the divalent ion SeO_4^{2-} over the range of pH tested; whereas monovalent biselenite ion $HSeO_3^-$ is prevalent at these pH values. At the low end of the pH range, neutral selenous acid may also play a role.

Elemental selenium is not measurably soluble in water. It has been reported that elemental selenium is slowly metabolized by several bacteria (Bacon and Ingledew 1989), and the translocation of elemental selenium into the soft tissue of the marine mollusk *Macoma balthica* has been reported (Luoma et al. 1992). The bioavailability of elemental selenium to *M. balthica* was assessed by feeding the organisms ^{75}Se-labeled sediments in which the elemental selenium was precipitated by microbial dissimilatory reduction. A 22% absorption efficiency of particulate elemental selenium was observed. In view of the insolubility of elemental selenium, uptake may be preceded by air oxidation, or in reducing environments thiols may facilitate the solubilization (Amaratunga and Milne 1994). Elemental selenium can be the dominant fraction in sediments (Zawislanski and McGrath 1998).

Selenium is reduced to hydrogen selenide, H_2Se, or other selenides at relatively low redox potentials. Hydrogen selenide by itself is not expected to exist in the aquatic environment since the Se^0/H_2Se couple falls even below the H^+/H_2 couple. Aqueous solutions of H_2Se are actually unstable in air due to its decomposition into elemental selenium and water. Under moderately reducing conditions, heavy metals are precipitated as the selenides, which have extremely low solubilities. The following are log K_s values of some heavy metal selenides of environmental interest: -11.5 (Mn^{2+}), -26.0 (Fe^{2+}), -60.8 (Cu^+), -48.1 (Cu^{2+}), -29.4 (Zn^{2+}), -35.2 (Cd^{2+}), and -64.5 (Hg^{2+}). The precipitation of selenium as heavy metal selenides can be an important factor affecting the cycling of the element in soils and natural waters.

2.0 ORGANOSELENIUM

Organic selenides (conventionally treated as Se(-II) species) in variable concentrations, usually in the form of free and combined selenomethionine and selenocysteine, are also present in natural surface waters (Fisher and Reinfelder 1991). Dissolved organic selenides may be an important source of selenium for phytoplankton cells, because they can account for ~80% of the dissolved selenium in open ocean surface waters, and for a significant fraction in many other environments as well (Cutter 1989; Cutter and Cutter 1995). Dissolved organoselenium levels of 14.2%, 65% and 66% were measured in samples (one meter depth) from Hyco Reservoir, NC; Robinson Impoundment, SC; and Catfish Lake, NC; respectively (Cutter 1986). The Hyco Reservoir organoselenium was identified as being protein bound.

Organoselenium concentrations were found to range from 10.4% (58.7 µg/L) to 53.7% (1.02 µg/L) of the total selenium present in Lake Creek and Benton Lake, MT surface waters (Zhang and Moore 1996). Organoselenium quite often is measured as the difference between total dissolved selenium

and the sum of selenite plus selenate, and is therefore not typically characterized. Much more work is needed in the area of specific identification and characterization of the nature of the organic selenides present in aquatic ecosystems. Organoselenium form(s) are much more bioavailable and probably play a very important role in selenium ecotoxic effects (e.g. Besser et al., 1993; Rosetta and Knight 1995).

3.0 DEPARTURE FROM THERMODYNAMIC EQUILIBRIUM

In the highly dynamic natural waters, there is often a departure from thermodynamic equilibrium. In the thermodynamic models, kinetic barriers to equilibrium and biological processes are not adequately considered, and the speciation of selenium in oxidized natural waters is not accurately predicted. Selenate is usually the predominate form in solution; however, selenite and organoselenium can both exist at concentrations higher than predicted (Faust 1981; Luoma et al. 1997). Bioaccumulation by microorganisms, bioproduction and release of organoselenium, and mineralization of particulate selenium forms contribute to the disequilibrium.

4.0 PHYSICAL DISTRIBUTION OF SPECIES IN SURFACE WATER

The physical distribution of various selenium species in surface waters is regulated by:

- sorption to or incorporation in suspended particulate matter (SPM), and
- complexation with inorganic and/or organic colloidal material, such as $(FeO\ OH)_n$ and humic substances (dissolved organic matter, DOM).

Both sorption to SPM and complexation with colloidal matter reduces the bioavailability of the selenium species. The average fraction of selenium associated with the suspended particulate phase (0.45μm filtration) as determined from eleven different studies of various surface waters was found to be 16% (0-39% range) of the total selenium, i.e., an average operationally defined dissolved selenium level of 84% (Table A-1). In the James River, VA, the dissolved inorganic and organic selenium was found to be 77% and 70% associated with colloidal matter, respectively (Takayangi and Wong 1984). A study of lake ecosystems in Finland (Wang et al. 1995) found that 52% of the dissolved selenium was associated with humic substances, and in a similar speciation study of Finnish stream waters, Lahermo et al. (1998) determined that 36% of the selenium was complexed with humic matter. Hence, in various waterbodies physical distribution as well as chemical speciation of selenium must be considered in relationship to bioavailability and aquatic toxicity.

Until recently, the organic selenium fraction has been routinely measured as the difference between total dissolved selenium and the sum of selenite and selenate. Unfortunately, the calculation of this important selenium fraction in water as the difference between the total and measurable inorganic fractions has not permitted this fraction to be fully characterized. New techniques are currently being

developed which should help the specific identification and characterization of the nature of the organic selenides present in aquatic systems. This work is particularly important because portions of the organic selenium fraction (e.g., selenomethionine) of total dissolved selenium in water have been shown to be much more bioavailable than the other forms of selenium, and therefore this work is also important for understanding the manifestation of selenium ecotoxic effects.

Table A-1. Suspended particulate and dissolved selenium as a function of total selenium in freshwater and marine aquatic ecosystems.

Reference	Waterbody	Particulate Se (% of Total)	Fraction dissolved, fd
Cutter 1989	Carquinez, CA	20 - 40	0.6 - 0.8
Cutter 1986	Hyco Reservoir, NC	0	1
Tanizaki et al. 1992	Japanese Rivers	16	0.84
Luoma et al. 1992	San Francisco Bay, CA	22 - 31	0.69-0.78
Cumbie and VanHorn, 1978	Belews Lake, NC	8	0.92
GLEC 1997	Unnamed Stream, Albright, WV	4	0.96
Wang et al. 1995	Finnish Lakes	10	0.9
Lahermo et al. 1998	Finnish Streams	8	0.92
Hamilton et al. 2001a,b	Adobe Creek, Fruita, CO	18	0.82
Hamilton et al. 2001a,b	North Pond, Fruita, CO	0	1
Hamilton et al. 2001a,b	Fish Ponds, Fruita, CO	7	0.93
Nakamoto and Hassler 1992	Merced River, CA	0	1
Nakamoto and Hassler 1992	Salt Slough, CA	4	0.96
Welsh and Maughan 1994	Cibola Lake, CA	39	0.62
Welsh and Maughan 1994	Hart Mine Marsh, Blythe, CA	6	0.94
Welsh and Maughan 1994	Colorado River, Blythe, CA	11	0.89
Welsh and Maughan 1994	Palo Verda Oxbow Lake, CA	33	0.67
Welsh and Maughan 1994	Palo Verda Outfall Drain, CA	0	1
Welsh and Maughan 1994	Pretty Water Lake, CA	21	0.79

APPENDIX B: CONVERSIONS

1.0 CONVERSION OF WET TO DRY TISSUE WEIGHT

1.1 Methodology

Conversion factors (*CF*) derived from selenium measurements were calculated using concentrations expressed as dry weights (µg/g dry weight). The majority of tissue and whole-body selenium concentrations were reported as dry weights. Measurements reported as wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type.

Species-specific percent moisture data for muscle tissue were available for bluegill (Gillespie and Baumann 1986; Nakamoto and Hassler 1992), rainbow trout (Seiler and Skorupa 2001), and for a composite average of nine fish species (May et al. 2000). Species specific percent moisture data for ovaries were available for bluegill (Gillespie and Baumann 1986; Nakamoto and Hassler 1992), fathead minnow (GEI Associates 2008; Rickwood et al. 2008), and rainbow trout (Seiler and Skorupa 2001). Species-specific % moisture data for whole-body tissues were available for bluegill (USGS NCBP).

Measurements reported as wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data were unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) were used. Table B-1a lists percent moisture by tissue type, species, data source, and the target species and study for which the % moisture data were used to convert from wet to dry weight. Table B-1b is a list of 38 freshwater fish species and their percent solids and moisture. Although these data were not needed for wet to dry weight conversion in any of the studies in this document, they are provided here as a potential resource.

Table B-1a. Percent moisture, by species and tissue type.

% Moisture Data Source		% Moisture by Tissue			Conversion Applied to	
Species	**Study**	**Whole-body**	**Muscle**	**Ovary**	**Species**	**Study**
Used in derivation of FCV						
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Brook trout	Holm et al. 2005
Fathead minnow	Average of GEI Assoc. 2008; Rickwood et al. 2008			75.30	Fathead minnow	Schultz and Hermanutz 1990
Bluegill	Average of Gillespie & Baumann 1986 and Nakamoto & Hassler 1992			76.00	Bluegill	Hermanutz et al. 1996
Avg of 9 spp	May et al. 2000		78.4		Striped bass	Coughlan and Velte 1989
Used in conversion of FCV in egg/ovary to whole-body Se concentrations						
Bluegill	USGS NCBP	74.80			Bluegill	Hermanutz et al. 1996
Bluegill	May et al. 2000		80.09		Bluegill	Hermanutz et al. 1996
Bluegill	Average of Gillespie & Baumann 1986 and Nakamoto & Hassler 1992			76.00	Bluegill	Hermanutz et al. 1996
Rainbow trout	May et al. 2000		77.54		Brook Trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Brook Trout	Holm et al. 2005
Rainbow trout	May et al. 2000		77.54		Rainbow Trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow Trout	Holm et al. 2005
Rainbow trout	May et al. 2000		77.54		Rainbow Trout	Casey & Siwik 2000
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow Trout	Casey & Siwik 2000

Table B-1b. Percent solids and moisture for whole body fish tissues by species.
Data provided by GEI Consultants (GEI 2014).

Species	Average % solids	Count	Min	Max	Avg % moisture
Black bullhead	23.18	6	18.4	27	76.82
Blacknose dace	26.25	44	21.3	31.2	73.75
Bluntnose minnow	25.2	3	23.8	25.9	74.8
Brook stickleback	24.18	57	19.3	27.8	75.82
Carp	21.8	6	21.1	22.8	78.2
Central Stoneroller	25.38	174	17.2	33.7	74.62
Common carp	24.54	62	17.4	43	75.64
Creek chub	23.29	306	16.5	29.3	76.71
Fantail darter	27.71	15	19.5	72.3	72.29
Fathead minnow	23.36	298	15.3	100	76.64
Green sunfish	23.87	150	7.9	29	76.13
Greenside darter	25.55	11	21.7	27	74.45
Johnny darter	28.3	1	--	--	71.7
Largemouth bass	24.26	64	20.6	28.8	75.74
Log perch	23.05	2	22.3	23.8	76.95
Longnose dace	26.75	17	23.4	31.3	73.25
Mimic shiner	24.9	2	24	25.8	75.1
Mosquitofish	23.96	8	22.5	24	76.04
Northern hogsucker	23.93	113	17	39	76.07
Plains killifish	24.5	9	23.3	26.1	75.5
Rainbow darter	27.17	85	12	33.3	72.83
Red shiner	26.93	46	20.9	34.8	73.07
Redside shiner	24.44	8	21.8	26.9	75.56
River chub	24.8	4	22.9	27.3	75.2
River redhorse	20.8	1	--	--	79.2
Rock bass	25.05	24	21.2	29.3	74.95
Rosyface shiner	30.25	2	27.6	32.9	69.75
Rosyside shiner	24.54	5	23.1	25.7	75.46
Sand shiner	26.03	83	20.7	30.7	73.97
Sauger	23	1	--	--	77
Silver shiner	23.4	7	22.3	24.6	76.6
Smallmouth bass	25.78	12	22.7	28.1	74.22
Speckled dace	26.04	35	21	31.2	73.96
Striped shiner	22.9	64	18.2	28.8	77.1
Sunfish	23.2	1	-	-	76.8
Variegated darter	27.45	13	21.7	30.3	72.55
White sucker	22.63	246	16.5	28.4	77.37
Yellow perch	26.02	5	24	28.4	73.98
Grand total	24.85	1990			75.15

2.0 DERIVATION OF TISSUE CONVERSION FACTORS

2.1 Methodology

EPA used a mechanistic bioaccumulation modeling approach to derive a mathematical relationship between the concentration of selenium in water to the concentration of selenium in the eggs and ovaries of fish. This approach characterizes selenium bioaccumulation as a series of steps representing the phase transformation of selenium from dissolved to particulate form, and then the trophic transfer of selenium through aquatic food webs to invertebrates and fish. The final step in this process is the transfer of selenium into eggs and ovary tissue.

Equation 1 quantitatively models the transfer of selenium through each environmental compartment as a series of site-specific and species-specific parameters. The parameter *CF* in Equation 1 represents the species-specific proportion of selenium in egg or ovary tissue relative to the average concentration of selenium in all body tissues and is given as:

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \text{(Equation 1)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g dw)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (µg/g dw).

EPA derived species-specific conversion factor (*CF*) values using the same methods that were used to derive species-specific *TTF* values from field data. To derive whole-body to egg-ovary *CF* values, the EPA defined matched pairs of selenium measurements from the whole-body and from the eggs or ovaries measured from the same individual fish or from matched composite samples. Egg-ovary concentration was defined as a measurement from either the eggs or the ovaries. If multiple measurements from both eggs and ovaries of the same individual or matched composite sample were available, the average value was used. EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using ordinary least squares (OLS) linear regression. If the regression resulted in a statistically significant ($P<0.05$) positive slope, EPA calculated the ratio of the egg-ovary to whole body selenium concentration for each matched pair of measurements and used the median as the *CF* value for that species.

EPA derived *CF* values from selenium measurements in units of µg/g dry weight. The majority of tissue and whole body selenium concentrations were reported as dry weights. Measurements reported as

wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data were unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) were used. A listing of percent moisture concentrations by species and target tissue are provided in Table B-1a.

For those species without sufficient data to directly calculate an egg-ovary to whole body *CF*, but which had sufficient data to calculate a conversion factor for either egg-ovary to muscle or whole body to muscle, EPA followed a two stage approach based on taxonomic similarity, similar to that described above. If a fish species had species specific egg-ovary to muscle conversion factor, but no whole body data with which to calculate an egg to whole body *CF*, then available data would be used to estimate a muscle to whole body conversion factor for that species based on taxonomic relatedness. The estimated muscle to whole body factor would be multiplied by the directly measured egg-ovary to muscle factor to estimate an egg-ovary to whole body *CF* for that species. For example, rainbow trout has a species specific egg-ovary to muscle conversion factor of 1.92, but does not have a species specific egg-ovary to whole body *CF*. Using the taxonomic approach described above, the most closely related taxa to rainbow trout with muscle to whole body conversion factors are in the class Actinopterygii. The median conversion factor for the 8 species within that class is 1.27. The final egg-ovary to whole body *CF* for rainbow trout is 2.44 (Table B-6), or 1.92 x 1.27.

The EPA developed species-specific egg-ovary to muscle and muscle to whole-body correction factors following the procedure described for whole-body to egg-ovary conversion factors. The EPA obtained matched pairs of selenium measurements in the whole-body and muscle filets and matched pairs of selenium measurements in muscle filets and whole-body from published scientific literature. EPA first confirmed a statistical relationship between the two tissue types for each species using OLS linear regression. If the regression resulted in a significant fit with a positive slope, the EPA calculated the ratio of each matched pair of measurements and then calculated the median ratio.

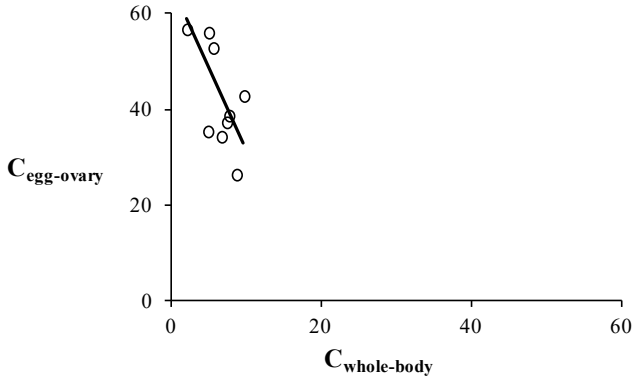
2.2 CF values calculated directly from whole-body and egg-ovary selenium measurements

$C_{whole\text{-}body}$ = Selenium concentration in all tissues (µg/g dw)
C_{egg} = Selenium concentration in eggs (µg/g dw)
C_{ovary} = Selenium concentration in ovary tissue (µg/g dw)
$C_{egg\text{-}ovary}$ = Average selenium concentration in eggs and ovaries $\left(\frac{C_{egg} + C_{o\,\mathrm{var}\,y}}{2}\right)$

Ratio = $\frac{C_{egg-o\,\mathrm{var}\,y}}{C_{whole-body}}$

Black bullhead (*Ameiurus melas*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	5.30	-	64.30	64.30	12.13
Osmundson et al. 2007	4.80	-	35.40	35.40	7.38
Osmundson et al. 2007	5.50	-	52.80	52.80	9.60
Osmundson et al. 2007	4.90	-	56.00	56.00	11.43
Osmundson et al. 2007	9.60	-	42.80	42.80	4.46
Osmundson et al. 2007	7.60	-	38.70	38.70	5.09
Osmundson et al. 2007	7.30	-	37.30	37.30	5.11
Osmundson et al. 2007	6.60	-	34.30	34.30	5.20
Osmundson et al. 2007	8.60	-	26.40	26.40	3.07
Osmundson et al. 2007	2.00	-	56.70	56.70	28.35
Osmundson et al. 2007	5.30	-	64.30	64.30	12.13



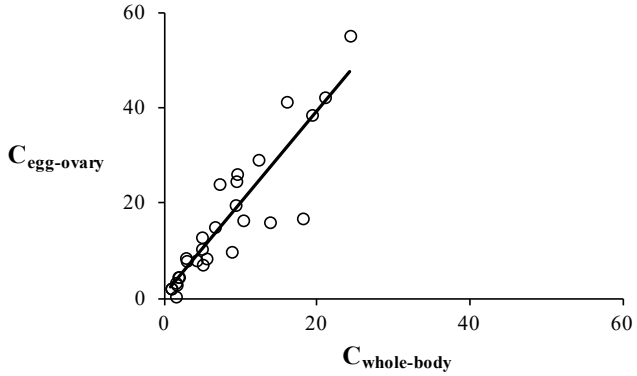
Median ratio: 6.29

R^2: 0.37
F: 4.67
df: 8
P: 0.046

Not used because negative slope.

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Coyle et al. 1993	0.90	1.90	2.10	2.00	2.22
Coyle et al. 1993	2.90	7.30	8.30	7.80	2.69
Coyle et al. 1993	4.90	13.00	12.50	12.75	2.60
Coyle et al. 1993	7.20	22.80	25.00	23.90	3.32
Coyle et al. 1993	16.00	41.30	41.00	41.15	2.57
Doroshov et al. 1992	1.60	2.80	-	2.80	1.75
Doroshov et al. 1992	5.50	8.30	-	8.30	1.51
Doroshov et al. 1992	9.30	19.50	-	19.50	2.10
Doroshov et al. 1992	19.30	38.40	-	38.40	1.99
Hermanutz et al. 1996	1.50	-	0.30	0.30	0.20
Hermanutz et al. 1996	18.10	-	16.70	16.70	0.92
Hermanutz et al. 1996	1.90	-	4.40	4.40	2.32
Hermanutz et al. 1996	2.80	-	8.40	8.40	3.00
Hermanutz et al. 1996	12.30	-	29.00	29.00	2.36
Hermanutz et al. 1996	9.40	-	24.50	24.50	2.61
Hermanutz et al. 1996	1.50	-	3.20	3.20	2.13
Hermanutz et al. 1996	4.90	-	10.30	10.30	2.10
Hermanutz et al. 1996	21.00	-	42.10	42.10	2.00
Hermanutz et al. 1996	24.30	-	55.00	55.00	2.26
Hermanutz et al. 1996	5.00	-	7.00	7.00	1.40
Hermanutz et al. 1996	9.50	-	26.00	26.00	2.74
Hermanutz et al. 1996	6.60	-	14.90	14.90	2.26
Hermanutz et al. 1996	1.80	-	4.40	4.40	2.44
Hermanutz et al. 1996	4.20	-	7.90	7.90	1.88
Hermanutz et al. 1996	10.30	-	16.30	16.30	1.58
Hermanutz et al. 1996	13.80	-	15.90	15.90	1.15
Osmundson et al. 2007	8.80	-	9.70	9.70	1.10

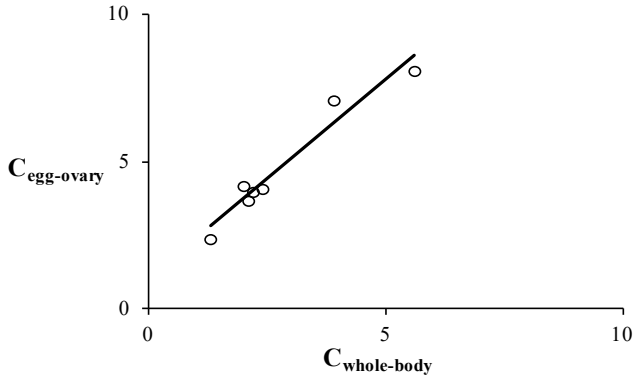


Median ratio: 2.13

R^2: 0.82
F: 110.9
df: 25
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	1.30	-	2.40	2.40	1.85
Osmundson et al. 2007	2.00	-	4.20	4.20	2.10
Osmundson et al. 2007	2.10	-	3.70	3.70	1.76
Osmundson et al. 2007	2.20	-	4.00	4.00	1.82
Osmundson et al. 2007	2.40	-	4.10	4.10	1.71
Osmundson et al. 2007	3.90	-	7.10	7.10	1.82
Osmundson et al. 2007	5.60	-	8.10	8.10	1.45



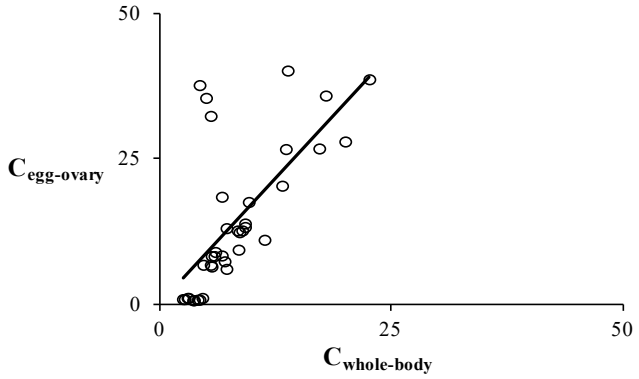
Median ratio: 1.82

R^2: 0.95
F: 88.9
df: 5
P: <0.001

Brown trout (*Salmo trutta*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Formation 2011 Saratoga fish hatchery	3.60	0.80	-	0.80	0.22
Formation 2011 Saratoga fish hatchery	4.10	0.90	-	0.90	0.22
Formation 2011 Saratoga fish hatchery	3.70	0.80	-	0.80	0.22
Formation 2011 Saratoga fish hatchery	4.30	0.90	-	0.90	0.21
Formation 2011 Saratoga fish hatchery	3.00	1.20	-	1.20	0.40
Formation 2011 Saratoga fish hatchery	3.10	1.20	-	1.20	0.39
Formation 2011 Saratoga fish hatchery	2.70	1.00	-	1.00	0.37
Formation 2011 Saratoga fish hatchery	2.50	1.00	-	1.00	0.40
Formation 2011 Saratoga fish hatchery	8.90	12.80	-	12.80	1.44
Formation 2011	13.80	40.30	-	40.30	2.92
Formation 2011	17.90	36.00	-	36.00	2.01
Formation 2011	13.60	26.80	-	26.80	1.97
Formation 2011	17.20	26.90	-	26.90	1.56
Formation 2011	6.70	18.60	-	18.60	2.78
Formation 2011	9.60	17.70	-	17.70	1.84
Formation 2011	22.60	38.80	-	38.80	1.72
Formation 2011	7.20	13.20	-	13.20	1.83
Formation 2011	9.20	13.40	-	13.40	1.46

Brown trout (*Salmo trutta*)					
Formation 2011	13.20	20.50	-	20.50	1.55
Formation 2011	8.60	12.50	-	12.50	1.45
Formation 2011	11.30	11.20	-	11.20	0.99
Formation 2011	20.00	28.10	-	28.10	1.41
Formation 2011	8.40	12.80	-	12.80	1.52
Formation 2011	5.60	8.40	-	8.40	1.50
Formation 2011	6.70	8.50	-	8.50	1.27
Formation 2011	5.90	8.40	-	8.40	1.42
Formation 2011	6.00	9.10	-	9.10	1.52
Formation 2011	7.00	7.50	-	7.50	1.07
Formation 2011	5.60	6.60	-	6.60	1.18
Formation 2011	4.70	6.90	-	6.90	1.47
Formation 2011	7.20	6.20	-	6.20	0.86
Formation 2011	9.20	14.00	-	14.00	1.52
Formation 2011	5.50	6.90	-	6.90	1.25
Formation 2011	8.50	9.50	-	9.50	1.12
Osmundson et al. 2007	4.60	-	1.20	1.20	0.26
Osmundson et al. 2007	4.30	-	37.80	37.80	8.79
Osmundson et al. 2007	5.00	-	35.60	35.60	7.12
Osmundson et al. 2007	5.50	-	32.50	32.50	5.91

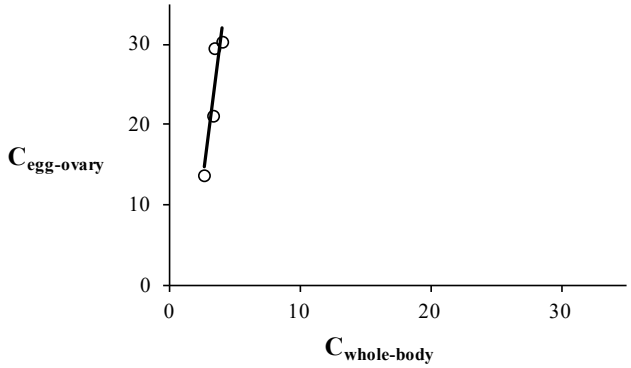


Median ratio: 1.45

R^2: 0.47
F: 31.3
df: 36
P: <0.001

Channel catfish (*Ictalurus punctatus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.40	-	29.50	29.50	8.68
Osmundson et al. 2007	3.30	-	21.10	21.10	6.39
Osmundson et al. 2007	2.60	-	13.70	13.70	5.27
Osmundson et al. 2007	4.00	-	30.30	30.30	7.58



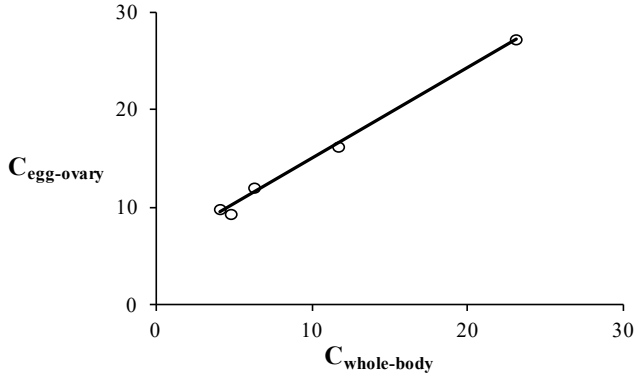
Median ratio: 6.98

R^2: 0.82
F: 9.1
df: 2
P: 0.099

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	6.30	-	12.10	12.10	1.92
Osmundson et al. 2007	4.80	-	9.40	9.40	1.96
Osmundson et al. 2007	11.70	-	16.30	16.30	1.39
Osmundson et al. 2007	23.10	-	27.30	27.30	1.18
Osmundson et al. 2007	4.10	-	9.90	9.90	2.41

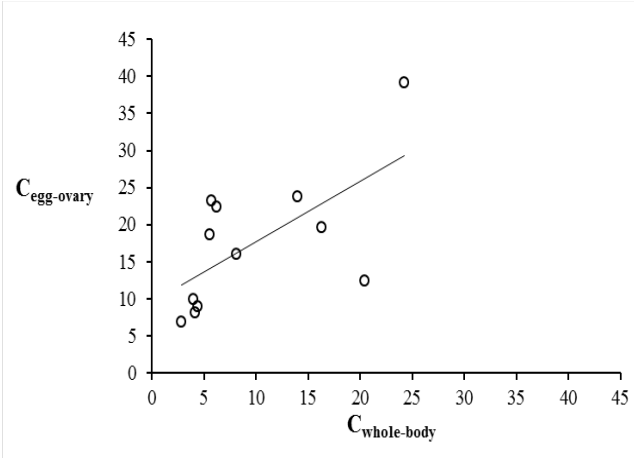


Median ratio: 1.92

R^2: 0.96
F: 584.8
df: 3
P: <0.001

Creek chub (*Semotilus atromaculatus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
GEI 2014	2.89	6.86	-	6.86	2.37
GEI 2014	4	9.94	-	9.94	2.49
GEI 2014	4.14	8.1	-	8.1	1.96
GEI 2014	4.46	8.98	-	8.98	2.01
GEI 2014	5.57	18.63	-	18.63	3.34
GEI 2014	6.23	22.35	-	22.35	3.59
GEI 2014	24.26	39.07	-	39.07	1.61
GEI 2014	20.49	12.38	-	12.38	0.60
GEI 2014	16.33	19.59	-	19.59	1.20
GEI 2014	14.03	23.78	-	23.78	1.69
GEI 2014	5.71	23.21	-	23.21	4.06
GEI 2014	8.17	16.03	-	16.03	1.96



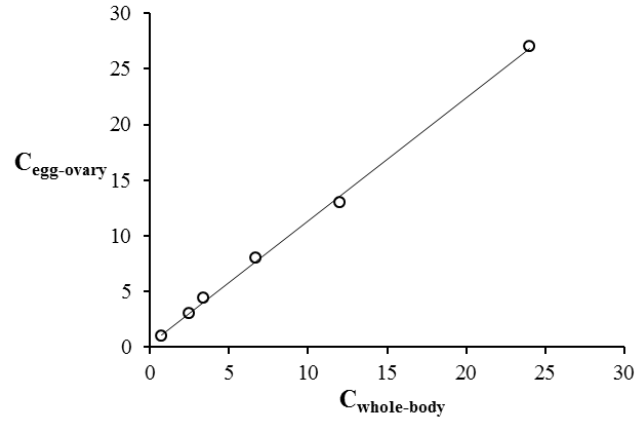
Median ratio: 1.99

R^2: 0.82
F: 7.09
df: 10
P: 0.012

Desert pupfish (*Cyprinodon macularius*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Besser et al. 2012	0.75	1	-	1	1.33
Besser et al. 2012	2.5	3	-	3	1.20
Besser et al. 2012	3.4	4.4	-	4.4	1.29
Besser et al. 2012	6.7	8	-	8	1.19
Besser et al. 2012	12	13	-	13	1.08
Besser et al. 2012	24	27	-	27	1.13

Desert pupfish (*Cyprinodon macularius*)



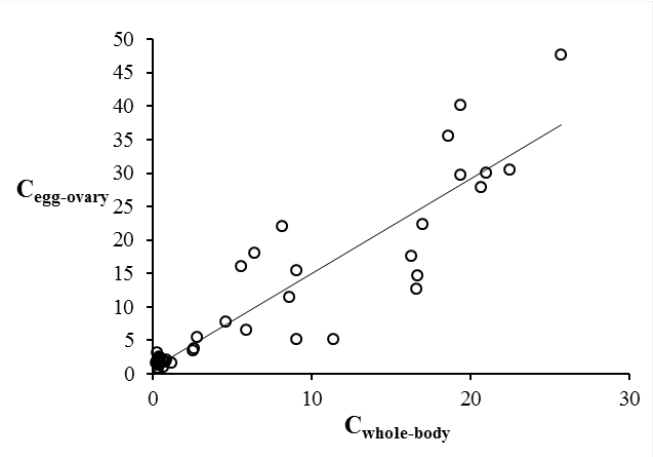
Median ratio: 1.20

R^2: 1.00
F: 194.3
df: 4
P: <0.001

Cutthroat trout (*Oncorhynchus clarkii*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Hardy 2005	0.70	1.00	-	1.00	1.43
Hardy 2005	2.60	3.80	-	3.80	1.46
Hardy 2005	2.80	5.50	-	5.50	1.96
Hardy 2005	6.40	18.00	-	18.00	2.81
Hardy 2005	1.20	1.60	-	1.60	1.33
Hardy 2005	4.60	7.80	-	7.80	1.70
Hardy 2005	5.90	6.60	-	6.60	1.12
Hardy 2005	9.10	5.10	-	5.10	0.56
Hardy 2005	11.40	5.20	-	5.20	0.46
Hardy 2005	5.60	16.00	-	16.00	2.86
Formation 2012	2.56	3.43	-	3.43	1.34
Formation 2012	16.3	17.6	-	17.6	1.08
Formation 2012	20.7	27.9	-	27.9	1.35
Formation 2012	19.4	29.7	-	29.7	1.53
Formation 2012	17	22.3	-	22.3	1.31
Formation 2012	16.7	14.6	-	14.6	0.87
Formation 2012	25.7	47.6	-	47.6	1.85
Formation 2012	8.17	22	-	22	2.69
Formation 2012	9.07	15.4	-	15.4	1.70
Formation 2012	8.63	11.4	-	11.4	1.32
Formation 2012	16.6	12.7	-	12.7	0.77
Formation 2012	19.4	40.1	-	40.1	2.07
Formation 2012	21	30	-	30	1.43
Formation 2012	18.6	35.6	-	35.6	1.91
Formation 2012	22.5	30.5	-	30.5	1.36
Formation 2012 Henry Lake fish hatchery	0.4	1.65	-	1.65	4.13

Cutthroat trout (*Oncorhynchus clarkii*)					
Formation 2012 Henry Lake fish hatchery	0.45	2.03	-	2.03	4.51
Formation 2012 Henry Lake fish hatchery	0.44	2.48	-	2.48	5.64
Formation 2012 Henry Lake fish hatchery	0.36	1.36	-	1.36	3.78
Formation 2012 Henry Lake fish hatchery	0.5	2.33	-	2.33	4.66
Formation 2012 Henry Lake fish hatchery	0.36	0.83	-	0.83	2.31
Formation 2012 Henry Lake fish hatchery	0.44	2.26	-	2.26	5.14
Formation 2012 Henry Lake fish hatchery	0.28	1.87	-	1.87	6.68
Formation 2012 Henry Lake fish hatchery	0.44	1.98	-	1.98	4.50
Formation 2012 Henry Lake fish hatchery	0.43	1.34	-	1.34	3.12
Formation 2012 Henry Lake fish hatchery	0.31	3.23	-	3.23	10.42
Formation 2012 Henry Lake fish hatchery	0.23	1.58	-	1.58	6.87
Formation 2012 Henry Lake fish hatchery	0.72	1.93	-	1.93	2.68
Formation 2012 Henry Lake fish hatchery	0.73	1.79	-	1.79	2.45
Formation 2012 Henry Lake fish hatchery	0.91	2.06	-	2.06	2.26
Formation 2012 Henry Lake fish hatchery	0.85	1.74	-	1.74	2.05



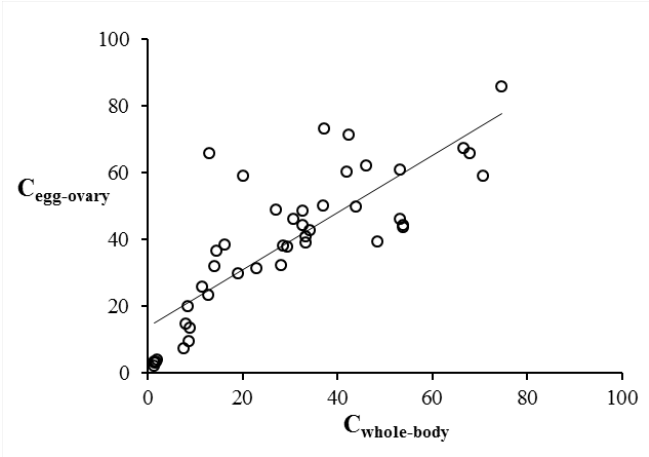
Median ratio: 1.96

R^2: 0.83
F: 194.3
df: 39
P: <0.001

Fathead minnow (*Pimephales promelas*)					
Study	**$C_{whole\text{-}body}$**	**C_{egg}**	**C_{ovary}**	**$C_{egg\text{-}ovary}$**	**Ratio**
GEI 2014	2.04	3.81	-	3.81	1.87
GEI 2014	1.39	2.23	-	2.23	1.60
GEI 2014	1.85	3.31	-	3.31	1.79
GEI 2014	1.32	3.43	-	3.43	2.60
GEI 2014	1.55	3.08	-	3.08	1.99
GEI 2014	37.13	50.06	-	50.06	1.35
GEI 2014	29.54	37.77	-	37.77	1.28
GEI 2014	33.32	40.82	-	40.82	1.23
GEI 2014	28.26	32.23	-	32.23	1.14
GEI 2014	30.74	46.21	-	46.21	1.50

Fathead minnow (*Pimephales promelas*)					
GEI 2014	53.17	60.84	-	60.84	1.14
GEI 2014	48.52	39.28	-	39.28	0.81
GEI 2014	53.81	44.28	-	44.28	0.82
GEI 2014	53.2	46.21	-	46.21	0.87
GEI 2014	54.01	43.51	-	43.51	0.81
GEI 2014	12.93	23.18	-	23.18	1.79
GEI 2014	8.19	14.67	-	14.67	1.79
GEI 2014	14.25	32.04	-	32.04	2.25
GEI 2014	8.65	19.95	-	19.95	2.31
GEI 2014	16.33	38.51	-	38.51	2.36
GEI 2014	7.69	7.39	-	7.39	0.96
GEI 2014	19.05	29.69	-	29.69	1.56
GEI 2014	8.78	9.55	-	9.55	1.09
GEI 2014	14.68	36.58	-	36.58	2.49
GEI 2014	9.02	13.63	-	13.63	1.51
GEI 2014	46.17	61.99	-	61.99	1.34
GEI 2014	41.97	60.07	-	60.07	1.43
GEI 2014	34.33	42.74	-	42.74	1.24
GEI 2014	33.4	38.89	-	38.89	1.16
GEI 2014	42.53	71.24	-	71.24	1.68
GEI 2014	74.56	85.87	-	85.87	1.15
GEI 2014	67.94	65.85	-	65.85	0.97
GEI 2014	70.85	58.91	-	58.91	0.83
GEI 2014	43.93	49.67	-	49.67	1.13
GEI 2014	66.57	67.39	-	67.39	1.01
GEI 2014	20.21	58.91	-	58.91	2.91
GEI 2014	13.08	65.85	-	65.85	5.03
GEI 2014	23.02	31.38	-	31.38	1.36
GEI 2014	11.55	25.72	-	25.72	2.23
GEI 2014	32.8	48.52	-	48.52	1.48
GEI 2014	27.17	48.9	-	48.9	1.80
GEI 2014	28.54	38.04	-	38.04	1.33
GEI 2014	37.2	73.16	-	73.16	1.97
GEI 2014	32.79	44.28	-	44.28	1.35
GEI 2014	46.17	61.99	-	61.99	1.87

Fathead minnow (*Pimephales promelas*)

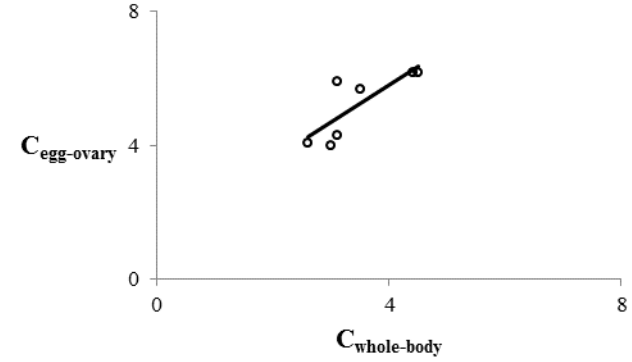


Median ratio: 1.40

R^2: 0.86
F: 81.4
df: 42
P: <0.001

Flannelmouth sucker (*Catostomus latipinnis*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.00	-	4.00	4.00	1.33
Osmundson et al. 2007	2.60	-	4.10	4.10	1.58
Osmundson et al. 2007	3.10	-	5.90	5.90	1.90
Osmundson et al. 2007	3.10	-	4.30	4.30	1.39
Osmundson et al. 2007	3.50	-	5.70	5.70	1.63
Osmundson et al. 2007	4.40	-	6.20	6.20	1.41
Osmundson et al. 2007	4.50	-	6.20	6.20	1.38



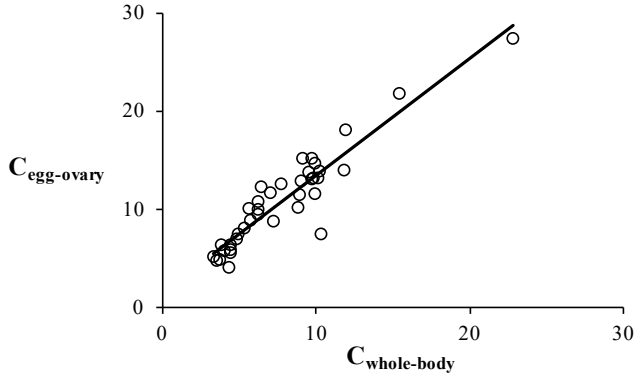
Median ratio: 1.41

R^2: 0.65
F: 9.2
df: 5
P: 0.021

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	22.80	-	27.40	27.40	1.20
Osmundson et al. 2007	8.80	-	10.20	10.20	1.16
Osmundson et al. 2007	15.40	-	21.80	21.80	1.42
Osmundson et al. 2007	4.80	-	7.00	7.00	1.46
Osmundson et al. 2007	5.70	-	8.90	8.90	1.56
Osmundson et al. 2007	4.40	-	6.40	6.40	1.45
Osmundson et al. 2007	3.80	-	6.40	6.40	1.68
Osmundson et al. 2007	11.90	-	18.10	18.10	1.52
Osmundson et al. 2007	6.40	-	12.30	12.30	1.92
Osmundson et al. 2007	9.50	-	13.80	13.80	1.45
Osmundson et al. 2007	9.10	-	15.20	15.20	1.67
Osmundson et al. 2007	6.20	-	10.80	10.80	1.74
Osmundson et al. 2007	7.00	-	11.70	11.70	1.67
Osmundson et al. 2007	7.70	-	12.60	12.60	1.64
Osmundson et al. 2007	6.20	-	10.00	10.00	1.61
Osmundson et al. 2007	10.20	-	13.90	13.90	1.36
Osmundson et al. 2007	9.70	-	15.20	15.20	1.57
Osmundson et al. 2007	9.90	-	14.70	14.70	1.48
Osmundson et al. 2007	7.20	-	8.80	8.80	1.22
Osmundson et al. 2007	9.00	-	12.90	12.90	1.43
Osmundson et al. 2007	9.70	-	13.10	13.10	1.35
Osmundson et al. 2007	8.90	-	11.50	11.50	1.29
Osmundson et al. 2007	9.80	-	13.20	13.20	1.35
Osmundson et al. 2007	9.90	-	11.60	11.60	1.17
Osmundson et al. 2007	10.30	-	7.50	7.50	0.73
Osmundson et al. 2007	5.30	-	8.10	8.10	1.53
Osmundson et al. 2007	10.10	-	13.20	13.20	1.31
Osmundson et al. 2007	11.80	-	14.00	14.00	1.19
Osmundson et al. 2007	3.30	-	5.20	5.20	1.58
Osmundson et al. 2007	4.00	-	5.80	5.80	1.45
Osmundson et al. 2007	4.30	-	4.10	4.10	0.95
Osmundson et al. 2007	3.70	-	4.90	4.90	1.32
Osmundson et al. 2007	6.20	-	9.50	9.50	1.53
Osmundson et al. 2007	3.50	-	4.80	4.80	1.37
Osmundson et al. 2007	4.40	-	5.60	5.60	1.27
Osmundson et al. 2007	5.60	-	10.10	10.10	1.80
Osmundson et al. 2007	4.90	-	7.50	7.50	1.53
Osmundson et al. 2007	4.40	-	5.90	5.90	1.34

Green sunfish (*Lepomis cyanellus*)

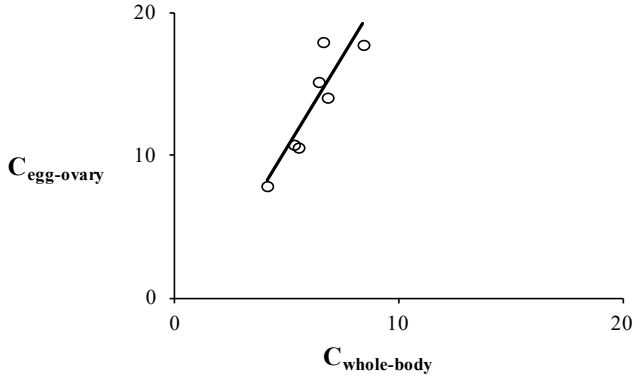


Median ratio: 1.45

R^2: 0.87
F: 240.0
df: 36
P: < 0.001

Roundtail chub (*Gila robusta*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	4.10	-	7.90	7.90	1.93
Osmundson et al. 2007	5.30	-	10.80	10.80	2.04
Osmundson et al. 2007	6.40	-	15.20	15.20	2.38
Osmundson et al. 2007	6.80	-	14.10	14.10	2.07
Osmundson et al. 2007	5.50	-	10.60	10.60	1.93
Osmundson et al. 2007	6.60	-	18.00	18.00	2.73
Osmundson et al. 2007	8.40	-	17.80	17.80	2.12

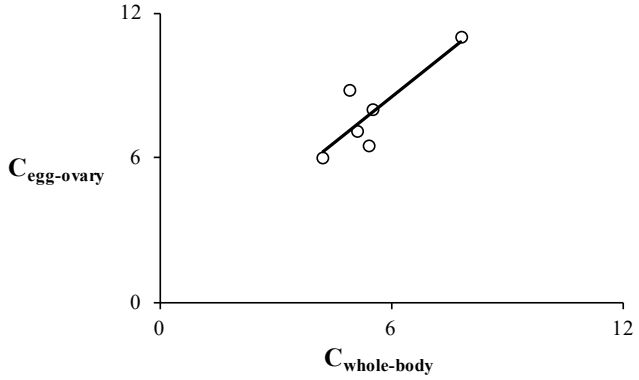


Median ratio: 2.07

R^2: 0.80
F: 20.4
df: 5
P: 0.004

Smallmouth bass (*Micropterus dolomieu*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	4.20	-	6.00	6.00	1.43
Osmundson et al. 2007	5.50	-	8.00	8.00	1.45
Osmundson et al. 2007	5.40	-	6.50	6.50	1.20
Osmundson et al. 2007	7.80	-	11.00	11.00	1.41
Osmundson et al. 2007	5.10	-	7.10	7.10	1.39
Osmundson et al. 2007	4.90	-	8.80	8.80	1.80



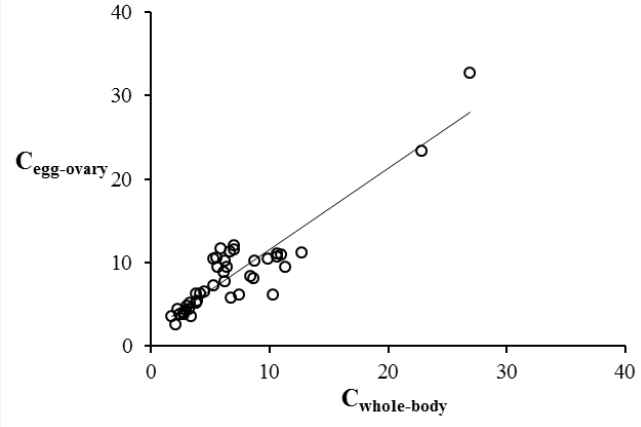
Median ratio: 1.42

R2: 0.73
F: 10.6
df: 4
P: 0.026

White sucker (*Catostomus commersonii*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.80	-	6.20	6.20	1.63
Osmundson et al. 2007	4.20	-	6.20	6.20	1.48
Osmundson et al. 2007	3.30	-	5.20	5.20	1.58
Osmundson et al. 2007	4.50	-	6.50	6.50	1.44
Osmundson et al. 2007	6.30	-	7.70	7.70	1.22
Osmundson et al. 2007	6.80	-	5.80	5.80	0.85
Osmundson et al. 2007	11.00	-	10.90	10.90	0.99
Osmundson et al. 2007	12.70	-	11.20	11.20	0.88
Osmundson et al. 2007	5.70	-	9.40	9.40	1.65
Osmundson et al. 2007	3.90	-	5.40	5.40	1.38
Osmundson et al. 2007	3.80	-	5.10	5.10	1.34
Osmundson et al. 2007	9.90	-	10.40	10.40	1.05
Osmundson et al. 2007	5.30	-	10.40	10.40	1.96
Osmundson et al. 2007	10.70	-	11.00	11.00	1.03
Osmundson et al. 2007	5.90	-	11.70	11.70	1.98
Osmundson et al. 2007	7.00	-	11.60	11.60	1.66
Osmundson et al. 2007	6.40	-	9.40	9.40	1.47
Osmundson et al. 2007	6.30	-	10.20	10.20	1.62
Osmundson et al. 2007	5.30	-	7.30	7.30	1.38
Osmundson et al. 2007	6.20	-	8.90	8.90	1.44

White sucker (*Catostomus commersonii*)					
Osmundson et al. 2007	5.60	-	10.50	10.50	1.88
Osmundson et al. 2007	8.80	-	10.20	10.20	1.16
Osmundson et al. 2007	8.70	-	8.10	8.10	0.93
Osmundson et al. 2007	11.40	-	9.50	9.50	0.83
Osmundson et al. 2007	10.70	-	10.70	10.70	1.00
Osmundson et al. 2007	8.40	-	8.30	8.30	0.99
Osmundson et al. 2007	7.00	-	12.00	12.00	1.71
Osmundson et al. 2007	7.50	-	6.10	6.10	0.81
Osmundson et al. 2007	10.30	-	6.10	6.10	0.59
Osmundson et al. 2007	6.70	-	11.30	11.30	1.69
Osmundson et al. 2007	2.10	-	2.60	2.60	1.24
Osmundson et al. 2007	1.80	-	3.60	3.60	2.00
Osmundson et al. 2007	3.20	-	4.40	4.40	1.38
Osmundson et al. 2007	2.30	-	4.40	4.40	1.91
Osmundson et al. 2007	3.10	-	4.80	4.80	1.55
Osmundson et al. 2007	3.00	-	4.30	4.30	1.43
Osmundson et al. 2007	2.80	-	4.10	4.10	1.46
Osmundson et al. 2007	2.50	-	3.80	3.80	1.52
Osmundson et al. 2007	3.40	-	3.60	3.60	1.06
Osmundson et al. 2007	2.80	-	3.80	3.80	1.36
GEI 2014	26.9	-	32.7	32.7	1.22
GEI 2014	22.9	-	23.3	23.3	1.02



Median ratio: 1.38

R^2: 0.83
F: 200.4
df: 40
P: < 0.001

Table B-2. Summary of egg-ovary to whole body conversion factors (*CF*) from matched pairs of whole-body and egg-ovary measurements.

Common name	Scientific name	Median ratio (*CF*)
Bluegill	*Lepomis macrochirus*	2.13
Bluehead sucker	*Catostomus discobolus*	1.82
Brown trout	*Salmo trutta*	1.45
Common carp	*Cyprinus carpio*	1.92
Creek chub	*Semotilus atromaculatus*	1.99
Cutthroat trout	*Oncorhynchus clarkii*	1.96
Desert pupfish	*Cyprinodon macularius*	1.20
Fathead minnow	*Pimephales promelas*	1.40
Flannelmouth sucker	*Catostomus latipinnis*	1.41
Green sunfish	*Lepomis cyanellus*	1.45
Roundtail chub	*Gila robusta*	2.07
Smallmouth bass	*Micropterus dolomieu*	1.42
White sucker	*Catostomus commersonii*	1.38

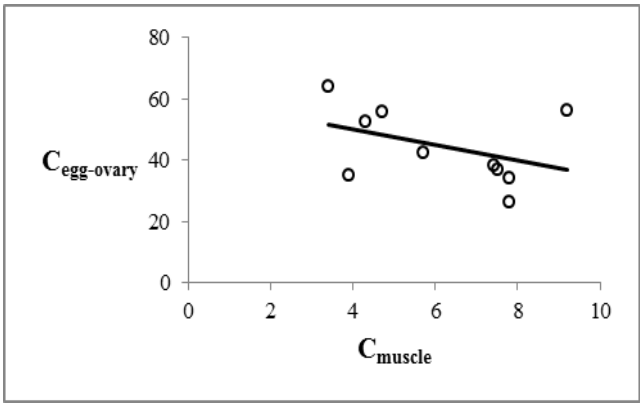
2.3 Muscle to egg-ovary conversion factors

C_{muscle} = Selenium concentration in muscle tissue only (µg/g dw)
C_{egg} = Selenium concentration in eggs (µg/g dw)
C_{ovary} = Selenium concentration in ovary tissue (µg/g dw)
$C_{egg\text{-}ovary}$ = Average selenium concentration in eggs and ovaries $\left(\frac{C_{egg} + C_{o\,\mathrm{var}\,y}}{2}\right)$

Ratio = $\frac{C_{egg-o\,\mathrm{var}\,y}}{C_{muscle}}$

Black bullhead (*Ameiurus melas*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.40	-	64.30	64.30	18.91
Osmundson et al. 2007	3.90	-	35.40	35.40	9.08
Osmundson et al. 2007	4.30	-	52.80	52.80	12.28
Osmundson et al. 2007	4.70	-	56.00	56.00	11.91
Osmundson et al. 2007	5.70	-	42.80	42.80	7.51
Osmundson et al. 2007	7.40	-	38.70	38.70	5.23
Osmundson et al. 2007	7.50	-	37.30	37.30	4.97
Osmundson et al. 2007	7.80	-	34.30	34.30	4.40
Osmundson et al. 2007	7.80	-	26.40	26.40	3.38
Osmundson et al. 2007	9.20	-	56.70	56.70	6.16



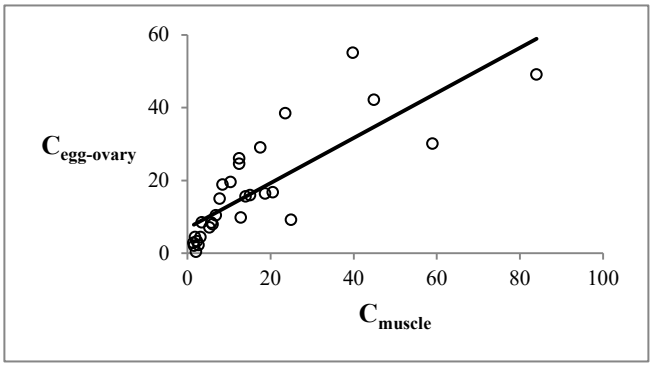
Median ratio: 6.84

R^2: 0.17
F: 1.65
df: 8
P: 0.250

Not used because P > 0.05 and negative slope.

Bluegill (*Lepomis macrochirus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Bryson et al. 1984	84.0	-	49.0	49.0	0.58
Bryson et al. 1985a (pt. 1)	59.0	-	30.0	30.0	0.51
Bryson et al. 1985a (pt. 1)	2.7	-	2.2	2.2	0.81
Bryson et al. 1985a (pt. 2)	25.0	-	9.1	9.1	0.36
Doroshov et al. 1992	1.5	2.8	-	2.8	1.87
Doroshov et al. 1992	5.8	8.3	-	8.3	1.43
Doroshov et al. 1992	10.4	19.5	-	19.5	1.88
Doroshov et al. 1992	23.6	38.4	-	38.4	1.63
Hermanutz et al. 1996	1.6	-	2.0	2.0	1.25
Hermanutz et al. 1996	8.5	-	18.8	18.8	2.21
Hermanutz et al. 1996	14	-	15.5	15.5	1.11
Hermanutz et al. 1996	2.1	-	0.3	0.3	0.14
Hermanutz et al. 1996	20.6	-	16.7	16.7	0.81
Hermanutz et al. 1996	1.9	-	4.4	4.4	2.32
Hermanutz et al. 1996	3.5	-	8.4	8.4	2.40
Hermanutz et al. 1996	17.6	-	29.0	29.0	1.65
Hermanutz et al. 1996	12.5	-	24.5	24.5	1.96
Hermanutz et al. 1996	2.3	-	3.2	3.2	1.39
Hermanutz et al. 1996	6.9	-	10.3	10.3	1.49
Hermanutz et al. 1996	44.9	-	42.1	42.1	0.94
Hermanutz et al. 1996	39.8	-	55.0	55.0	1.38
Hermanutz et al. 1996	5.3	-	7.0	7.0	1.32
Hermanutz et al. 1996	12.5	-	26.0	26.0	2.08
Hermanutz et al. 1996	7.8	-	14.9	14.9	1.91
Hermanutz et al. 1996	3.2	-	4.4	4.4	1.38
Hermanutz et al. 1996	6.1	-	7.9	7.9	1.30
Hermanutz et al. 1996	18.7	-	16.3	16.3	0.87
Hermanutz et al. 1996	15.1	-	15.9	15.9	1.05
Osmundson et al. 2007	12.9	-	9.7	9.7	0.75

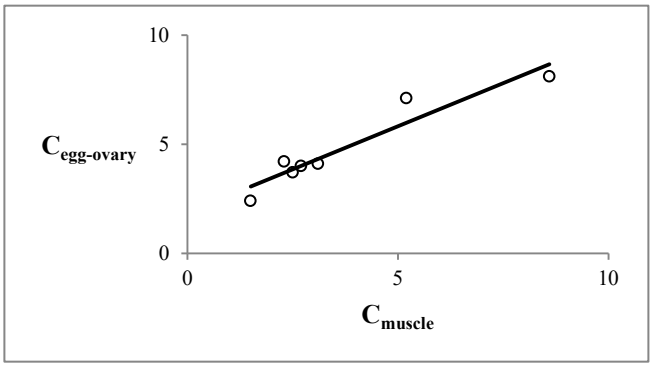


Median ratio: 1.38

R^2: 0.65
F: 50.37
df: 27
P: <0.001

Bluehead sucker (*Catostomus discobolus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	1.5	-	2.4	2.4	1.60
Osmundson et al. 2007	2.3	-	4.2	4.2	1.83
Osmundson et al. 2007	2.5	-	3.7	3.7	1.48
Osmundson et al. 2007	2.7	-	4	4	1.48
Osmundson et al. 2007	3.1	-	4.1	4.1	1.32
Osmundson et al. 2007	5.2	-	7.1	7.1	1.37
Osmundson et al. 2007	8.6	-	8.1	8.1	0.94



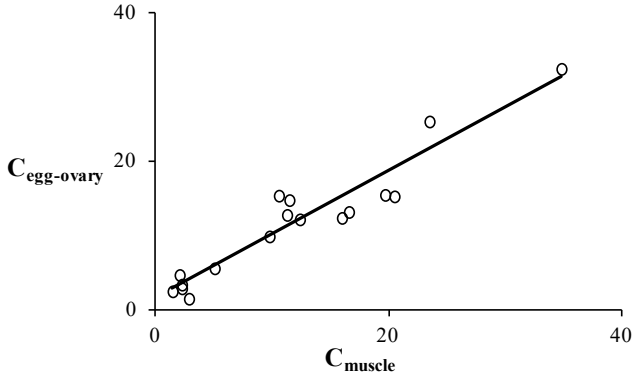
Median ratio: 1.48

R^2: 0.91
F: 47.70
df: 5
P: <0.001

Brook trout (*Salvelinus fontinalis*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Holm et al. 2005	2.80	1.50	-	1.50	0.54
Holm et al. 2005	1.40	2.50	-	2.50	1.79
Holm et al. 2005	2.20	3.40	-	3.40	1.55
Holm et al. 2005	2.00	4.70	-	4.70	2.35
Holm et al. 2005	2.20	2.90	-	2.90	1.32
Holm et al. 2005	5.00	5.60	-	5.60	1.12
Holm et al. 2005	9.70	9.90	-	9.90	1.02
Holm et al. 2005	10.50	15.40	-	15.40	1.47
Holm et al. 2005	11.20	12.80	-	12.80	1.14
Holm et al. 2005	11.40	14.80	-	14.80	1.30
Holm et al. 2005	12.30	12.20	-	12.20	0.99
Holm et al. 2005	15.90	12.40	-	12.40	0.78
Holm et al. 2005	16.50	13.20	-	13.20	0.80
Holm et al. 2005	19.60	15.50	-	15.50	0.79
Holm et al. 2005	20.40	15.30	-	15.30	0.75
Holm et al. 2005	23.40	25.40	-	25.40	1.09
Holm et al. 2005	34.70	32.50	-	32.50	0.94

Brook trout (*Salvelinus fontinalis*)

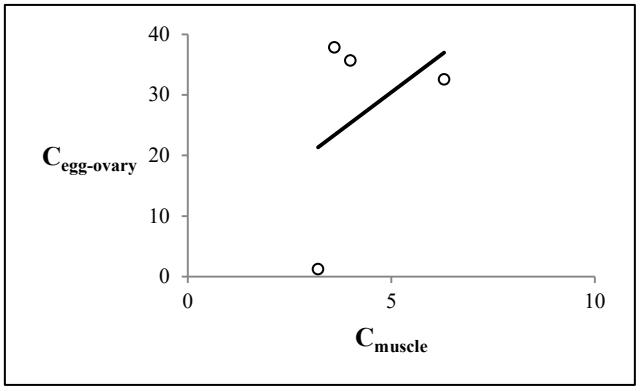


Median ratio: 1.09

R^2: 0.91
F: 152.3
df: 15
P: < 0.001

Brown trout (*Salmo trutta*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.2	-	1.2	1.2	0.38
Osmundson et al. 2007	3.6	-	37.8	37.8	10.50
Osmundson et al. 2007	4	-	35.6	35.6	8.90
Osmundson et al. 2007	6.3	-	32.5	32.5	5.16



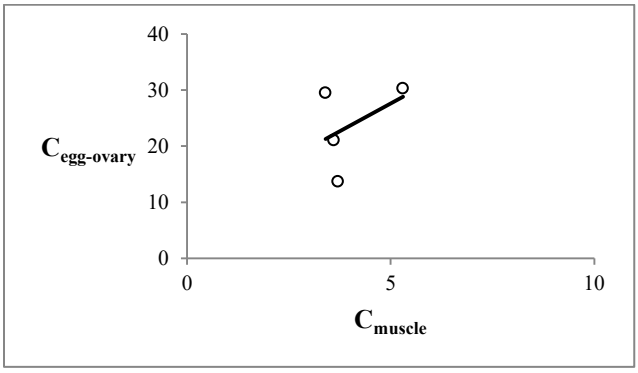
Median ratio: 7.03

R^2: 0.17
F: 0.40
df: 2
P: 0.71

Not used because P > 0.05.

Channel catfish (*Ictaluris punctatus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.4	-	29.5	29.5	8.68
Osmundson et al. 2007	3.6	-	21.1	21.1	5.86
Osmundson et al. 2007	3.7	-	13.7	13.7	3.70
Osmundson et al. 2007	5.3	-	30.3	30.3	5.72



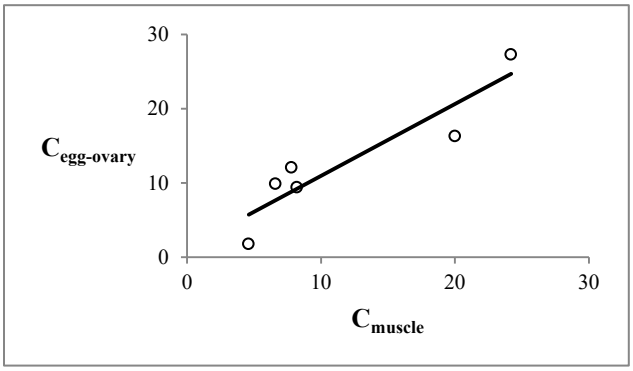
Median ratio: 5.79

R^2: 0.20
F: 0.49
df: 2
P: 0.67

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Garcia-Hernandez 2000	4.6	-	1.8	1.8	0.39
Osmundson et al. 2007	7.8	-	12.1	12.1	1.55
Osmundson et al. 2007	8.2	-	9.4	9.4	1.15
Osmundson et al. 2007	20	-	16.3	16.3	0.82
Osmundson et al. 2007	24.2	-	27.3	27.3	1.13
Osmundson et al. 2007	6.6	-	9.9	9.9	1.50



Median ratio: 1.14

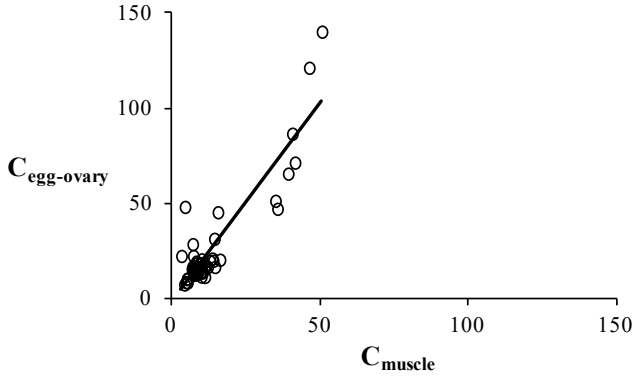
R^2: 0.84
F: 21.7
df: 4
P: 0.007

Cutthroat trout (*Oncorhynchus clarkii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Golder 2005	6.80	-	28.20	28.20	4.15
Golder 2005	4.20	-	47.80	47.80	11.38
Golder 2005	3.00	-	22.00	22.00	7.33
Golder 2005	4.90	-	9.80	9.80	2.00
Golder 2005	4.50	-	8.20	8.20	1.82
Golder 2005	4.00	-	7.00	7.00	1.75
Golder 2005	5.00	-	10.00	10.00	2.00
Golder 2005	5.00	-	10.00	10.00	2.00
Golder 2005	5.00	-	8.00	8.00	1.60
Golder 2005	8.40	-	16.20	16.20	1.93
Golder 2005	8.30	-	18.30	18.30	2.20
Golder 2005	7.00	-	14.30	14.30	2.04
Golder 2005	6.60	-	14.30	14.30	2.17
Golder 2005	8.40	-	14.70	14.70	1.75
Golder 2005	9.80	-	16.40	16.40	1.67
Golder 2005	8.50	-	15.90	15.90	1.87
Golder 2005	16.00	-	20.00	20.00	1.25
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	8.00	-	19.00	19.00	2.38
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	9.00	-	16.00	16.00	1.78
Golder 2005	7.00	-	13.00	13.00	1.86
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	8.00	-	14.00	14.00	1.75
Golder 2005	9.80	-	20.20	20.20	2.06
Golder 2005	7.00	-	22.00	22.00	3.14
Golder 2005	9.00	-	16.00	16.00	1.78
Golder 2005	7.00	-	12.00	12.00	1.71
Golder 2005	8.00	-	13.00	13.00	1.63
Golder 2005	10.00	-	14.00	14.00	1.40
Kennedy et al. 2000	41.30	75.40	66.80	71.10	1.72
Kennedy et al. 2000	15.30	58.40	31.60	45.00	2.94
Kennedy et al. 2000	14.10	30.60	31.40	31.00	2.20
Kennedy et al. 2000	12.50	20.20	18.50	19.35	1.55
Kennedy et al. 2000	13.70	19.40	19.50	19.45	1.42
Kennedy et al. 2000	14.30	16.20	16.20	16.20	1.13
Kennedy et al. 2000	9.50	16.10	19.30	17.70	1.86
Kennedy et al. 2000	9.40	14.40	22.00	18.20	1.94
Kennedy et al. 2000	8.70	13.20	17.00	15.10	1.74
Kennedy et al. 2000	9.50	12.60	13.60	13.10	1.38
Kennedy et al. 2000	10.20	12.30	14.50	13.40	1.31

Cutthroat trout (*Oncorhynchus clarkii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Kennedy et al. 2000	10.70	10.50	20.60	15.55	1.45
Kennedy et al. 2000	6.60	9.90	21.50	15.70	2.38
Kennedy et al. 2000	9.70	9.10	13.20	11.15	1.15
Kennedy et al. 2000	10.90	8.50	13.40	10.95	1.00
Kennedy et al. 2000	6.90	13.20	20.30	16.75	2.43
Rudolph et al. 2007	7.70	13.90	-	13.90	1.81
Rudolph et al. 2007	8.20	12.50	-	12.50	1.52
Rudolph et al. 2007	8.00	15.00	-	15.00	1.88
Rudolph et al. 2007	8.10	14.90	-	14.90	1.84
Rudolph et al. 2007	6.60	15.20	-	15.20	2.30
Rudolph et al. 2007	8.50	12.90	-	12.90	1.52
Rudolph et al. 2007	7.20	12.30	-	12.30	1.71
Rudolph et al. 2007	7.30	16.70	-	16.70	2.29
Rudolph et al. 2007	7.60	13.10	-	13.10	1.72
Rudolph et al. 2007	8.70	15.60	-	15.60	1.79
Rudolph et al. 2007	8.20	13.90	-	13.90	1.70
Rudolph et al. 2007	7.90	15.10	-	15.10	1.91
Rudolph et al. 2007	7.60	12.30	-	12.30	1.62
Rudolph et al. 2007	11.80	16.10	-	16.10	1.36
Rudolph et al. 2007	40.40	86.30	-	86.30	2.14
Rudolph et al. 2007	46.10	121.00	-	121.00	2.62
Rudolph et al. 2007	50.40	140.00	-	140.00	2.78
Rudolph et al. 2007	34.70	51.00	-	51.00	1.47
Rudolph et al. 2007	39.00	65.30	-	65.30	1.67
Rudolph et al. 2007	35.40	46.80	-	46.80	1.32
Rudolph et al. 2007	11.30	16.90	-	16.90	1.50
Rudolph et al. 2007	13.40	20.60	-	20.60	1.54

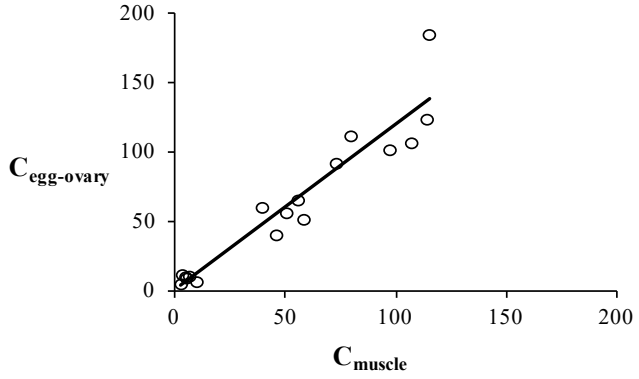


Median ratio: 1.81

R^2: 0.82
F: 308.3
df: 67
P: < 0.001

Dolly Varden (*Salvelinus malma*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Golder 2009	73.00	92.30	-	92.30	1.26
Golder 2009	45.90	40.70	-	40.70	0.89
Golder 2009	107.00	107.00	-	107.00	1.00
Golder 2009	97.20	102.00	-	102.00	1.05
Golder 2009	114.00	124.00	-	124.00	1.09
Golder 2009	115.00	185.00	-	185.00	1.61
Golder 2009	79.60	112.00	-	112.00	1.41
Golder 2009	9.90	7.00	-	7.00	0.71
Golder 2009	3.40	12.10	-	12.10	3.56
Golder 2009	5.30	9.60	-	9.60	1.81
Golder 2009	2.80	5.40	-	5.40	1.93
Golder 2009	4.90	10.50	-	10.50	2.14
Golder 2009	6.60	11.00	-	11.00	1.67
Golder 2009	55.70	65.80	-	65.80	1.18
Golder 2009	58.30	51.90	-	51.90	0.89
Golder 2009	39.50	60.50	-	60.50	1.53
Golder 2009	50.50	56.60	-	56.60	1.12

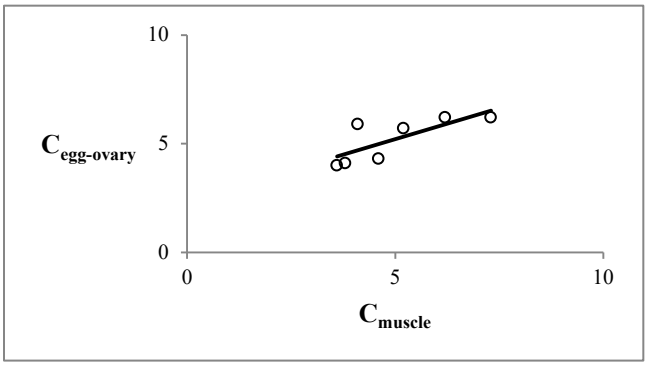


Median ratio: 1.26

R^2: 0.90
F: 140.3
df: 15
P: < 0. 001

Flannelmouth sucker (*Catostomus latipinnis*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.6	-	4.0	4.0	1.11
Osmundson et al. 2007	3.8	-	4.1	4.1	1.08
Osmundson et al. 2007	4.1	-	5.9	5.9	1.44
Osmundson et al. 2007	4.6	-	4.3	4.3	0.93
Osmundson et al. 2007	5.2	-	5.7	5.7	1.10
Osmundson et al. 2007	6.2	-	6.2	6.2	1.00
Osmundson et al. 2007	7.3	-	6.2	6.2	0.85



Median ratio: 1.08

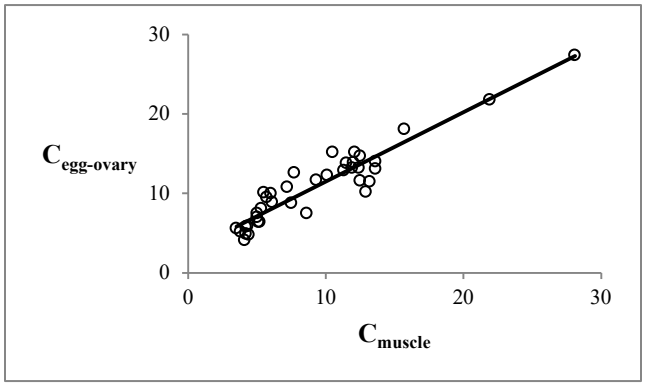
R^2: 0.58
F: 6.92
df: 5
P: 0.036

Green sunfish (*Lepomis cyanellus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	28.1	-	27.4	27.4	0.98
Osmundson et al. 2007	12.9	-	10.2	10.2	0.79
Osmundson et al. 2007	21.9	-	21.8	21.8	1.00
Osmundson et al. 2007	5	-	7	7	1.40
Osmundson et al. 2007	6.1	-	8.9	8.9	1.46
Osmundson et al. 2007	5.2	-	6.4	6.4	1.23
Osmundson et al. 2007	5.1	-	6.4	6.4	1.25
Osmundson et al. 2007	15.7	-	18.1	18.1	1.15
Osmundson et al. 2007	10.1	-	12.3	12.3	1.22
Osmundson et al. 2007	11.5	-	13.8	13.8	1.20
Osmundson et al. 2007	10.5	-	15.2	15.2	1.45
Osmundson et al. 2007	7.2	-	10.8	10.8	1.50
Osmundson et al. 2007	9.3	-	11.7	11.7	1.26
Osmundson et al. 2007	7.7	-	12.6	12.6	1.64
Osmundson et al. 2007	6	-	10	10	1.67
Osmundson et al. 2007	12	-	13.9	13.9	1.16
Osmundson et al. 2007	12.1	-	15.2	15.2	1.26
Osmundson et al. 2007	12.5	-	14.7	14.7	1.18
Osmundson et al. 2007	7.5	-	8.8	8.8	1.17
Osmundson et al. 2007	11.3	-	12.9	12.9	1.14

Green sunfish (*Lepomis cyanellus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	13.6	-	13.1	13.1	0.96
Osmundson et al. 2007	13.2	-	11.5	11.5	0.87
Osmundson et al. 2007	12.4	-	13.2	13.2	1.06
Osmundson et al. 2007	12.5	-	11.6	11.6	0.93
Osmundson et al. 2007	8.6	-	7.5	7.5	0.87
Osmundson et al. 2007	5.3	-	8.1	8.1	1.53
Osmundson et al. 2007	11.9	-	13.2	13.2	1.11
Osmundson et al. 2007	13.6	-	14	14	1.03
Osmundson et al. 2007	3.8	-	5.2	5.2	1.37
Osmundson et al. 2007	4.2	-	5.8	5.8	1.38
Osmundson et al. 2007	4.1	-	4.1	4.1	1.00
Osmundson et al. 2007	4.2	-	4.9	4.9	1.17
Osmundson et al. 2007	5.7	-	9.5	9.5	1.67
Osmundson et al. 2007	4.4	-	4.8	4.8	1.09
Osmundson et al. 2007	3.5	-	5.6	5.6	1.60
Osmundson et al. 2007	5.5	-	10.1	10.1	1.84
Osmundson et al. 2007	5	-	7.5	7.5	1.50
Osmundson et al. 2007	4.3	-	5.9	5.9	1.37

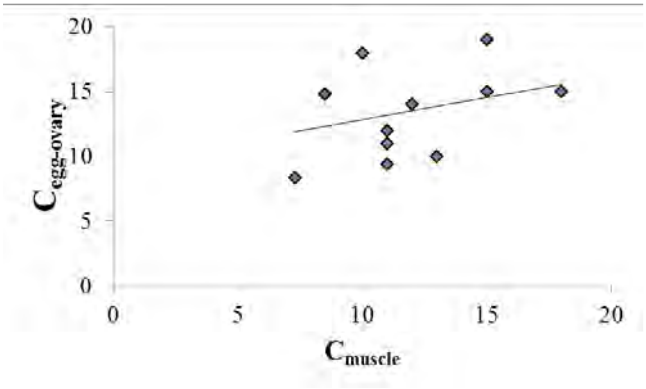


Median ratio: 1.21

R^2: 0.89
F: 281.4
df: 36
P: <0.001

Largemouth bass (*Micropterus salmoides*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Carolina Power & Light 1997	8.48	-	14.79	14.79	1.74
Carolina Power & Light 1997	8.48	-	14.79	14.79	1.74
Carolina Power & Light 1997	7.29	-	8.35	8.35	1.15
Carolina Power & Light 1997	15	-	19	19	1.27
Carolina Power & Light 1997	15	-	15	15	1.00
Carolina Power & Light 1997	12	-	14	14	1.17
Carolina Power & Light 1997	10	-	18	18	1.80
Carolina Power & Light 1997	18	-	15	15	0.83
Carolina Power & Light 1997	18	-	15	15	0.83
Carolina Power & Light 1997	11	-	12	12	1.09
Carolina Power & Light 1997	11	-	9.4	9.4	0.85
Carolina Power & Light 1997	13	-	10	10	0.77
Carolina Power & Light 1997	11	-	11	11	1.00

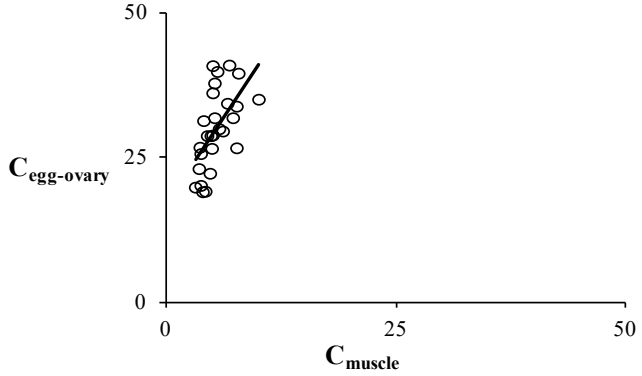


Median ratio: 1.09

R^2: 0.14
F: 1.74
df: 11
P: 0.22
Not used because P>0.05

Mountain whitefish (*Prosopium williamsoni*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Golder 2005	3.60	-	26.90	26.90	7.47
Golder 2005	3.70	-	25.80	25.80	6.97
Golder 2005	3.10	-	20.00	20.00	6.45
Golder 2005	4.20	-	19.30	19.30	4.60
Golder 2005	3.90	-	19.20	19.20	4.92
Golder 2005	3.50	-	23.20	23.20	6.63
Golder 2005	5.20	-	38.00	38.00	7.31
Golder 2005	5.00	-	41.00	41.00	8.20
Golder 2005	5.20	-	32.00	32.00	6.15
Golder 2005	7.60	-	34.00	34.00	4.47
Golder 2005	7.20	-	32.00	32.00	4.44
Golder 2005	5.50	-	40.00	40.00	7.27
Golder 2005	7.80	-	39.70	39.70	5.09
Golder 2005	3.70	-	20.30	20.30	5.49
Golder 2005	4.70	-	22.40	22.40	4.77
Golder 2005	4.40	-	28.90	28.90	6.57
Golder 2005	5.70	-	30.10	30.10	5.28
Golder 2005	4.00	-	31.50	31.50	7.88
Golder 2005	10.00	-	35.20	35.20	3.52
Golder 2005	4.90	-	26.70	26.70	5.45
Golder 2005	7.60	-	26.80	26.80	3.53
Golder 2005	6.10	-	29.70	29.70	4.87
Golder 2005	6.80	-	41.10	41.10	6.04
Golder 2005	5.00	-	29.00	29.00	5.80
Golder 2005	6.60	-	34.50	34.50	5.23
Golder 2005	5.00	-	36.30	36.30	7.26
Golder 2005	4.80	-	28.90	28.90	6.02

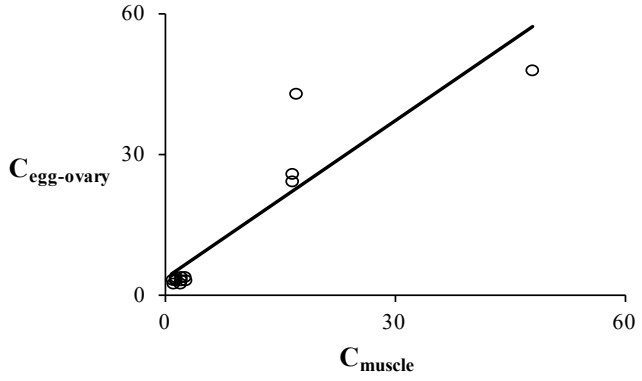


Median ratio: 5.80

R^2: 0.33
F: 12.4
df: 25
P: <0.001

Northern pike (*Esox lucius*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Muscatello et al. 2006	0.90	3.50	-	3.50	3.89
Muscatello et al. 2006	1.90	2.70	-	2.70	1.42
Muscatello et al. 2006	2.60	3.40	-	3.40	1.31
Muscatello et al. 2006	1.30	3.70	-	3.70	2.85
Muscatello et al. 2006	1.00	2.70	-	2.70	2.70
Muscatello et al. 2006	17.00	43.20	-	43.20	2.54
Muscatello et al. 2006	16.50	24.50	-	24.50	1.48
Muscatello et al. 2006	16.50	26.10	-	26.10	1.58
Muscatello et al. 2006	2.00	3.40	-	3.40	1.70
Muscatello et al. 2006	2.00	4.10	-	4.10	2.05
Muscatello et al. 2006	1.30	4.10	-	4.10	3.15
Muscatello et al. 2006	2.50	4.10	-	4.10	1.64
Muscatello et al. 2006	1.30	3.40	-	3.40	2.62
Muscatello et al. 2006	47.80	48.20	-	48.20	1.01



Median ratio: 1.88

R^2: 0.83
F: 58.9
df: 12
P: <0.001

Rainbow trout (*Oncorhynchus mykiss*)

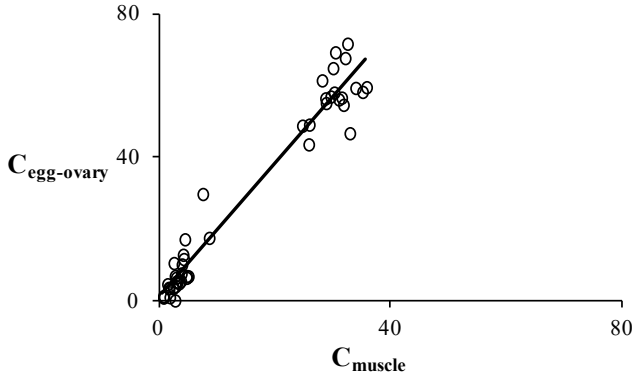
Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.10	11.60	-	11.60	2.83
Casey and Siwik 2000	3.80	10.10	-	10.10	2.66
Casey and Siwik 2000	2.60	0.10	-	0.10	0.04
Casey and Siwik 2000	3.30	4.90	-	4.90	1.48
Casey and Siwik 2000	2.30	3.60	-	3.60	1.57
Casey and Siwik 2000	2.80	5.30	-	5.30	1.89
Casey and Siwik 2000	2.30	3.70	-	3.70	1.61
Casey and Siwik 2000	2.80	6.40	-	6.40	2.29
Casey and Siwik 2000	3.00	5.20	-	5.20	1.73
Casey and Siwik 2000	4.90	6.80	-	6.80	1.39
Casey and Siwik 2000	1.50	3.60	-	3.60	2.40
Casey and Siwik 2000	2.60	6.90	-	6.90	2.65

Rainbow trout (*Oncorhynchus mykiss*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.60	6.90	-	6.90	1.50
Casey and Siwik 2000	4.60	6.40	-	6.40	1.39
Casey and Siwik 2000	3.60	5.50	-	5.50	1.53
Casey and Siwik 2000	2.40	10.50	-	10.50	4.38
Casey and Siwik 2000	3.70	7.60	-	7.60	2.05
Casey and Siwik 2000	2.70	4.10	-	4.10	1.52
Casey and Siwik 2000	0.70	1.10	-	1.10	1.57
Casey and Siwik 2000	0.60	0.90	-	0.90	1.50
Casey and Siwik 2000	0.60	1.30	-	1.30	2.17
Casey and Siwik 2000	28.60	56.30	-	56.30	1.97
Casey and Siwik 2000	30.90	56.00	-	56.00	1.81
Casey and Siwik 2000	32.40	71.50	-	71.50	2.21
Casey and Siwik 2000	28.00	61.30	-	61.30	2.19
Casey and Siwik 2000	31.70	54.50	-	54.50	1.72
Casey and Siwik 2000	29.50	56.80	-	56.80	1.93
Casey and Siwik 2000	30.10	57.90	-	57.90	1.92
Casey and Siwik 2000	29.90	64.70	-	64.70	2.16
Casey and Siwik 2000	32.80	46.60	-	46.60	1.42
Casey and Siwik 2000	31.40	56.50	-	56.50	1.80
Casey and Siwik 2000	32.00	67.50	-	67.50	2.11
Casey and Siwik 2000	35.70	59.40	-	59.40	1.66
Casey and Siwik 2000	24.60	48.70	-	48.70	1.98
Casey and Siwik 2000	30.30	69.10	-	69.10	2.28
Casey and Siwik 2000	25.70	43.50	-	43.50	1.69
Casey and Siwik 2000	35.00	58.10	-	58.10	1.66
Casey and Siwik 2000	33.80	59.20	-	59.20	1.75
Casey and Siwik 2000	28.70	55.00	-	55.00	1.92
Casey and Siwik 2000	25.80	49.00	-	49.00	1.90
Holm et al. 2005	1.70	1.00	-	1.00	0.59
Holm et al. 2005	1.60	3.50	-	3.50	2.19
Holm et al. 2005	1.30	4.60	-	4.60	3.54
Holm et al. 2005	4.00	12.80	-	12.80	3.20
Holm et al. 2005	4.30	17.10	-	17.10	3.98
Holm et al. 2005	8.50	17.50	-	17.50	2.06
Holm et al. 2005	7.40	29.70	-	29.70	4.01

Rainbow trout (*Oncorhynchus mykiss*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio



Median ratio: 1.92

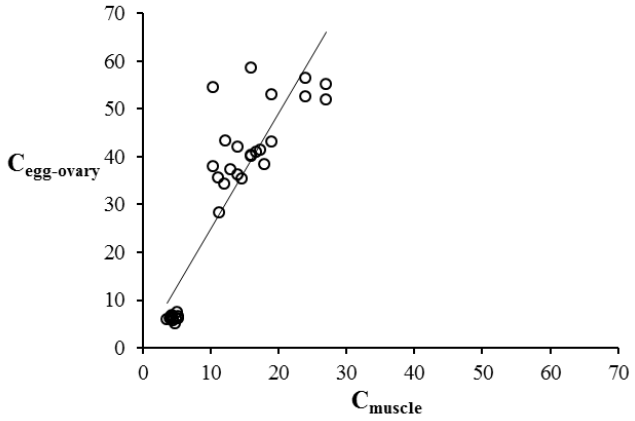
R^2: 0.96
F: 990.0
df: 45
P: <0. 001

Razorback sucker (*Xyrauchen texanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Hamilton et al. 2001a	5	7.5	-	7.5	1.50
Hamilton et al. 2001a	4	6.1	-	6.1	1.53
Hamilton et al. 2001a	4.2	6.6	-	6.6	1.57
Hamilton et al. 2001a	4.4	6.2	-	6.2	1.41
Hamilton et al. 2001a	4.5	5.8	-	5.8	1.29
Hamilton et al. 2001a	4.3	6.8	-	6.8	1.58
Hamilton et al. 2001a	11.1	35.5	-	35.5	3.20
Hamilton et al. 2001a	12.2	43.4	-	43.4	3.56
Hamilton et al. 2001a	10.4	54.5	-	54.5	5.24
Hamilton et al. 2001a	11.3	28.2	-	28.2	2.50
Hamilton et al. 2001a	10.4	38	-	38	3.65
Hamilton et al. 2001a	17.3	41.3	-	41.3	2.39
Hamilton et al. 2001a	13	37.2	-	37.2	2.86
Hamilton et al. 2001a	16.7	40.9	-	40.9	2.45
Hamilton et al. 2001a	14.6	35.3	-	35.3	2.42
Hamilton et al. 2001a	12.1	34.3	-	34.3	2.83
Hamilton et al. 2001b	4.7	5	-	5	1.06
Hamilton et al. 2001b	5.3	6.2	-	6.2	1.17
Hamilton et al. 2001b	3.6	5.9	-	5.9	1.64
Hamilton et al. 2001b	5.3	6.5	-	6.5	1.23
Hamilton et al. 2001b	4.1	6.35	-	6.35	1.55
Hamilton et al. 2001b	4.9	6.1	-	6.1	1.24
Hamilton et al. 2001b	16	40.1	-	40.1	2.51
Hamilton et al. 2001b	18	38.4	-	38.4	2.13
Hamilton et al. 2001b	16	40.2	-	40.2	2.51
Hamilton et al. 2001b	19	43.1	-	43.1	2.27

Razorback sucker (*Xyrauchen texanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Hamilton et al. 2001b	14	41.9	-	41.9	2.99
Hamilton et al. 2001b	14	36.2	-	36.2	2.59
Hamilton et al. 2001b	24	56.5	-	56.5	2.35
Hamilton et al. 2001b	27	51.8	-	51.8	1.92
Hamilton et al. 2001b	24	52.6	-	52.6	2.19
Hamilton et al. 2001b	27	55.1	-	55.1	2.04
Hamilton et al. 2001b	19	53	-	53	2.79
Hamilton et al. 2001b	16	58.5	-	58.5	3.66
Waddell and May 1995 [a]	4.40	3.70	-	3.70	×
Waddell and May 1995 [a]	7.10	4.70	-	4.70	×
Waddell and May 1995 [a]	32.00	10.60	-	10.60	×



Median ratio: 2.31

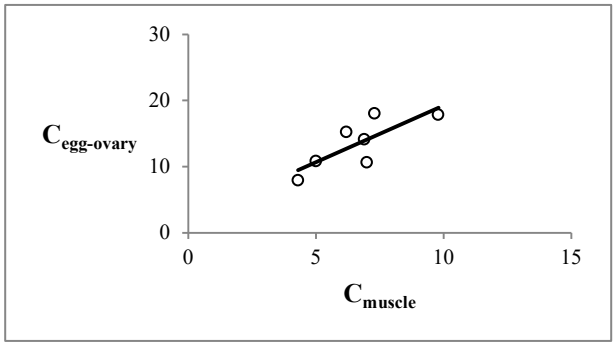
R^2: 0.80
F: 125.6
df: 32
P: <0.001

[a] Data from this study were excluded because results were atypical.

Roundtail chub (*Gila robusta*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	4.3	-	7.9	7.9	1.84
Osmundson et al. 2007	5	-	10.8	10.8	2.16
Osmundson et al. 2007	6.2	-	15.2	15.2	2.45
Osmundson et al. 2007	6.9	-		14.1	2.04
Osmundson et al. 2007	7	-	10.6	10.6	1.51
Osmundson et al. 2007	7.3	-	18	18	2.47
Osmundson et al. 2007	9.8	-	17.8	17.8	1.82

Roundtail chub (*Gila robusta*)

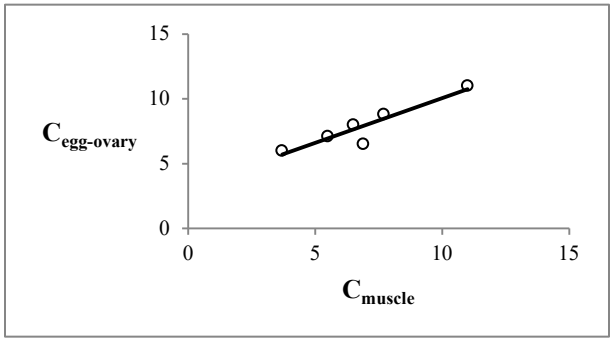


Median ratio: 2.04

R^2: 0.62
F: 8.27
df: 5
P: 0.026

Smallmouth bass (*Micropterus dolomieu*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.7	-	6.0	6.0	1.62
Osmundson et al. 2007	6.5	-	8.0	8.0	1.23
Osmundson et al. 2007	6.9	-	6.5	6.5	0.94
Osmundson et al. 2007	11			11	1.00
Osmundson et al. 2007	5.5	-	7.1	7.1	1.29
Osmundson et al. 2007	7.7	-	8.8	8.8	1.14

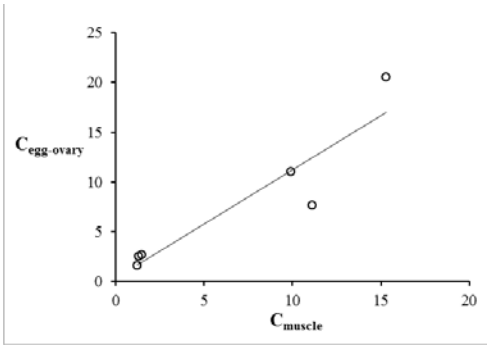


Median ratio: 1.19

R^2: 0.85
F: 23.5
df: 4
P: 0.006

White Sturgeon (*Acipenser transmontanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Linville 2006	1.28	2.46	-	2.46	2.46
Linville 2006	1.22	1.61	-	1.61	1.61
Linville 2006	1.48	2.68	-	2.68	2.68
Linville 2006	9.93	11	-	11	11
Linville 2006	15.3	20.5	-	20.5	20.5
Linville 2006	11.1	7.61	-	7.61	7.61



Median ratio: 1.33

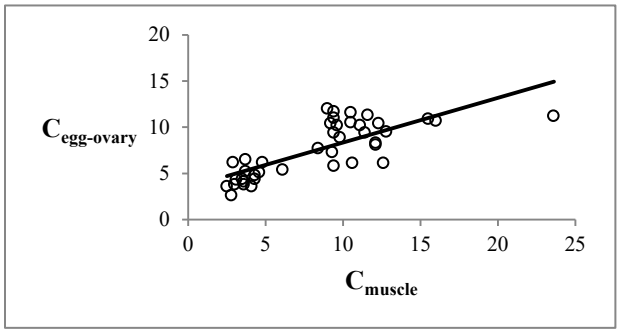
R^2: 0.86
F: 24.96
df: 4
P: 0.006

White Sucker (*Catostomus commersonii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	2.9	-	6.2	6.2	2.14
Osmundson et al. 2007	4.8	-	6.2	6.2	1.29
Osmundson et al. 2007	3.7	-	5.2	5.2	1.41
Osmundson et al. 2007	3.7	-	6.5	6.5	1.76
Osmundson et al. 2007	8.4	-	7.7	7.7	0.92
Osmundson et al. 2007	9.4	-	5.8	5.8	0.62
Osmundson et al. 2007	15.5	-	10.9	10.9	0.70
Osmundson et al. 2007	23.6	-	11.2	11.2	0.47
Osmundson et al. 2007	9.4	-	9.4	9.4	1.00
Osmundson et al. 2007	6.1	-	5.4	5.4	0.89
Osmundson et al. 2007	4.6	-	5.1	5.1	1.11
Osmundson et al. 2007	12.3	-	10.4	10.4	0.85
Osmundson et al. 2007	9.2	-	10.4	10.4	1.13
Osmundson et al. 2007	9.4	-	11	11	1.17
Osmundson et al. 2007	9.4	-	11.7	11.7	1.24
Osmundson et al. 2007	10.5	-	11.6	11.6	1.10
Osmundson et al. 2007	11.4	-	9.4	9.4	0.82
Osmundson et al. 2007	9.6	-	10.2	10.2	1.06
Osmundson et al. 2007	9.3	-	7.3	7.3	0.78
Osmundson et al. 2007	9.8	-	8.9	8.9	0.91
Osmundson et al. 2007	10.5	-	10.5	10.5	1.00

White Sucker (*Catostomus commersonii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	11.1	-	10.2	10.2	0.92
Osmundson et al. 2007	12.1	-	8.1	8.1	0.67
Osmundson et al. 2007	12.8	-	9.5	9.5	0.74
Osmundson et al. 2007	16.0	-	10.7	10.7	0.67
Osmundson et al. 2007	12.1	-	8.3	8.3	0.69
Osmundson et al. 2007	9.0	-	12	12	1.33
Osmundson et al. 2007	10.6	-	6.1	6.1	0.58
Osmundson et al. 2007	12.6	-	6.1	6.1	0.48
Osmundson et al. 2007	11.6	-	11.3	11.3	0.97
Osmundson et al. 2007	2.8	-	2.6	2.6	0.93
Osmundson et al. 2007	2.5	-	3.6	3.6	1.44
Osmundson et al. 2007	4.3	-	4.4	4.4	1.02
Osmundson et al. 2007	3.5	-	4.4	4.4	1.26
Osmundson et al. 2007	4.3	-	4.8	4.8	1.12
Osmundson et al. 2007	3.1	-	4.3	4.3	1.39
Osmundson et al. 2007	3.6	-	4.1	4.1	1.14
Osmundson et al. 2007	3.0	-	3.8	3.8	1.27
Osmundson et al. 2007	4.1	-	3.6	3.6	0.88
Osmundson et al. 2007	3.6	-	3.8	3.8	1.06



Median ratio: 1.00

R^2: 0.59
F: 53.92
df: 38
P: < 0.001

Table B-3. Summary of egg-ovary to muscle conversion factors.

Common name	Scientific name	Median ratio
Bluegill	*Lepomis macrochirus*	1.38
Bluehead sucker	*Catostomus discobolus*	1.48
Brook trout	*Salvelinus fontinalis*	1.09
Common carp	*Cyprinus carpio*	1.14
Cutthroat trout	*Oncorhynchus clarkii*	1.81
Dolly Varden	*Salvelinus malma*	1.26
Flannelmouth sucker	*Catostomus latipinnis*	1.08
Green sunfish	*Lepomis cyanellus*	1.21
Mountain whitefish	*Prosopium williamsoni*	5.80
Northern pike	*Esox lucius*	1.88
Rainbow trout	*Oncorhynchus mykiss*	1.92
Razorback sucker	*Xyrauchen texanus*	2.31
Roundtail chub	*Gila robusta*	2.04
Smallmouth bass	*Micropterus dolomieu*	1.19
White sturgeon	*Acipenser transmontanus*	1.33
White sucker	*Catostomus commersonii*	1.00

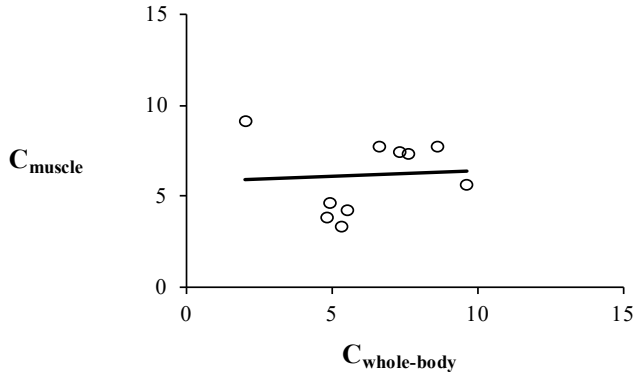
2.4 Muscle to whole-body conversion factors

$C_{whole\text{-}body}$ = Selenium concentration in all tissues (µg/g dw)
C_{muscle} = Selenium concentration in muscle tissue only (µg/g dw)

$$\text{Ratio} = \frac{C_{muscle}}{C_{whole-body}}$$

Black bullhead (*Ameiurus melas*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	5.30	3.40	0.64
Osmundson et al. 2007	4.80	3.90	0.81
Osmundson et al. 2007	5.50	4.30	0.78
Osmundson et al. 2007	4.90	4.70	0.96
Osmundson et al. 2007	9.60	5.70	0.59
Osmundson et al. 2007	7.60	7.40	0.97
Osmundson et al. 2007	7.30	7.50	1.03
Osmundson et al. 2007	6.60	7.80	1.18
Osmundson et al. 2007	8.60	7.80	0.91
Osmundson et al. 2007	2.00	9.20	4.60



Median ratio: 0.93

R^2: 0.00
F: 0.03
df: 8
P: 0.973

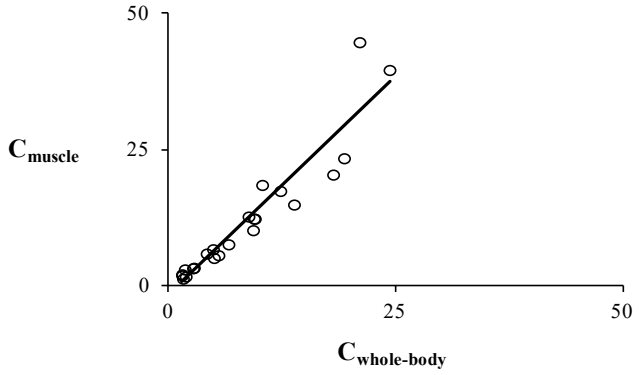
Not used because P > 0.05.

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Doroshov et al. 1992	1.60	1.50	0.94
Doroshov et al. 1992	5.50	5.80	1.05
Doroshov et al. 1992	9.30	10.40	1.12
Doroshov et al. 1992	19.30	23.60	1.22
Hermanutz et al. 1996	1.50	2.10	1.40
Hermanutz et al. 1996	18.10	20.60	1.14
Hermanutz et al. 1996	1.90	1.90	1.00
Hermanutz et al. 1996	2.80	3.50	1.25

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Hermanutz et al. 1996	12.30	17.60	1.43
Hermanutz et al. 1996	9.40	12.50	1.33
Hermanutz et al. 1996	1.50	2.30	1.53
Hermanutz et al. 1996	4.90	6.90	1.41
Hermanutz et al. 1996	21.00	44.90	2.14
Hermanutz et al. 1996	24.30	39.80	1.64
Hermanutz et al. 1996	2.70	3.40	1.26
Hermanutz et al. 1996	5.00	5.30	1.06
Hermanutz et al. 1996	9.50	12.50	1.32
Hermanutz et al. 1996	6.60	7.80	1.18
Hermanutz et al. 1996	1.80	3.20	1.78
Hermanutz et al. 1996	4.20	6.10	1.45
Hermanutz et al. 1996	10.30	18.70	1.82
Hermanutz et al. 1996	13.80	15.10	1.09
Osmundson et al. 2007	8.80	12.90	1.47

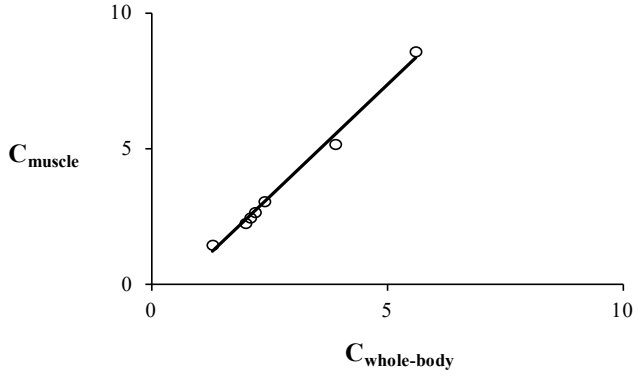


Median ratio: 1.32

R^2: 0.89
F: 172.2
df: 21
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	1.30	1.50	1.15
Osmundson et al. 2007	2.00	2.30	1.15
Osmundson et al. 2007	2.10	2.50	1.19
Osmundson et al. 2007	2.20	2.70	1.23
Osmundson et al. 2007	2.40	3.10	1.29
Osmundson et al. 2007	3.90	5.20	1.33
Osmundson et al. 2007	5.60	8.60	1.54

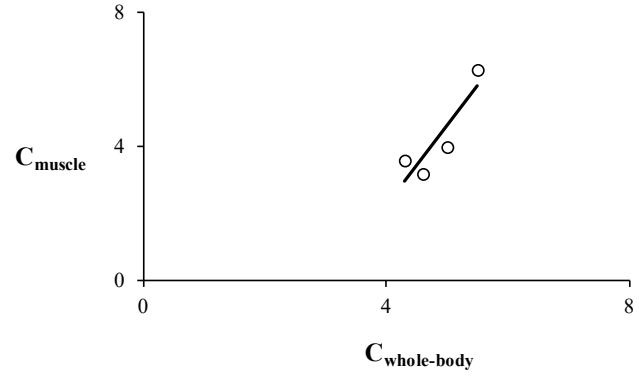


Median ratio: 1.23

R^2: 0.99
F: 682.9
df: 5
P: <0.001

Brown trout (*Salmo trutta*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.60	3.20	0.70
Osmundson et al. 2007	4.30	3.60	0.84
Osmundson et al. 2007	5.00	4.00	0.80
Osmundson et al. 2007	5.50	6.30	1.15



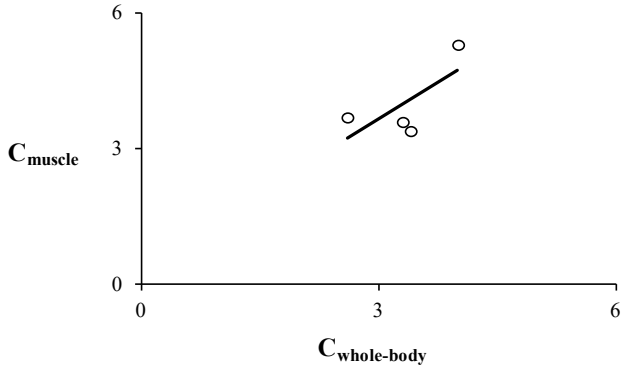
Median ratio: 0.82

R^2: 0.78
F: 7.2
df: 2
P: 0.122

Not used because P > 0.05.

Channel catfish (*Ictalurus punctatus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.40	3.40	1.00
Osmundson et al. 2007	3.30	3.60	1.09
Osmundson et al. 2007	2.60	3.70	1.42
Osmundson et al. 2007	4.00	5.30	1.33



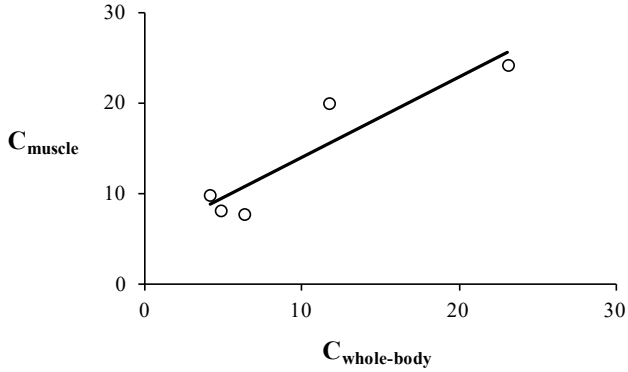
Median ratio: 1.21

R^2: 0.49
F: 2.0
df: 2
P: 0.338

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	6.30	7.80	1.24
Osmundson et al. 2007	4.80	8.20	1.71
Osmundson et al. 2007	11.70	20.00	1.71
Osmundson et al. 2007	23.10	24.20	1.05
Osmundson et al. 2007	4.10	6.60	1.61

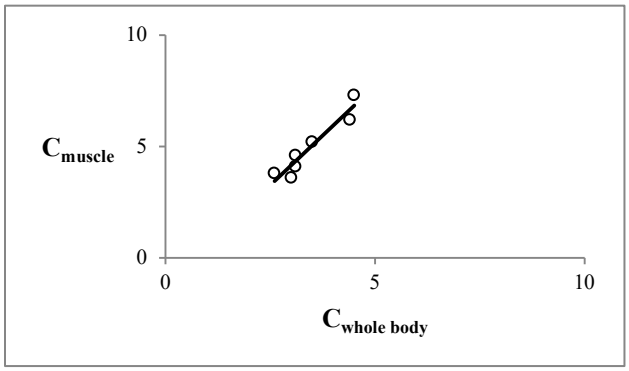


Median ratio: 1.61

R^2: 0.85
F: 17.6
df: 3
P: 0.017

Flannelmouth sucker (*Catostomus latipinnis*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.0	3.6	1.20
Osmundson et al. 2007	2.6	3.8	1.46
Osmundson et al. 2007	3.1	4.1	1.32
Osmundson et al. 2007	3.1	4.6	1.48
Osmundson et al. 2007	3.5	5.2	1.49
Osmundson et al. 2007	4.4	6.2	1.41
Osmundson et al. 2007	4.5	7.3	1.62



Median ratio: 1.46

R^2: 0.91
F: 50.1
df: 5
P: <0.001

Green sunfish (*Lepomis cyanellus*)

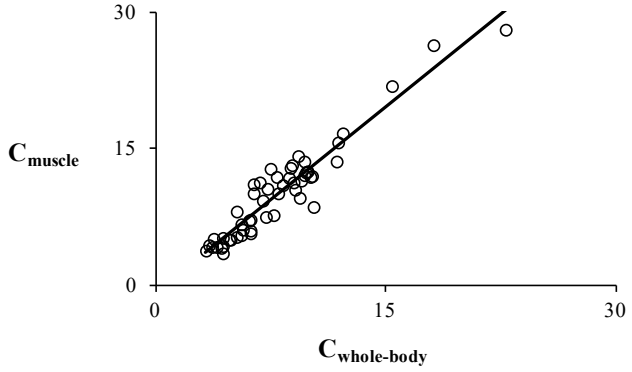
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	22.80	28.10	1.23
Osmundson et al. 2007	8.80	12.90	1.47
Osmundson et al. 2007	15.40	21.90	1.42
Osmundson et al. 2007	4.80	5.00	1.04
Osmundson et al. 2007	5.70	6.10	1.07
Osmundson et al. 2007	4.40	5.20	1.18
Osmundson et al. 2007	3.80	5.10	1.34
Osmundson et al. 2007	11.90	15.70	1.32
Osmundson et al. 2007	6.40	10.10	1.58
Osmundson et al. 2007	9.50	11.50	1.21
Osmundson et al. 2007	9.10	10.50	1.15
Osmundson et al. 2007	6.20	7.20	1.16
Osmundson et al. 2007	7.00	9.30	1.33
Osmundson et al. 2007	7.70	7.70	1.00
Osmundson et al. 2007	6.20	6.00	0.97
Osmundson et al. 2007	10.20	12.00	1.18
Osmundson et al. 2007	9.70	12.10	1.25
Osmundson et al. 2007	9.90	12.50	1.26
Osmundson et al. 2007	7.20	7.50	1.04
Osmundson et al. 2007	9.00	11.30	1.26

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	9.70	13.60	1.40
Osmundson et al. 2007	8.90	13.20	1.48
Osmundson et al. 2007	9.80	12.40	1.27
Osmundson et al. 2007	9.90	12.50	1.26
Osmundson et al. 2007	10.30	8.60	0.83
Osmundson et al. 2007	5.30	5.30	1.00
Osmundson et al. 2007	10.10	11.90	1.18
Osmundson et al. 2007	11.80	13.60	1.15
Osmundson et al. 2007	3.30	3.80	1.15
Osmundson et al. 2007	4.00	4.20	1.05
Osmundson et al. 2007	4.30	4.10	0.95
Osmundson et al. 2007	3.70	4.20	1.14
Osmundson et al. 2007	6.20	5.70	0.92
Osmundson et al. 2007	3.50	4.40	1.26
Osmundson et al. 2007	4.40	3.50	0.80
Osmundson et al. 2007	5.60	5.50	0.98
Osmundson et al. 2007	4.90	5.00	1.02
Osmundson et al. 2007	4.40	4.30	0.98
Osmundson et al. 2007	8.00	10.10	1.26
Osmundson et al. 2007	7.90	11.90	1.51
Osmundson et al. 2007	6.40	11.10	1.73
Osmundson et al. 2007	8.70	11.80	1.36
Osmundson et al. 2007	8.30	11.00	1.33
Osmundson et al. 2007	6.10	7.10	1.16
Osmundson et al. 2007	5.60	6.70	1.20
Osmundson et al. 2007	18.10	26.40	1.46
Osmundson et al. 2007	9.40	9.60	1.02
Osmundson et al. 2007	12.20	16.70	1.37
Osmundson et al. 2007	5.30	8.10	1.53
Osmundson et al. 2007	7.30	10.60	1.45
Osmundson et al. 2007	9.30	14.20	1.53
Osmundson et al. 2007	6.80	11.30	1.66
Osmundson et al. 2007	7.50	12.80	1.71

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio

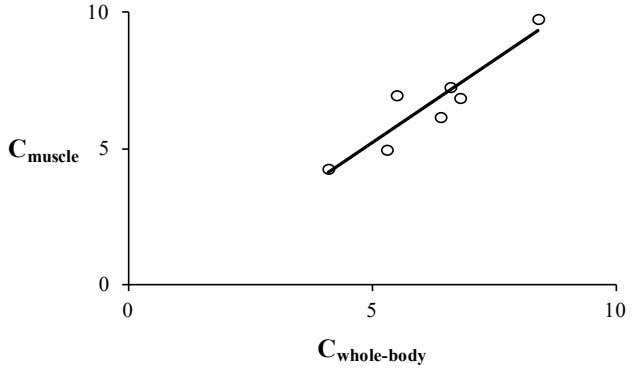


Median ratio: 1.23

R^2: 0.91
F: 501.6
df: 51
P: < 0.001

Roundtail chub (*Gila robusta*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.10	4.30	1.05
Osmundson et al. 2007	5.30	5.00	0.94
Osmundson et al. 2007	6.40	6.20	0.97
Osmundson et al. 2007	6.80	6.90	1.01
Osmundson et al. 2007	5.50	7.00	1.27
Osmundson et al. 2007	6.60	7.30	1.11
Osmundson et al. 2007	8.40	9.80	1.17

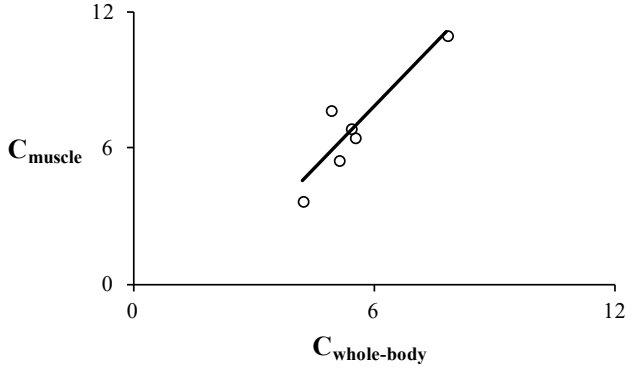


Median ratio: 1.05

R^2: 0.86
F: 29.6
df: 5
P: 0.002

Smallmouth bass (*Micropterus dolomieu*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.20	3.70	0.88
Osmundson et al. 2007	5.50	6.50	1.18
Osmundson et al. 2007	5.40	6.90	1.28
Osmundson et al. 2007	7.80	11.0	1.41
Osmundson et al. 2007	5.10	7.10	1.08
Osmundson et al. 2007	4.90	8.80	1.57



Median ratio: 1.23

R^2: 0.83
F: 20.2
df: 4
P: 0.008

White sucker (*Catostomus commersonii*)

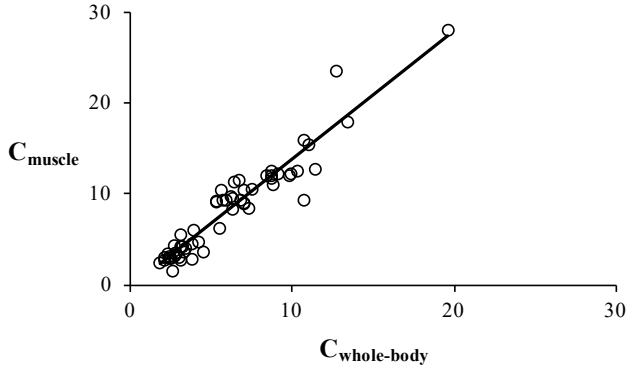
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.80	2.90	0.76
Osmundson et al. 2007	4.20	4.80	1.14
Osmundson et al. 2007	3.30	3.70	1.12
Osmundson et al. 2007	4.50	3.70	0.82
Osmundson et al. 2007	6.30	8.40	1.33
Osmundson et al. 2007	6.80	9.40	1.38
Osmundson et al. 2007	11.00	15.50	1.41
Osmundson et al. 2007	12.70	23.60	1.86
Osmundson et al. 2007	5.70	9.40	1.65
Osmundson et al. 2007	3.90	6.10	1.56
Osmundson et al. 2007	3.80	4.60	1.21
Osmundson et al. 2007	9.90	12.30	1.24
Osmundson et al. 2007	5.30	9.20	1.74
Osmundson et al. 2007	10.70	9.40	0.88
Osmundson et al. 2007	5.90	9.40	1.59
Osmundson et al. 2007	7.00	10.50	1.50
Osmundson et al. 2007	6.40	11.40	1.78
Osmundson et al. 2007	6.30	9.60	1.52
Osmundson et al. 2007	5.30	9.30	1.75
Osmundson et al. 2007	6.20	9.80	1.58

White sucker (*Catostomus commersonii*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	5.60	10.50	1.88
Osmundson et al. 2007	8.80	11.10	1.26
Osmundson et al. 2007	8.70	12.10	1.39
Osmundson et al. 2007	11.40	12.80	1.12
Osmundson et al. 2007	10.70	16.00	1.50
Osmundson et al. 2007	8.40	12.10	1.44
Osmundson et al. 2007	7.00	9.00	1.29
Osmundson et al. 2007	7.50	10.60	1.41
Osmundson et al. 2007	10.30	12.60	1.22
Osmundson et al. 2007	6.70	11.60	1.73
Osmundson et al. 2007	2.10	2.80	1.33
Osmundson et al. 2007	1.80	2.50	1.39
Osmundson et al. 2007	3.20	4.30	1.34
Osmundson et al. 2007	2.30	3.50	1.52
Osmundson et al. 2007	3.10	4.30	1.39
Osmundson et al. 2007	3.00	3.10	1.03
Osmundson et al. 2007	2.80	3.60	1.29
Osmundson et al. 2007	2.50	3.00	1.20
Osmundson et al. 2007	3.40	4.10	1.21
Osmundson et al. 2007	2.80	3.60	1.29
Osmundson et al. 2007	3.10	5.60	1.81
Osmundson et al. 2007	5.50	6.30	1.15
Osmundson et al. 2007	7.00	9.10	1.30
Osmundson et al. 2007	7.30	8.50	1.16
Osmundson et al. 2007	2.40	3.00	1.25
Osmundson et al. 2007	2.70	4.40	1.63
Osmundson et al. 2007	2.70	3.20	1.19
Osmundson et al. 2007	2.60	1.60	0.62
Osmundson et al. 2007	19.60	28.10	1.43
Osmundson et al. 2007	9.80	12.10	1.23
Osmundson et al. 2007	8.70	11.80	1.36
Osmundson et al. 2007	8.70	12.60	1.45
Osmundson et al. 2007	9.10	12.30	1.35
Osmundson et al. 2007	13.40	18.00	1.34
Osmundson et al. 2007	3.10	2.80	0.90
Osmundson et al. 2007	2.40	3.20	1.33
Osmundson et al. 2007	2.10	3.10	1.48
Osmundson et al. 2007	3.20	4.30	1.34
Osmundson et al. 2007	2.80	3.40	1.21

White sucker (*Catostomus commersonii*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio



Median ratio: 1.34

R^2: 0.91
F: 561.3
df: 57
P: < 0.001

Table B-4. Muscle to whole-body correction factor.

Common name	Scientific name	Median ratio
Bluegill	*Lepomis macrochirus*	1.32
Bluehead sucker	*Catostomus discobolus*	1.23
Common carp	*Cyprinus carpio*	1.61
Flannelmouth sucker	*Catostomus latipinnis*	1.46
Green sunfish	*Lepomis cyanellus*	1.23
Roundtail chub	*Gila robusta*	1.05
Smallmouth bass	*Micropterus dolomieu*	1.23
White sucker	*Catostomus commersonii*	1.34

Table B-5. Directly calculated final egg-ovary to whole body conversion factors (*CF*).

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Species				
Bluegill	2.13			2.13
Bluehead sucker	1.82			1.82
Brook trout		1.09	1.27	1.38
Brown trout	1.45			1.45
Common carp	1.92			1.92
Creek chub	1.99			1.99
Cutthroat trout	1.96			1.96

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Desert pupfish	1.20			1.20
Dolly Varden		1.26	1.27	1.61
Fathead minnow	1.40			1.40
Flannelmouth sucker	1.41			1.41
Green sunfish	1.45			1.45
Mountain whitefish		5.80	1.27	7.39
Northern pike		1.88	1.27	2.39
Rainbow trout		1.92	1.27	2.44
Razorback sucker		2.31	1.34	3.11
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sturgeon		1.33	1.27	1.69
White sucker	1.38			1.38
Genus				
Catostomus				1.41
Gila				2.07
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				1.96
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				1.95
Salmonidae				1.71
Order				
Cyprinodontiformes				1.20
Perciformes				1.45

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Class				
Actinopterygii				1.45

Table B-6. All EPA-derived egg-ovary to whole body (CF), egg-ovary to muscle, and muscle to whole body conversion factors directly calculated or estimated using taxonomic classification. (See main text for explanation of the taxonomic classification approach).

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
alligator gar	Atractosteus spatula				Lepistosteiformes	Lepisosteidae	Atractosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bigmouth buffalo	Ictiobus cyprinellus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
black bullhead	Ameiurus melas				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
black crappie	Pomoxis nigromaculatus				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
black redhorse	Moxostoma duquesnei				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
blacknose dace	Rhinichthys atratulus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
blue catfish	Ictalurus furcatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bluegill	Lepomis macrochirus	2.13	1.38	1.32	Perciformes	Centrarchidae	Lepomis	2.13	Exact match	1.38	Exact match	1.32	Exact match	2.13	Exact match
bluehead sucker	Catostomus discobolus	1.82	1.48	1.23	Cypriniformes	Catostomidae	Catostomus	1.82	Exact match	1.48	Exact match	1.23	Exact match	1.82	Exact match
brassy minnow	Hybognathus hankinsoni				Cypriniformes	Cyprinidae	Hybognathus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
brook stickleback	Culaea inconstans				Gasterosteiformes	Gasterosteidae	Culaea	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brook trout	Salvelinus fontinalis		1.09		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.09	Exact match	1.27	All fish	1.38	E-O/WB * M/WB
brown bullhead	Ameiurus nebulosus				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brown trout	Salmo trutta	1.45			Salmoniformes	Salmonidae	Salmo	1.45	Exact match	1.81	Family Salmonidae	1.27	All fish	1.45	Exact match
burbot	Lota lota				Gadiformes	Lotidae	Lota	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bullhead					Siluriformes	Ictaluridae		1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
chain pickerel	Esox				Esociformes	Esocidae	Esox	1.45	All fish	1.88	Genus Esox	1.27	All fish	2.39	E-O/WB * M/WB
channel catfish	Ictalurus punctatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
common carp	Cyprinus carpio	1.92	1.14	1.61	Cypriniformes	Cyprinidae	Cyprinus	1.92	Exact match	1.14	Exact match	1.61	Exact match	1.92	Exact match
common snook	Centropomus undecimalis				Perciformes	Centropomidae	Centropomus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
crappie	Pomoxis sp.				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
creek chub	Semotilus atromaculatus	1.99			Cypriniformes	Cyprinidae	Semotilus	1.99	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.99	Family Cyprinidae
cutthroat trout	Oncorhynchus clarkii	1.96	1.81		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Exact match	1.81	Exact match	1.27	All fish	1.96	Exact match

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
desert pupfish	Cyprinodon macularius	1.20			Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.20	Exact match	1.35	All fish	1.27	All fish	1.20	Exact match
Dolly Varden	Salvelinus malma		1.26		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.26	Exact match	1.27	All fish	1.61	E-O/WB * M/WB
fathead minnow	Pimephales promelas	1.40			Cypriniformes	Cyprinidae	Pimephales	1.40	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.40	Family Cyprinidae
flannelmouth sucker	Catostomus latipinnis	1.41	1.08	1.46	Cypriniformes	Catostomidae	Catostomus	1.41	Exact match	1.08	Exact match	1.46	Exact match	1.41	Exact match
flathead catfish	Pylodictis				Siluriformes	Ictaluridae	Pylodictus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
flathead chub	Platygobio gracilis				Cypriniformes	Cyprinidae	Platygobio	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
freshwater drum	Aplodinotus grunniens				Perciformes	Sciaenidae	Aplodinotus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
goldeye	Hiodon alosoides				Hiodontiformes	Hiodontidae	Hiodon	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
gizzard shad	Dorosoma cepedianum				Clupeiformes	Clupeidae	Dorosoma	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
green sunfish	Lepomis cyanellus	1.45	1.21	1.23	Perciformes	Centrarchidae	Lepomis	1.45	Exact match	1.21	Exact match	1.23	Exact match	1.45	Exact match
iowa darter	Etheostoma exile				Perciformes	Percidae	Etheostoma	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
Japanese medaka	Oryzias latipes				Beloniformes	Adrianichthyidae	Oryzias	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
kokanee salmon	Oncorhynchus nerka				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
largemouth bass	Micropterus salmoides				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
largescale sucker	Catostomus macrocheilus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
longnose dace	Rhinichthys cataractae				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
longnose sucker	Catostomus catostomus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
mosquitofish	Gambusia sp.				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
mottled sculpin	Cottus bairdi				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
mountain whitefish	Prosopium williamsoni		5.80		Salmoniformes	Salmonidae	Prosopium	1.71	Family Salmonidae	5.80	Exact match	1.27	All fish	7.39	E-O/WB * M/WB
ninespine stickleback	Pungitius pungitius				Gasterosteiformes	Gasterosteidae	Pungitius	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
northern pike	Esox lucius		1.88		Esociformes	Esocidae	Esox	1.45	All fish	1.88	Exact match	1.27	All fish	2.39	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
northern pikeminnow	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern plains killifish	Fundulus kansae				Cyprinodontiformes	Fundulidae	Fundulus	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
northern redbelly dace	Chrosomus eos				Cypriniformes	Cyprinidae	Chrosomus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern squawfish	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
quillback	Carpiodes cyprinus				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rainbow trout	Oncorhynchus mykiss		1.92		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.92	Exact match	1.27	All fish	2.44	E-O/WB * M/WB
razorback sucker	Xyrauchen texanus		2.31		Cypriniformes	Catostomidae	Xyrauchen	1.41	Family Catostomidae	2.31	Exact match	1.34	Family Catostomidae	3.11	E-O/WB * M/WB
red shiner	Cyprinella lutrensis				Cypriniformes	Cyprinidae	Cyprinella	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
redbreast sunfish	Lepomis auritus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redear sunfish	Lepomis microlophus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redside shiner	Richardsonius balteatus				Cypriniformes	Cyprinidae	Richardsonius	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
river carpsucker	Carpiodes carpio				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
river redhorse	Moxostoma carinatum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rock bass	Ambloplites rupestris				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
roundtail chub	Gila robusta	2.07	2.04	1.05	Cypriniformes	Cyprinidae	Gila	2.07	Exact match	2.04	Exact match	1.05	Exact match	2.07	Exact match
sacramento perch	Archoplites interruptus				Perciformes	Centrarchidae	Archoplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
sacramento pikeminnow	Ptychocheilus grandis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sailfin molly	Poecilia latipinna				Cyprinodontiformes	Poeciliidae	Poecilia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
sand shiner	Notropis stramineus				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sauger	Sander canadensis				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
sculpin	Cottus sp.				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
shadow bass	Ambloplites ariommus				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
shorthead redhorse	Moxostoma macrolepidotum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
silver carp	Hypophthalmichthys molitrix				Cypriniformes	Cyprinidae	Hypophthalmichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
smallmouth bass	Micropterus dolomieu	1.42	1.19	1.23	Perciformes	Centrarchidae	Micropterus	1.42	Exact match	1.19	Exact match	1.23	Exact match	1.42	Exact match
smallmouth buffalo	Ictiobus bubalus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
speckled dace	Rhinichthys osculus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spottail shiner	Notropis hudsonius				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spotted bass	Micropterus punctulatus				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
spotted gar	Lepisosteus oculatus				Lepistosteiformes	Lepisosteidae	Lepisosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
stonecat	Noturus flavus				Siluriformes	Ictaluridae	Noturus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
striped bass	Morone saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
striped mullet	Mugil cephalus				Mugiliformes	Mugilidae	Mugil	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
sucker					Cypriniformes	Catostomidae		1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
tilapia					Perciformes	Cichlidae		1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
trout species	Oncorhynchus sp.				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
tui chub	Gila bicolor				Cypriniformes	Cyprinidae	Gila	2.07	Genus Gila	2.04	Genus Gila	1.05	Genus Gila	2.07	Genus Gila
utah sucker	Catostomus ardens				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
walleye	Sander vitreus				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
western mosquitofish	Gambusia affinis				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
white bass	Morone chrysops				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
white crappie	Pomoxis annularis				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
white sturgeon	Acipenser transmontanus		1.33		Acipenseriformes	Acipenseridae	Acipenser	1.45	All fish	1.33	Exact match	1.27	All fish	1.69	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
white sucker	Catostomus commersonii	1.38	1.00	1.34	Cypriniformes	Catostomidae	Catostomus	1.38	Exact match	1.00	Exact match	1.34	Exact match	1.38	Exact match
wiper	Morone chrysops x Moron saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
yellow bullhead	Ameiurus natalis				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
yellow perch	Perca flavescens				Perciformes	Percidae	Perca	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes

3.0 DERIVATION OF TROPHIC TRANSFER FACTOR VALUES

3.1 Methodology

Taxa specific trophic transfer factors (TTF) to quantify the degree of biomagnification across a given trophic level were calculated from either physiological parameters measured in laboratory studies or from field measurements of paired selenium concentrations in consumer species and their food. TTFs from both approaches were used to calculate translated water concentrations; however, when TTF data of similar quality are available from both approached, as was the case with bluegill, field-derived TTF data are used.

Physiological data consisted of assimilation efficiencies (AE), measured as either a percentage or a proportion, ingestion rates (IR), measured as grams of Se per grams of food consumed per day, and efflux rate constant (k_e), measured as 1/day. All available data were collected for a particular species, and then the TTF for that species was calculated using the equation:

$$\text{TTF} = \frac{AE \; x \; IR}{k_e}$$

Where AE, IR, and K_e were estimated as the median value of all available data for that parameter for that species.

The majority of TTF were calculated using paired whole-body Se measurements from organisms collected at the same site in the field. TTFs for trophic level 2 organisms were determined using the equation:

$$TTF^{TL2} = \frac{C_{tissue}^{TL2}}{C_{food}^{TL2}}$$

Where C_{food}^{TL2} equals the average Se concentration in particulate matter, defined as the average of C_{algae}, $C_{detritus}$, and $C_{sediment}$. Of the three types of particulate matter potentially assumed by TL2 organisms (e.g., the majority of invertebrates), $C_{sediment}$ correlated relatively poorly to C_{tissue}^{TL2}, when compared to C_{algae} and $C_{detritus}$. In order to minimize potentially erroneous TTF calculations based solely on sediment Se concentrations, while note completely discounting the importance of organic matter in sediments as a potential food source, $C_{sediment}$ was included in $C_{particulate}$ calculations only when either C_{algae} or $C_{detritus}$ data were also available.

TTFs for trophic level 3 organisms were determined using the equation:

$$TTF^{TL3} = \frac{C_{tissue}^{TL3}}{C_{food}^{TL3}}$$

Where C_{food}^{TL3} equals the average whole-body Se concentration in invertebrates collected at the same site as their potential predator species. The majority of trophic level 3 organisms are fish species, but damselflies and dragonflies of the order Odonata are also trophic level 3 organisms, and TTF^{TL3} values were calculated for those species as well.

For all field derived data used to determine TTFs, EPA first confirmed a statistical relationship between whole-body selenium concentrations for each species and its food using OLS linear regression. If the regression resulted in a statistically significant ($P<0.05$) positive slope, EPA calculated the TTF as the median ratio of the paired concentration data.

3.2 TTF values from physiological coefficients

AE (%) = Assimilation efficiency
IR ($g\ g^{-1}\ d^{-1}$) = Ingestion rate
k_e (d^{-1}) = Efflux rate constant
TTF = $\frac{AE\ x\ IR}{K_e}$

3.2.1 Invertebrates

Baltic macoma (*Macoma balthica*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
22.5				Luoma et al. 1992
91.0				Luoma et al. 1992
84.0				Luoma et al. 1992
95.0				Luoma et al. 1992
78.0		0.03		Reinfelder et al. 1997
74.0		0.03		Reinfelder et al. 1997
92.3				Schleckat et al. 2002
58.0				Schleckat et al. 2002
85.8				Schleckat et al. 2002
64.9				Schleckat et al. 2002
90.4				Schleckat et al. 2002
Median Values and TTF				
84.0	0.27[a]	0.03	7.56	

[a] Value taken from *Mytilus edulis*

Short-necked clam (*Ruditapes philippinarum*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
70.0		0.013		Zhang et al. 1990
52.0		0.013		Zhang et al. 1990
Median Values and TTF				
61.0	0.27[a]	0.013	12.67	

[a] Value taken from *Mytilus edulis*

Quahog (*Mercenaria mercenaria*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
100.1				Reinfelder and Fisher 1994
92.0		0.01		Reinfelder et al. 1997
Median Values and TTF				
96.1	0.27[a]	0.01	25.93	

[a] Value taken from *Mytilus edulis*

Eastern Oyster (*Crassostrea virginica*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.005		Okazaki and Panietz 1981
105.4				Reinfelder and Fisher 1994
70.0		0.070		Reinfelder et al. 1997
Median Values and TTF				
87.7	0.27a	0.038	6.31	

[a] Value taken from *Mytilus edulis*

Common mussel (*Mytilus edulis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
86.0		0.02		Reinfelder et al. 1997
75.0		0.05		Reinfelder et al. 1997
60.7				Wang and Fisher 1996
48.0				Wang and Fisher 1996
13.7				Wang and Fisher 1996
55.1				Wang and Fisher 1996
55.8				Wang and Fisher 1996
71.9				Wang and Fisher 1996
71.5				Wang and Fisher 1996
27.9				Wang and Fisher 1996
84.4				Wang and Fisher 1996
81.0				Wang and Fisher 1996
79.4				Wang and Fisher 1996

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
63.0		0.037		Wang and Fisher 1996
61.5		0.05		Wang and Fisher 1996
69.0		0.027		Wang and Fisher 1996
81.0		0.022		Wang and Fisher 1997
82.0		0.020		Wang and Fisher 1997
72.0		0.018		Wang and Fisher 1997
78.0		0.055		Wang et al. 1995
76.0		0.065		Wang et al. 1995
71.0		0.058		Wang et al. 1995
33.9				Wang et al. 1996
27.5				Wang et al. 1996
				Wang et al. 1996
				Wang et al. 1996
	0.27	0.022		Wang et al. 1996
		0.026		Wang et al. 1996
		0.019		Wang et al. 1996
Median Values and TTF				
71.3	0.27	0.026	7.30	

Asian clam (*Corbicula fluminea*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.05	0.006		Lee et al. 2006
Median Values and TTF				
55.0	0.05	0.006	4.58	

Zebra mussel (*Dreissena polymorpha*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
18.0				Roditi and Fisher 1999
24.0				Roditi and Fisher 1999
46.0				Roditi and Fisher 1999
40.0				Roditi and Fisher 1999
41.0				Roditi and Fisher 1999
7.7				Roditi and Fisher 1999
23.0				Roditi and Fisher 1999
28.0				Roditi and Fisher 1999
	0.40			Roditi and Fisher 1999
		0.026		Roditi and Fisher 1999
Median Values and TTF				
26.0	0.40	0.026	4.00	

Water flea (*Daphnia magna*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
	0.08			Goulet et al. 2007
	0.34			Goulet et al. 2007
57.9				Yu and Wang 2002b
43.0				Yu and Wang 2002b
39.8				Yu and Wang 2002b
33.0				Yu and Wang 2002b
41.4				Yu and Wang 2002b
41.5				Yu and Wang 2002b
38.0				Yu and Wang 2002b
24.5				Yu and Wang 2002b
		0.101		Yu and Wang 2002b
		0.12		Yu and Wang 2002b
		0.131		Yu and Wang 2002b
		0.134		Yu and Wang 2002b
		0.108		Yu and Wang 2002b
		0.112		Yu and Wang 2002b
Median Values and TTF				
40.6	0.21	0.12	0.74	

Copepod (*Temora longicornis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.42	0.115		Wang and Fisher 1998
Median Values and TTF				
55.0	0.42	0.115	2.01	

Copepod (Small, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.35	

Copepod (Large, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
52.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.41	

Blackworm (*Lumbriculus variegatus*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.009		Riedel and Cole 2001
		0.006		Riedel and Cole 2001
24.0	0.067	0.013		Riedel and Cole 2001
9.0	0.067	0.009		Riedel and Cole 2001
Median Values and TTF				
16.5	0.067	0.0086	1.29	

Mayfly (*Centroptilum triangulifer*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
38.0	0.72	0.25		Riedel and Cole 2001
40.0	0.72	0.19		Riedel and Cole 2001
Median Values and TTF				
39.0	0.72	0.22	1.28	

a – not used because field TTF data available

3.2.2 Vertebrates

Bluegill (*Lepomis macrochirus*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
34.0				Besser et al. 1993
22.0				Besser et al. 1993
24.0				Besser et al. 1993
36.0				Besser et al. 1993
30.0				Besser et al. 1993
32.0				Besser et al. 1993
43.0				Besser et al. 1993
40.0				Besser et al. 1993
37.0		0.041		Besser et al. 1993
		0.031		Besser et al. 1993
		0.034		Besser et al. 1993
36.0		0.031		Besser et al. 1993
		0.038		Besser et al. 1993
		0.038		Besser et al. 1993
	0.008			Whitledge and Haywood 2000
	0.042			Whitledge and Haywood 2000
Median Values and TTF				
35.0	0.025	0.036	1.156[a]	

[a] Not used because of availability of acceptable field-based TTF data

Fathead Minnow (*Pimephales promelas*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0				Presser and Luoma 2010
	0.050			Bertram and Brooks 1986
		0.029		Bertram and Brooks 1986
		0.019		Bertram and Brooks 1986
		0.3		Bertram and Brooks 1986
		0.014		Bertram and Brooks 1986
		0.013		Bertram and Brooks 1986
		0.016		Bertram and Brooks 1986
		0.012		Bertram and Brooks 1986
		0.026		Bertram and Brooks 1986
		0.018		Bertram and Brooks 1986
		0.025		Bertram and Brooks 1986
Median Values and TTF				
50.0	0.050	0.0185	1.35	

Striped Bass (*Morone saxatilis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
33	0.17	0.09		Baines et al. 2002
42	0.5	0.08		Baines et al. 2002
	0.12			Buckel and Stoner 2004
	0.16			Buckel and Stoner 2004
	0.11			Buckel and Stoner 2004
	0.08			Buckel and Stoner 2004
Median Values and TTF				
37.5	0.335	0.085	1.48	

TTF calculated from only Baines et al. (2002) because it had complete data.

3.3 TTF values from field data

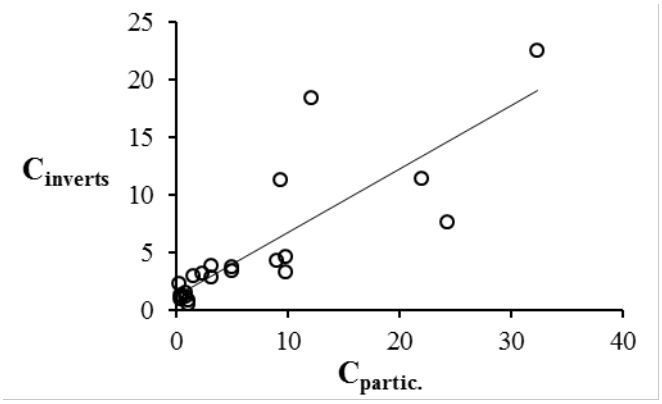
3.3.1 Invertebrates

C_{alg} = Selenium concentration in algae (mg/kg)
C_{det} = Selenium concentration in detritus (mg/kg)
C_{sed} = Selenium concentration in sediment (mg/kg)
C_{invert} = Selenium concentration in invertebrate tissue (mg/kg)
C_{part} = Average selenium concentration in particulate material $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$
Ratio = $\frac{C_{invert}}{C_{part}}$

Scuds (Amphipoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	18.40	1.52
Birkner 1978	20	3.00		41.00	22.00	11.40	0.52
Birkner 1978	7	0.18		2.80	1.49	2.90	1.95
Birkner 1978	19	16.80		1.20	9.00	4.30	0.48
Birkner 1978	30	17.30		47.30	32.30	22.50	0.70
Birkner 1978	3	0.10		0.30	0.20	2.30	11.50
Birkner 1978	22	4.60		44.00	24.30	7.60	0.31
Birkner 1978	23	7.80		10.80	9.30	11.30	1.22
Lambing et al. 1994	S46	2.30			2.30	3.20	1.39
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.44	0.40
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.86	0.79
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.60	0.47
Saiki et al. 1993	GT5	4.50	14.95		9.73	3.30	0.34
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.40	0.69
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.70	0.76
Saiki et al. 1993	SJR2	1.25	5.00		3.13	3.80	1.22
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.80	0.90
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.10	1.30
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.89	2.47
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42

Scuds (Amphipoda)

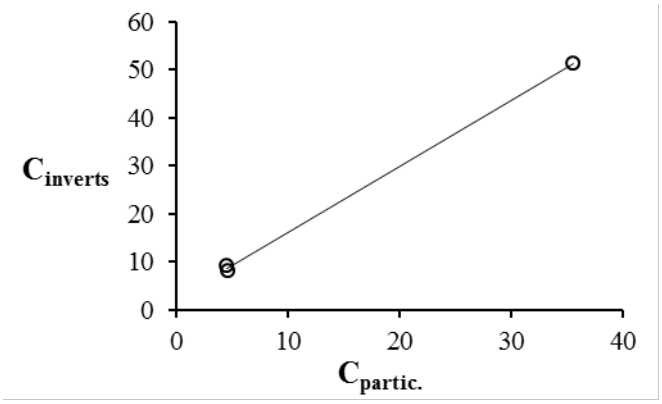


Median ratio: 1.22

R^2: 0.69
F: 46.9
df: 21
P: < 0.001

Earthworms and Leeches (Annelida)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Lemly 1985	Badin Lake	8.20		0.91	4.56	8.10	1.78
Lemly 1985	Belews Lake	62.70		8.27	35.49	51.15	1.44
Lemly 1985	High Rock Lake	8.25		0.79	4.52	9.05	2.00



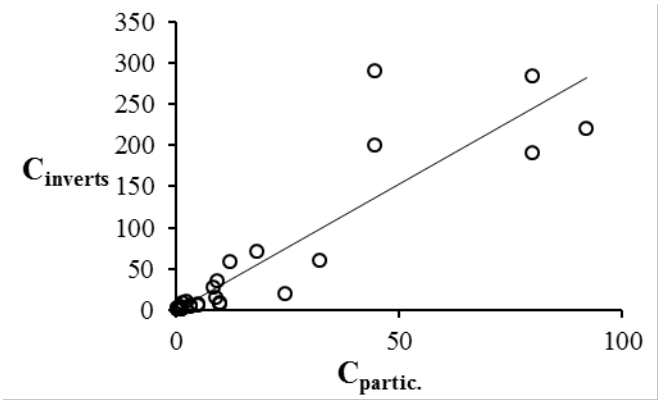
Median ratio: 1.78

R^2: 1.00
F: 2426
df: 1
P: < 0.001

Midges (Chironomidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	58.20	4.81
Birkner 1978	19	16.80		1.20	9.00	15.30	1.70
Birkner 1978	30	17.30		47.30	32.30	59.30	1.84
Birkner 1978	3	0.10		0.30	0.20	2.50	12.50
Birkner 1978	22	4.60		44.00	24.30	18.80	0.77
Birkner 1978	27	10.35		6.50	8.43	26.70	3.17
Birkner 1978	12	2.30		0.30	1.30	7.70	5.92
Birkner 1978	23	7.80		10.80	9.30	34.20	3.68
Grasso et al. 1995	17	1.87		0.40	1.14	2.07	1.82
Lambing et al. 1994	S46	2.30			2.30	9.70	4.22
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	71.00	3.91
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	200.0	4.48
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	290.0	6.49
Saiki and Lowe 1987	Kesterson Pond 8	136.5	92.00	6.05	92.00	220.0	2.39
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	190.0	2.38
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	284.0	3.55
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.74	4.18
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.30	3.13
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	3.00	3.37
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.30	1.46
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.58	0.53
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.00	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	8.90	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	7.20	0.74
Saiki et al. 1993	GT4	1.39	8.40		4.90	5.40	1.10
Saiki et al. 1993	GT4	1.39	8.40		4.90	6.90	1.41
Saiki et al. 1993	SJR2	1.25	5.00		3.13	6.00	1.92
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.10	1.31
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.47	1.31
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.00	2.78
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.53	1.16
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.84	1.85

Midges (Chironomidae)

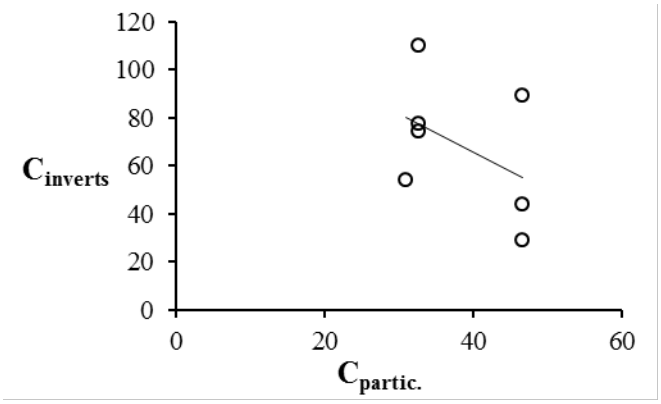


Median ratio: 1.90

R^2: 0.82
F: 144.0
df: 32
P: < 0.001

Beetles (Coleoptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	77.60	2.38
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	74.10	2.27
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	110.00	3.37
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	54.00	1.75
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	89.10	1.92
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	28.80	0.62
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	43.70	0.94



Median ratio: 1.92

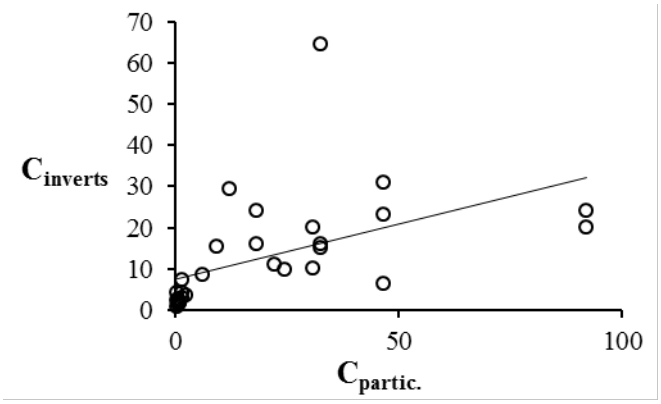
R^2: 0.20
F: 1.24
df: 5
P: 0.36
Not used because P > 0.05 and negative slope.

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	18	7.60		4.30	5.95	8.40	1.41
Birkner 1978	29	8.80		15.40	12.10	29.40	2.43

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	4.20	2.82
Birkner 1978	3	0.10		0.30	0.20	4.20	21.00
Birkner 1978	22	4.60		44.00	24.30	9.90	0.41
Birkner 1978	12	2.30		0.30	1.30	7.30	5.62
Birkner 1978	23	7.80		10.80	9.30	15.50	1.67
Lambing et al. 1994	S46	2.30			2.30	3.40	1.48
Rinella et al. 1994	G	0.84		0.50	0.67	1.38	2.06
Rinella et al. 1994	A	2.21		0.40	1.31	2.98	2.28
Rinella et al. 1994	Q	1.42		0.50	0.96	2.00	2.08
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	24.00	1.32
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	16.00	0.88
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	20.00	0.22
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	24.00	0.26
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	2.15	5.17
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	0.87	2.10
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.76	1.98
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.53	1.72
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.90	0.49
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	64.60	1.98
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.10	0.46
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	20.00	0.65
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	10.00	0.32
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	23.00	0.49
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	30.90	0.66
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	6.46	0.14
Rinella and Schuler 1992	18	0.59			0.59	2.70	4.58



Median ratio: 1.48

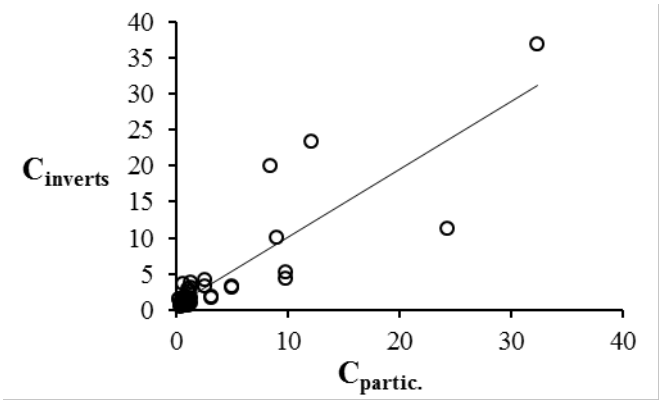
R^2: 0.25
F: 9.17
df: 27
P: < 0.001

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	23.30	1.93
Birkner 1978	19	16.80		1.20	9.00	10.10	1.12
Birkner 1978	30	17.30		47.30	32.30	36.80	1.14
Birkner 1978	22	4.60		44.00	24.30	11.30	0.47
Birkner 1978	27	10.35		6.50	8.43	20.00	2.37
Butler et al. 1993	SP2	1.60		0.50	1.05	2.60	2.48
Butler et al. 1993	SP2	1.60		0.50	1.05	2.90	2.76
Butler et al. 1995	AK	0.45		0.20	0.33	0.76	2.34
Butler et al. 1995	AK	0.45		0.20	0.33	0.79	2.43
Butler et al. 1995	DD	0.88		0.70	0.79	0.62	0.78
Butler et al. 1995	DD	0.88		0.70	0.79	1.10	1.39
Butler et al. 1995	HD1	0.59			0.59	0.86	1.46
Butler et al. 1995	HD1	0.59			0.59	0.79	1.34
Butler et al. 1995	HD2	0.45		0.20	0.32	0.96	2.98
Butler et al. 1995	HD2	0.45		0.20	0.32	1.00	3.10
Butler et al. 1995	ME2	1.11		1.10	1.10	1.10	1.00
Butler et al. 1995	ME2	1.11		1.10	1.10	1.40	1.27
Butler et al. 1995	ME4	1.04		0.50	0.77	1.30	1.69
Butler et al. 1995	ME4	1.04		0.50	0.77	1.80	2.35
Butler et al. 1995	ME3	0.82		0.40	0.61	1.40	2.30
Butler et al. 1995	ME3	0.82		0.40	0.61	3.70	6.07
Butler et al. 1995	NW	3.45		1.60	2.53	4.20	1.66
Butler et al. 1995	NW	3.45		1.60	2.53	3.30	1.31
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.40	6.83
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.50	7.32
Butler et al. 1997	CHK	1.19			1.19	0.90	0.76
Butler et al. 1997	MN2	0.79			0.79	0.83	1.06
Butler et al. 1997	MUD2	1.30			1.30	3.10	2.38
Butler et al. 1997	MUD2	1.30			1.30	3.80	2.92
Butler et al. 1997	TRH	1.25			1.25	0.98	0.78
Butler et al. 1997	TRH	1.25			1.25	1.60	1.28
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.67	0.62
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.83	0.76
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.20	0.53
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.40	0.45
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.10	0.63
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.20	0.65
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.70	0.54
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.90	0.61

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	SJR3	0.45	1.25		0.85	0.77	0.91
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.30	1.53
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.50	1.39
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.74	2.06
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.87	1.91
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.85	1.87

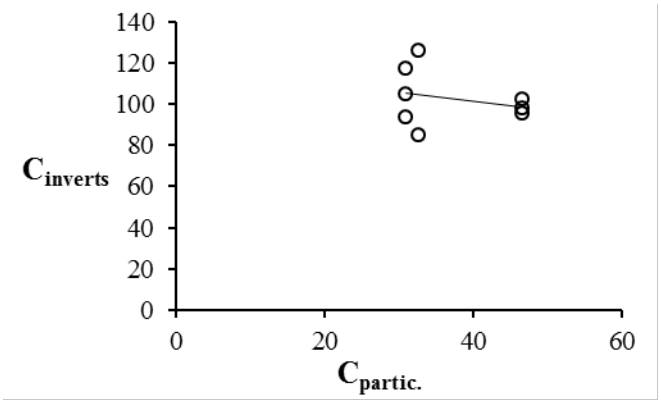


Median ratio: 1.46

R^2: 0.74
F: 130.8
df: 45
P: < 0.001

True flies (Diptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	126.00	3.87
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	85.10	2.61
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	117.00	3.79
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	93.30	3.02
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	105.00	3.40
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	95.50	2.05
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	97.70	2.10
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	102.00	2.19

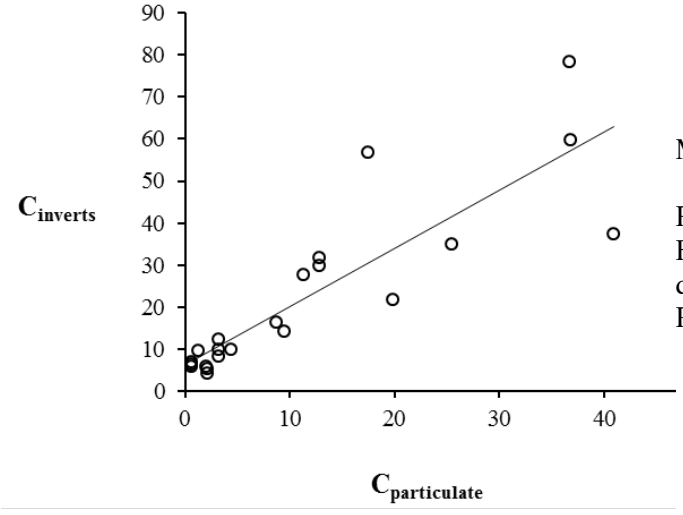


Median ratio: 2.81

R^2: 0.07
F: 0.46
df: 6
P: 0.65
Not used because P > 0.05 and negative slope.

Mayflies (Ephemeroptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Rinella et al. 1994	A	2.21		0.40	1.31	9.65	7.39
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.40	10.67
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	8.20	2.56
Casey 2005	Deerlick Creek		1.00	0.20	0.60	5.70	9.50
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	9.70	3.03
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.80	11.33
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	12.30	3.84
Conley et al. 2009	Plate 10A	4.40			4.40	9.70	2.20
Conley et al. 2009	Plate 20A	25.50			25.50	34.80	1.36
Conley et al. 2009	Plate 20B	17.50			17.50	56.70	3.24
Conley et al. 2009	Plate 20C	8.70			8.70	16.20	1.86
Conley et al. 2009	Plate 20D	11.30			11.30	27.50	2.43
Conley et al. 2009	Plate 5A	2.20			2.20	4.20	1.91
Conley et al. 2009	Plate 5B	2.00			2.00	5.70	2.85
Conley et al. 2011	2x-High	40.90			40.90	37.30	0.91
Conley et al. 2011	2x-Low	9.50			9.50	14.10	1.48
Conley et al. 2011	2x-Medium	19.90			19.90	21.60	1.09
Conley et al. 2013	Control	2.20			2.20	5.10	2.32
Conley et al. 2013	Selenate-high	36.80			36.80	59.80	1.63
Conley et al. 2013	Selenate-low	12.80			12.80	31.70	2.48
Conley et al. 2013	Selenite-high	36.70			36.70	78.40	2.14
Conley et al. 2013	Selenite-low	12.80			12.80	29.80	2.33

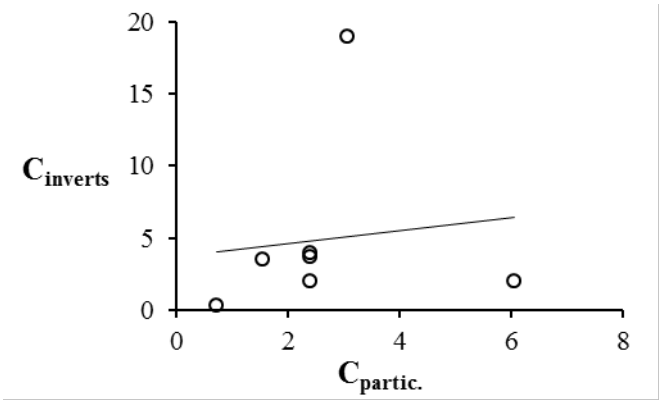


Median ratio: 2.38

R^2: 0.75
F: 59.19
df: 20
P: <0.001

Snails (Gastropoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Butler et al. 1995	WC	3.30		1.50	2.40	3.70	1.54
Butler et al. 1995	WC	3.30		1.50	2.40	3.90	1.63
Butler et al. 1995	WC	3.30		1.50	2.40	2.00	0.83
Butler et al. 1997	DCP1	1.00		2.10	1.55	3.50	2.26
Butler et al. 1997	MNP2	5.40		6.70	6.05	2.00	0.33
Butler et al. 1997	CHP	4.00		2.10	3.05	19.00	6.23
Butler et al. 1997	LCHP1	0.33		1.10	0.72	0.32	0.45



Median ratio: 1.54

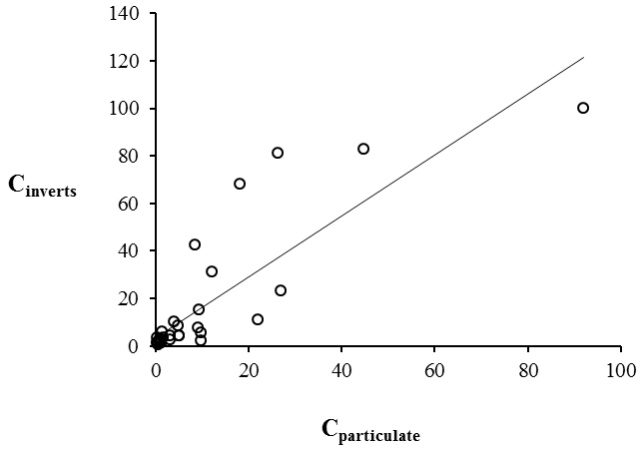
R^2: 0.01
F: 0.07
df: 5
P: 0.93
Not used because P > 0.05.

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	31.30	2.59
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	3.30	2.22
Birkner 1978	19	16.80		1.20	9.00	7.70	0.86
Birkner 1978	3	0.10		0.30	0.20	3.40	17.00
Birkner 1978	27	10.35		6.50	8.43	42.50	5.04
Birkner 1978	12	2.30		0.30	1.30	5.80	4.46
Birkner 1978	23	7.80		10.80	9.30	15.40	1.66
Bowie et al. 1996	Hyco Reservoir	27.0			27.0	23.0	0.85
Lambing et al. 1988	12	1.40		0.30	0.85	2.60	3.06
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	68.30	3.76
Saiki and Lowe 1987	Kesterson Pond 2	152.70	44.65	34.82	44.65	83.00	1.86
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	100.00	1.09
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.46	3.51
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	2.90	3.26
Saiki and Lowe 1987	Volta Wasteway	0.87	2.03	0.24	0.87	2.80	3.21
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.20	1.10
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.50	1.38

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	GT5	4.50	14.95		9.73	2.40	0.25
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.40	0.56
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.50	0.92
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.40	0.90
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.60	0.83
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.30	1.38
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.80	2.12
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.40	3.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.63	1.38
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.40	3.08



Median ratio: 1.89

R^2: 0.71
F: 76.3
df: 31
P: < 0.001

Special case of Odonates (Damselflies and Dragonflies) consuming invertebrates

n = Number of invertebrate food species co-occurring with an Odonate species.

C_{part} = Average selenium concentration in particulate material (mg/kg): $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$

C_{food} = Median selenium concentration in all invertebrate tissues that co-occur with an Odonate species (mg/kg)

C_{damsel} = Selenium concentration in damselfly tissue (mg/kg)

C_{dragon} = Selenium concentration in dragonfly tissue (mg/kg)

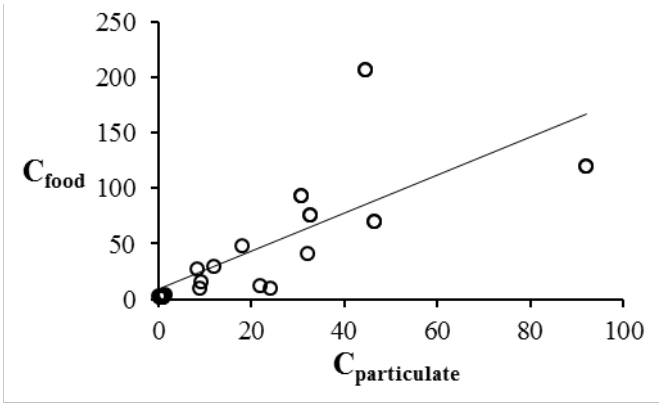
Ratio = $\frac{C_{food}}{C_{part}}$, $\frac{C_{damsel}}{C_{food}}$, or $\frac{C_{dragon}}{C_{food}}$

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Saiki and Lowe 1987	Kesterson Pond 11	dragonflies	4	18.15	47.5	2.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Wasteway	dragonflies	2	0.87	1.83	2.10
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Birkner 1978	29	damselflies	3	12.10	29.4	2.43
Birkner 1978	20	damselflies	2	22.00	11.2	0.51
Birkner 1978	7	damselflies	2	1.49	3.55	2.39
Birkner 1978	19	damselflies	2	9.00	9.8	1.09
Birkner 1978	30	damselflies	2	32.30	40.9	1.27
Birkner 1978	3	damselflies	3	0.20	2.5	12.50
Birkner 1978	22	damselflies	3	24.30	9.9	0.41
Birkner 1978	27	damselflies	1	8.43	26.7	3.17
Birkner 1978	23	damselflies	3	9.30	15.5	1.67
Grasso et al. 1995	17	damselflies	1	1.14	2.07	1.82



Median ratio: 2.21

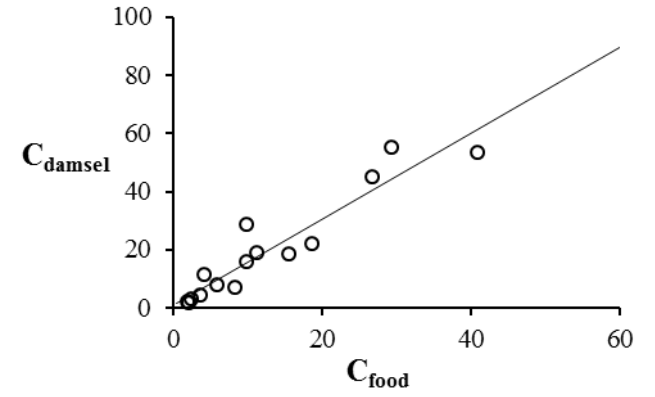
R^2: 0.54
F: 28.7
df: 24
P: < 0.001

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	29	29.4	55	1.87
Birkner 1978	4	1.95	1.8	0.92
Birkner 1978	25	18.7	21.9	1.17
Birkner 1978	20	11.2	18.7	1.67
Birkner 1978	7	3.55	4.4	1.24
Birkner 1978	19	9.8	28.4	2.90
Birkner 1978	6	4.2	11.1	2.64
Birkner 1978	30	40.9	53.3	1.30
Birkner 1978	3	2.5	3.1	1.24
Birkner 1978	22	9.9	15.8	1.60

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	27	26.7	45.1	1.69
Birkner 1978	23	15.5	18.4	1.19
Birkner 1978	11	5.9	7.7	1.31
Grasso et al. 1995	17	2.07	1.75	0.85
Grasso et al. 1995	9	8.2	6.98	0.85



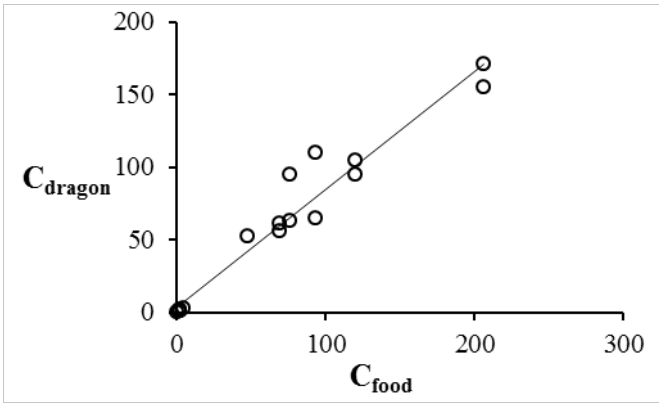
Median ratio: 1.30 x 2.21
(damselfly food to particulate) = 2.88

R^2: 0.89
F: 104.4
df: 13
P: <0.001

Dragonflies (Zygoptera)

Study	Site	C_{food}	C_{dragon}	Ratio
Mason et al. 2000	BK	1.845	1.665	0.90
Mason et al. 2000	HCRT	4.305	2.81	0.65
Saiki and Lowe 1987	Kesterson Pond 11	47.5	53	1.12
Saiki and Lowe 1987	Kesterson Pond 2	206.5	155	0.75
Saiki and Lowe 1987	Kesterson Pond 2	206.5	171	0.83
Saiki and Lowe 1987	Kesterson Pond 8	120	95.5	0.80
Saiki and Lowe 1987	Kesterson Pond 8	120	105	0.88
Saiki and Lowe 1987	Volta Pond 26	1.52	1.4	0.92
Saiki and Lowe 1987	Volta Pond 26	1.52	1.42	0.93
Saiki and Lowe 1987	Volta Pond 7	1.53	1.2	0.78
Saiki and Lowe 1987	Volta Pond 7	1.53	1.4	0.92
Saiki and Lowe 1987	Volta Wasteway	1.83	2.5	1.37
Schuler et al. 1990	Kesterson Pond 11	75.85	63.1	0.83
Schuler et al. 1990	Kesterson Pond 11	75.85	95.5	1.26
Schuler et al. 1990	Kesterson Pond 2	93.3	110	1.18
Schuler et al. 1990	Kesterson Pond 2	93.3	65	0.70
Schuler et al. 1990	Kesterson Pond 7	69.2	61.7	0.89
Schuler et al. 1990	Kesterson Pond 7	69.2	56.2	0.81
Sorenson & Schwarzbach 1991	5	0.42	0.49	1.17

Dragonflies (Zygoptera)



Median ratio: 0.89 x 2.21
(damselfly food to particulate) = 1.97

R^2: 0.95
F: 343.5
df: 17
P: <0.001

3.3.2 Vertebrates

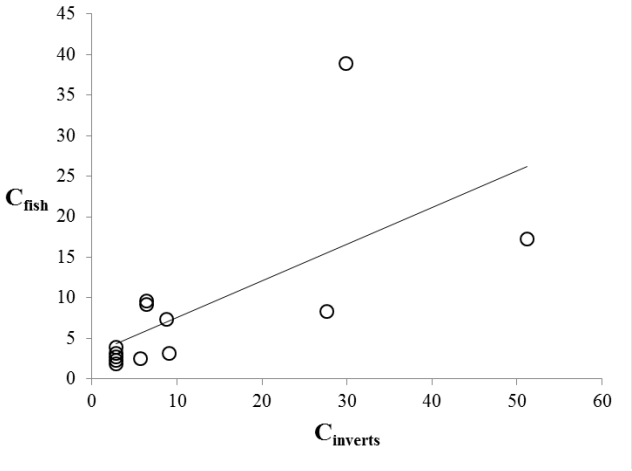
C_{invert} = Selenium concentration in invertebrate tissue (µg/g)
C_{fish} = Average selenium concentration in the whole-body of fish (µg/g)

Ratio = $\frac{C_{fish}}{C_{invert}}$

Black bullhead (*Ameiurus melas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	39.00	1.31
GEI 2013	SWA1	2.81	2.37	0.84
GEI 2013	SWA1	2.81	2.73	0.97
GEI 2013	SWA1	2.81	3.96	1.41
GEI 2013	SWA1	2.81	1.95	0.70
GEI 2013	SWA1	2.81	3.21	1.14
Mueller et al. 1991	R2	6.40	9.70	1.52
Mueller et al. 1991	R2	6.40	9.20	1.44
Mueller et al. 1991	R1	8.70	7.40	0.85
Lemly 1985	Badin Lake	5.70	2.58	0.45
Lemly 1985	Belews Lake	51.15	17.32	0.34
Lemly 1985	High Rock Lake	9.05	3.24	0.36
GEI 2014	SC-6	27.54	8.42	0.31

Black bullhead (*Ameiurus melas*)



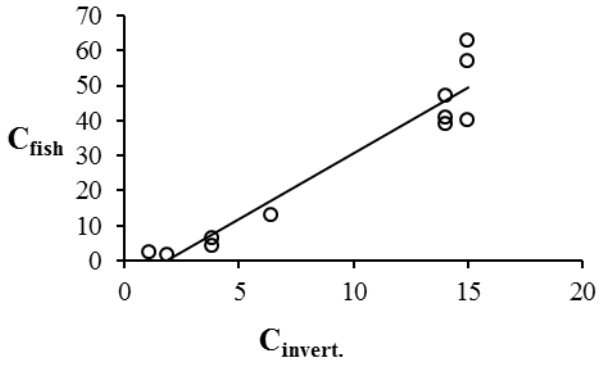
Median ratio: 0.85

R^2: 0.44
F: 8.52
df: 11
P: 0.006

Black crappie (*Pomoxis nigromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	Totten Reservoir	1.07	2.50	2.35
Butler et al. 1995	Summit Reservoir	1.85	1.70	0.92
Peterson et al. 1991	Ocean Lake, west side	3.83	4.20	1.10
Peterson et al. 1991	Ocean Lake, west side	3.83	6.32	1.65
Mueller et al. 1991	Lake Meredith near Ordway, CO	6.40	13.00	2.03
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	39.00	2.79
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	41.00	2.93
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	47.00	3.36
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	40.00	2.67
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	57.00	3.80
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	63.00	4.20

Black crappie (*Pomoxis nigromaculatus*)



Median ratio: 2.67

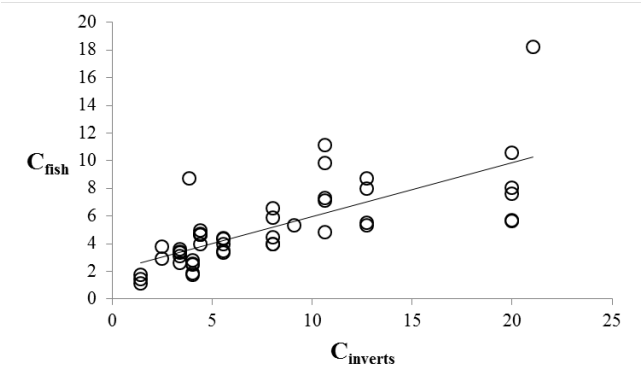
R^2: 0.92
F: 97.9
df: 9
P: < 0.001

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Cabin Creek, C-CC1	4.40	4.65	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	4.74	1.08
GEI 2014	Cabin Creek, C-CC1	4.40	4.95	1.12
GEI 2014	Cabin Creek, C-CC1	4.40	4.69	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	3.98	0.90
GEI 2014	Cabin Creek, C-CC2	5.56	3.46	0.62
GEI 2014	Cabin Creek, C-CC2	5.56	3.38	0.61
GEI 2014	Cabin Creek, C-CC2	5.56	3.95	0.71
GEI 2014	Cabin Creek, C-CC2	5.56	4.36	0.78
GEI 2014	Cabin Creek, C-CC2	5.56	4.39	0.79
GEI 2014	Coal Fork, C-CF1	3.39	3.58	1.06
GEI 2014	Coal Fork, C-CF1	3.39	3.09	0.91
GEI 2014	Coal Fork, C-CF1	3.39	3.37	0.99
GEI 2014	Coal Fork, C-CF1	3.39	2.64	0.78
GEI 2014	Coal Fork, C-CF1	3.39	3.42	1.01
GEI 2014	Hazy Creek, C-HC1	8.03	3.99	0.50
GEI 2014	Hazy Creek, C-HC1	8.03	5.88	0.73
GEI 2014	Hazy Creek, C-HC1	8.03	4.46	0.56
GEI 2014	Hazy Creek, C-HC1	8.03	6.55	0.82
GEI 2014	Hazy Creek, C-HC1	8.03	3.98	0.50
GEI 2014	Laurel Fork, C-LF1	12.73	5.36	0.42
GEI 2014	Laurel Fork, C-LF1	12.73	7.99	0.63
GEI 2014	Laurel Fork, C-LF1	12.73	8.72	0.68
GEI 2014	Laurel Fork, C-LF1	12.73	5.49	0.43
GEI 2014	Tenmile Fork, C-TF1	20.00	7.62	0.38
GEI 2014	Tenmile Fork, C-TF1	20.00	10.56	0.53

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Tenmile Fork, C-TF1	20.00	8.02	0.40
GEI 2014	Tenmile Fork, C-TF1	20.00	5.63	0.28
GEI 2014	Tenmile Fork, C-TF1	20.00	5.68	0.28
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.81	0.70
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.86	0.46
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.78	0.44
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.47	0.61
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.55	0.63
GEI 2014	Lukey Fork, H-LF1	9.09	5.32	0.59
GEI 2014	Mud River, H-MR3	3.86	8.72	2.26
GEI 2014	Mud River, H-MR6	2.49	3.80	1.53
GEI 2014	Mud River, H-MR6	2.49	2.93	1.18
GEI 2014	Sugartree Branch, H-SB1	10.62	9.82	0.92
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	11.14	1.05
GEI 2014	Sugartree Branch, H-SB1	10.62	4.85	0.46
GEI 2014	Sugartree Branch, H-SB1	10.62	7.16	0.67
GEI 2014	Stanley Fork, H-SF1	21.05	18.21	0.87



Median ratio: 0.71

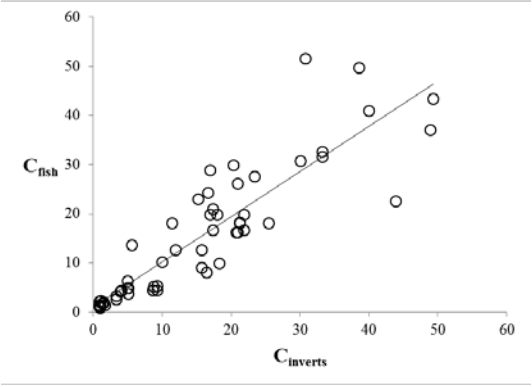
R^2: 0.52
F: 48.97
df: 45
P: < 0.001

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.30	2.16
Hermanutz et al. 1996	MSO II	16.63	24.29	1.46
Hermanutz et al. 1996	MSO III	5.55	13.77	2.48
Hermanutz et al. 1996	MSO I	21.19	18.28	0.86
Hermanutz et al. 1996	MSO I	21.19	18.13	0.86
Hermanutz et al. 1996	MSO II	17.30	20.99	1.21
Hermanutz et al. 1996	MSO II	5.05	4.88	0.97
Hermanutz et al. 1996	MSO I	0.87	1.55	1.78
Hermanutz et al. 1996	MSO II	1.70	1.55	0.91
Hermanutz et al. 1996	MSO III	1.20	1.83	1.52
Hermanutz et al. 1996	MSO III	10.00	10.32	1.03
Hermanutz et al. 1996	MSO III	3.95	4.21	1.06
Hermanutz et al. 1996	MSO II	17.30	16.76	0.97
Hermanutz et al. 1996	MSO II	5.05	3.86	0.76
Mueller et al. 1991	R1	8.70	5.20	0.60
Saiki et al. 1993	ET6	0.85	2.20	2.60
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.40	1.31
Saiki et al. 1993	GT5	4.90	5.00	1.02
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.30	1.06
Saiki et al. 1993	SJR2	3.30	3.30	1.00
Saiki et al. 1993	SJR2	3.30	2.70	0.82
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR3	1.50	1.90	1.27
Saiki et al. 1993	SJR1	0.95	0.87	0.92
Saiki et al. 1993	SJR1	0.95	1.40	1.48
Saiki et al. 1993	ET7	0.86	1.20	1.40
Saiki et al. 1993	ET7	0.86	1.20	1.40
Crutchfield 2000	transect 3	21.80	19.91	0.91
Crutchfield 2000	transect 3	21.80	16.72	0.77
Crutchfield 2000	transect 3	17.90	19.91	1.11
Crutchfield 2000	transect 3	20.70	16.26	0.79
Crutchfield 2000	transect 3	20.35	29.87	1.47
Crutchfield 2000	transect 3	23.40	27.59	1.18
Crutchfield 2000	transect 3	15.20	23.10	1.52
Crutchfield 2000	transect 3	16.95	28.96	1.71
Crutchfield 2000	transect 3	16.95	19.91	1.17
Crutchfield 2000	transect 3	11.95	12.69	1.06
Crutchfield 2000	transect 3	11.40	18.09	1.59
Crutchfield 2000	transect 3	9.25	4.56	0.49
Crutchfield 2000	transect 3	9.25	5.40	0.58

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 4	30.70	51.60	1.68
Crutchfield 2000	transect 4	30.00	30.78	1.03
Crutchfield 2000	transect 4	33.20	31.69	0.95
Crutchfield 2000	transect 4	48.90	37.09	0.76
Crutchfield 2000	transect 4	38.55	49.78	1.29
Crutchfield 2000	transect 4	49.30	43.40	0.88
Crutchfield 2000	transect 4	43.90	22.65	0.52
Crutchfield 2000	transect 4	33.25	32.60	0.98
Crutchfield 2000	transect 4	25.40	18.09	0.71
Crutchfield 2000	transect 4	20.90	16.26	0.78
Crutchfield 2000	transect 4	20.90	26.22	1.25
Crutchfield 2000	transect 4	15.70	12.69	0.81
Crutchfield 2000	transect 4	15.70	9.04	0.58
Crutchfield 2000	transect 4	16.45	8.13	0.49
Crutchfield 2000	transect 4	18.25	9.96	0.55
Bowie et al. 1996	Hyco Reservoir	40.00	41.00	1.03



Median ratio: 1.03

R^2: 0.80
F: 226.0
df: 58
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

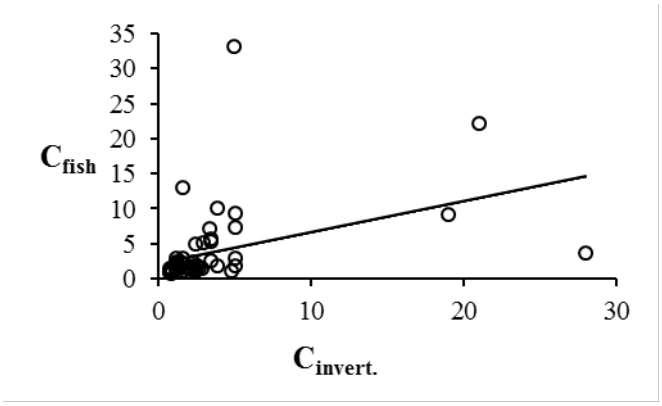
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	AK	0.78	0.94	1.21
Butler et al. 1995	HD1	0.83	0.83	1.01
Butler et al. 1995	HD1	0.83	0.86	1.04
Butler et al. 1995	HD1	0.83	1.20	1.45
Butler et al. 1995	HD1	0.83	1.40	1.70
Butler et al. 1995	DD	0.86	0.64	0.74
Butler et al. 1995	DD	0.86	0.88	1.02
Butler et al. 1995	DD	0.86	1.30	1.51

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	D1	1.20	2.80	2.33
Butler et al. 1993	B1	1.25	1.90	1.52
Butler et al. 1993	B1	1.25	2.20	1.76
Butler et al. 1995	ME2	1.25	0.83	0.66
Butler et al. 1995	ME2	1.25	1.30	1.04
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	SD	1.40	1.50	1.07
Butler et al. 1995	SD	1.40	1.80	1.29
Butler et al. 1993	D2	1.45	1.60	1.10
Butler et al. 1993	D2	1.45	2.30	1.59
Butler et al. 1993	P1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	RB3	1.60	13.00	8.13
Butler et al. 1995	YJ2	1.65	0.96	0.58
Butler et al. 1995	YJ2	1.65	2.80	1.70
Butler et al. 1994	NFK3	2.00	1.40	0.70
Butler et al. 1997	MN2	2.20	1.20	0.55
Butler et al. 1997	MUD	2.30	1.80	0.78
Butler et al. 1997	MUD	2.30	2.30	1.00
Butler et al. 1997	CHK	2.40	1.20	0.50
Butler et al. 1997	CHK	2.40	1.60	0.67
Butler et al. 1993	U1	2.45	4.80	1.96
Butler et al. 1995	SJ1	2.50	0.94	0.38
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.80	0.71
Butler et al. 1997	MN3	2.70	1.50	0.56
Butler et al. 1997	MN1	2.90	1.40	0.48
Butler et al. 1993	SP1	2.95	5.10	1.73
Butler et al. 1993	SP2	3.40	7.10	2.09
Butler et al. 1997	MUD2	3.45	2.50	0.72
Butler et al. 1997	MUD2	3.45	5.20	1.51
Butler et al. 1997	MUD2	3.45	5.60	1.62
Butler et al. 1993	F2	3.90	10.00	2.56
Butler et al. 1991	4	3.90	1.80	0.46
Butler et al. 1993	F2	4.80	0.94	0.20
Butler et al. 1994	BSW1	5.00	33.00	6.60
Butler et al. 1997	WBR	5.05	1.80	0.36
Butler et al. 1997	WBR	5.05	2.80	0.55
Butler et al. 1995	NW	5.10	7.20	1.41
Butler et al. 1995	NW	5.10	9.30	1.82

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	LZA1	19.00	9.00	0.47
Butler et al. 1994	RB1	21.00	22.00	1.05
Butler et al. 1994	GUN2	28.00	3.60	0.13



Median ratio: 1.04

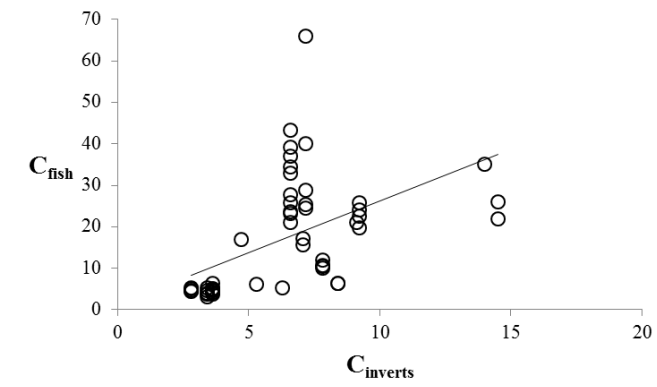
R^2: 0.16
F: 9.6
df: 51
P: < 0.001

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWA1	2.81	4.40	1.57
GEI 2013	SWA1	2.81	4.59	1.64
GEI 2013	SWA1	2.81	4.66	1.66
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.21	1.86
GEI 2013	SWA1	3.64	3.69	1.02
GEI 2013	SWA1	3.64	4.16	1.14
GEI 2013	SWA1	3.64	4.21	1.16
GEI 2013	SWA1	3.64	4.62	1.27
GEI 2013	SWA1	3.64	4.78	1.31
GEI 2013	SWA1	3.64	4.98	1.37
GEI 2013	SWA1	3.64	5.06	1.39
GEI 2013	SWA1	3.64	6.28	1.73
Lambing et al. 1994	S38	4.70	17.00	3.62
Lambing et al. 1994	S37	5.30	6.10	1.15
Lambing et al. 1994	S36	6.30	5.30	0.84
GEI 2014	Dry Creek, DC-2	3	3.14	0.92
GEI 2014	Dry Creek, DC-2	3	4.03	1.18
GEI 2014	Dry Creek, DC-2	3	3.76	1.10
GEI 2014	Dry Creek, DC-2	3	5.31	1.55
GEI 2014	Dry Creek, DC-2	3	4.59	1.34
GEI 2014	Dry Creek, DC-3	7	28.89	4.02
GEI 2014	Dry Creek, DC-3	7	66.07	9.20

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7	24.43	3.40
GEI 2014	Dry Creek, DC-3	7	25.36	3.53
GEI 2014	Dry Creek, DC-3	7	40.17	5.59
GEI 2014	Dry Creek, DC-3	9	25.80	2.80
GEI 2014	Dry Creek, DC-3	9	24.14	2.62
GEI 2014	Dry Creek, DC-3	9	22.46	2.43
GEI 2014	Dry Creek, DC-3	9	19.86	2.15
GEI 2013	SW2-1	6.60	21.14	3.21
GEI 2013	SW2-1	6.60	23.21	3.52
GEI 2013	SW2-1	6.60	23.64	3.58
GEI 2013	SW2-1	6.60	25.89	3.93
GEI 2013	SW2-1	6.60	27.71	4.20
GEI 2013	SW2-1	6.60	32.97	5.00
GEI 2013	SW2-1	6.60	34.54	5.24
GEI 2013	SW2-1	6.60	37.05	5.62
GEI 2013	SW2-1	6.60	39.26	5.95
GEI 2013	SW2-1	6.60	43.38	6.58
GEI 2013	SWB	7.06	15.74	2.23
GEI 2013	SWB	7.06	17.15	2.43
GEI 2013	SW1	7.82	9.96	1.27
GEI 2013	SW1	7.82	10.38	1.33
GEI 2013	SW1	7.82	10.58	1.35
GEI 2013	SW1	7.82	11.98	1.53
GEI 2013	SW11	8.41	6.36	0.76
GEI 2013	SW11	8.41	6.45	0.77
GEI 2013	SW2-1	9.14	21.09	2.31
Lambing et al. 1994	S34	14.00	35.00	2.50
Lambing et al. 1994	S11	14.50	22.00	1.52
Lambing et al. 1994	S11	14.50	26.00	1.79

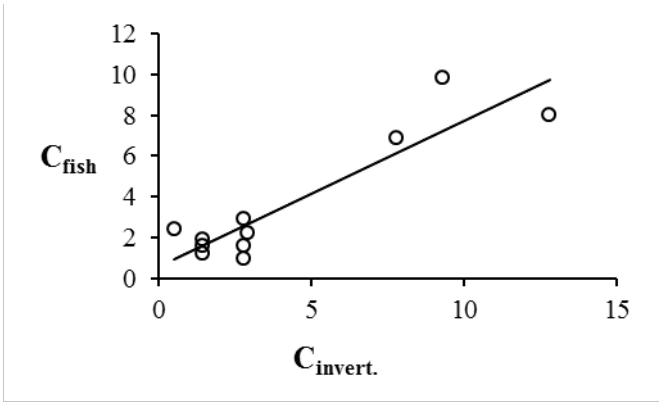


Median ratio: 1.79

R^2: 0.27
F: 18.48
df: 50
P: < 0.001

Brook trout (*Salvelinus fontinalis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	2.40	4.80
Mason et al. 2000	BK	1.43	1.21	0.84
Mason et al. 2000	BK	1.43	1.57	1.10
Mason et al. 2000	BK	1.43	1.90	1.33
Mason et al. 2000	HCRT	2.81	0.99	0.35
Mason et al. 2000	HCRT	2.81	1.59	0.57
Mason et al. 2000	HCRT	2.81	2.95	1.05
Butler et al. 1997	MN1	2.90	2.20	0.76
Hamilton and Buhl 2005	LGC	7.80	6.90	0.88
Hamilton and Buhl 2005	UGC	9.30	9.80	1.05
Hamilton and Buhl 2004	DVC	12.80	8.00	0.63
Hamilton and Buhl 2004	USC	0.50	2.40	4.80

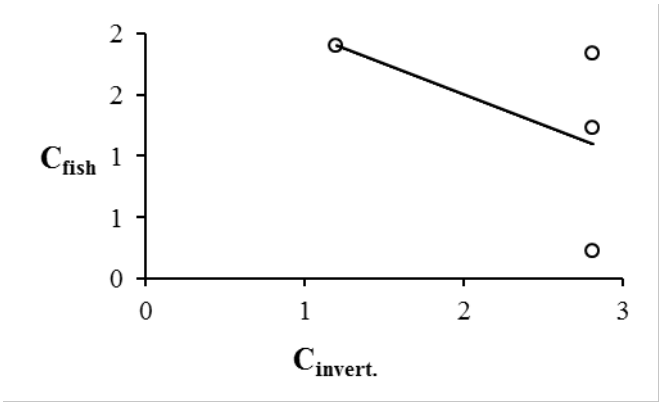


Median ratio: 0.88

R^2: 0.83
F: 43.6
df: 9
P: < 0.001

Brown bullhead (*Ameiurus nebulosus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Rinella and Schuler 1992		1.20	1.90	1.58
Mason et al. 2000	HCRT	2.81	0.22	0.08
Mason et al. 2000	HCRT	2.81	1.23	0.44
Mason et al. 2000	HCRT	2.81	1.83	0.65



Median ratio: 0.55

R^2: 0.27
F: 0.73
df: 2
P: 0.58
Not used because P > 0.05 and negative slope

Brown trout (*Salmo trutta*)

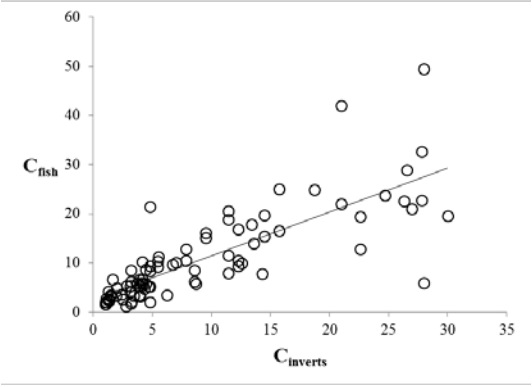
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.00	0.42
Butler et al. 1991	12	2.80	5.40	1.93
Butler et al. 1991	4	3.90	3.30	0.85
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1991	3	6.20	3.50	0.56
Butler et al. 1993	SP2	3.40	3.40	1.00
Butler et al. 1993	SP2	2.75	1.20	0.44
Butler et al. 1993	B2	1.35	2.40	1.78
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B1	1.25	4.20	3.36
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.20	2.21
Butler et al. 1993	P1	1.95	3.30	1.69
Butler et al. 1993	LP2	1.00	1.70	1.70
Butler et al. 1993	LP2	1.00	2.10	2.10
Butler et al. 1993	LP2	1.00	1.60	1.60
Butler et al. 1993	LP3	1.12	2.10	1.88
Butler et al. 1993	LP3	1.12	2.80	2.51
Butler et al. 1993	LP4	3.20	1.80	0.56

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1993	R2	3.90	6.70	1.72
Butler et al. 1993	R2	3.70	5.90	1.59
Butler et al. 1993	ST2	4.10	6.00	1.46
Butler et al. 1994	GUN2	28.00	49.45	1.77
Butler et al. 1994	GUN2	28.00	5.90	0.21
Butler et al. 1994	HCC1	21.00	21.98	1.05
Butler et al. 1994	HCC1	21.00	42.00	2.00
Butler et al. 1994	NFK3	2.00	5.00	2.50
Butler et al. 1994	SMF	4.80	21.44	4.47
Butler et al. 1994	SMF	4.80	5.26	1.10
Butler et al. 1994	SMF	4.80	8.40	1.75
Butler et al. 1994	SMF	4.80	9.40	1.96
Formation 2012	CC-1A	12.24	10.51	0.86
Formation 2012	CC-1A	12.24	9.33	0.76
Formation 2012	CC-1A	12.57	9.95	0.79
Formation 2012	CC-1A	12.24	16.85	1.38
Formation 2012	CC-1A	13.55	14.03	1.04
Formation 2012	CC-3A	5.45	10.44	1.92
Formation 2012	CC-3A	5.45	9.20	1.69
Formation 2012	CC-3A	5.48	11.25	2.05
Formation 2012	CC-3A	14.50	15.38	1.06
Formation 2012	CC-3A	14.50	19.68	1.36
Formation 2012	CC-150	4.46	5.83	1.31
Formation 2012	CC-150	4.46	8.67	1.94
Formation 2012	CC-150	4.70	5.20	1.11
Formation 2012	CC-150	7.03	10.14	1.44
Formation 2012	CC-150	14.32	7.83	0.55
Formation 2012	CC-350	3.16	6.28	1.99
Formation 2012	CC-350	3.16	8.53	2.70
Formation 2012	CC-350	4.20	5.78	1.38
Formation 2012	CC-350	11.45	11.50	1.00
Formation 2012	CC-350	11.45	7.95	0.69
Formation 2012	CC-75	3.11	4.05	1.30
Formation 2012	CC-75	3.11	5.35	1.72
Formation 2012	CC-75	3.97	3.18	0.80
Formation 2012	CC-75	4.16	10.32	2.48
Formation 2012	CC-75	4.16	6.60	1.59
Formation 2012	DC-600	8.53	8.54	1.00
Formation 2012	DC-600	8.53	6.20	0.73
Formation 2012	DC-600	8.65	5.85	0.68
Formation 2012	DC-600	7.83	12.83	1.64

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	7.83	10.54	1.35
Formation 2012	HS	15.70	16.52	1.05
Formation 2012	HS	15.70	25.00	1.59
Formation 2012	HS	18.70	24.90	1.33
Formation 2012	HS	27.80	32.63	1.17
Formation 2012	HS	27.80	22.80	0.82
Formation 2012	HS-3	11.40	20.60	1.81
Formation 2012	HS-3	11.40	18.83	1.65
Formation 2012	HS-3	13.41	17.89	1.33
Formation 2012	HS-3	24.70	23.68	0.96
Formation 2012	HS-3	26.55	28.97	1.09
Formation 2012	LSV-2C	22.62	19.45	0.86
Formation 2012	LSV-2C	22.62	12.78	0.56
Formation 2012	LSV-2C	26.31	22.67	0.86
Formation 2012	LSV-2C	30.00	19.53	0.65
Formation 2012	LSV-2C	26.95	20.96	0.78
Formation 2012	LSV-4	9.54	16.20	1.70
Formation 2012	LSV-4	9.54	15.18	1.59
Formation 2012	SFTC-1	2.42	3.68	1.52
Formation 2012	SFTC-1	3.21	2.25	0.70
Formation 2012	SFTC-1	1.63	6.70	4.11
Formation 2012	SFTC-1	2.49	2.64	1.06
Hamilton and Buhl 2005	CC	6.70	9.70	1.45
McDonald and Strosher 1998	ER 747	4.29	4.80	1.12

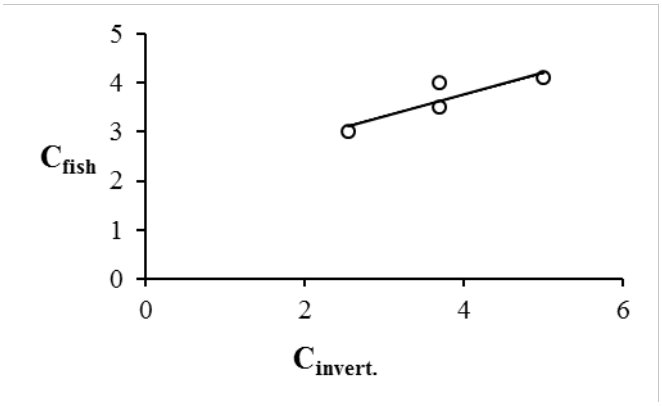


Median ratio: 1.38

R^2: 0.64
F: 151.8
df: 85
P: < 0.001

Bullhead (*Ameiurus sp.*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME3	2.55	3.00	1.18
Butler et al. 1993	R2	3.70	3.50	0.95
Butler et al. 1993	R2	3.70	4.00	1.08
Butler et al. 1994	BSW1	5.00	4.10	0.82



Median ratio: 1.01

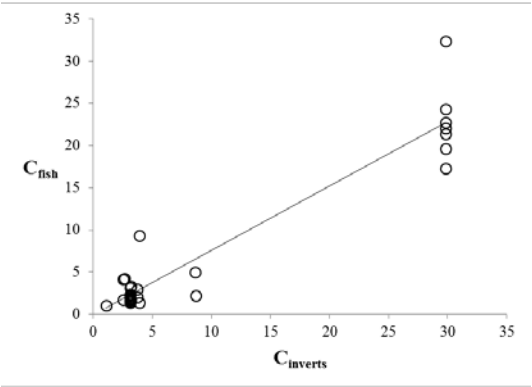
R^2: 0.77
F: 6.58
df: 2
P: 0.13
Not used because P > 0.05

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	21.36	0.72
Butler et al. 1991	7	29.80	22.05	0.74
Butler et al. 1991	7	29.80	17.27	0.58
Butler et al. 1991	7	29.80	19.62	0.66
Butler et al. 1991	7	29.80	22.76	0.76
Butler et al. 1991	7	29.80	24.33	0.82
Butler et al. 1991	7	29.80	32.40	1.09
Butler et al. 1993	LP4	3.20	1.65	0.52
Butler et al. 1993	LP4	3.20	3.30	1.03
Butler et al. 1993	R2	3.90	9.30	2.39
Butler et al. 1993	R2	3.90	1.33	0.34
Butler et al. 1993	R2	3.70	2.04	0.55
Butler et al. 1993	R2	3.70	3.00	0.81
Butler et al. 1995	SJ1	2.50	1.73	0.69
Butler et al. 1995	SJ1	2.50	4.10	1.64
Butler et al. 1995	TT	1.07	1.00	0.94
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	5.00	0.58
Mueller et al. 1991	R1	8.70	2.20	0.25
Roddy et al. 1991	18	3.10	1.40	0.45
Roddy et al. 1991	18	3.10	1.60	0.52
Roddy et al. 1991	18	3.10	1.70	0.55

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	1.90	0.61
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	1.50	0.48
Roddy et al. 1991	18	3.10	1.70	0.55
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	3.10	1.00



Median ratio: 0.74

R^2: 0.91
F: 332.8
df: 32
P: < 0.001

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	10.30	2.15
Butler et al. 1991	7	29.80	25.80	0.87
Butler et al. 1991	7	29.80	31.00	1.04
Butler et al. 1991	7	29.80	40.00	1.34
Butler et al. 1991	7	29.80	50.00	1.68
Butler et al. 1991	9	4.10	3.90	0.95
Butler et al. 1991	3	6.20	2.20	0.35
Butler et al. 1993	D2	1.45	3.70	2.55
Butler et al. 1993	F2	7.50	5.80	0.77
Butler et al. 1993	R2	4.30	5.00	1.16
Butler et al. 1993	R2	3.90	4.80	1.23
Butler et al. 1993	R2	3.70	3.30	0.89
Butler et al. 1994	GUN2	28.00	63.00	2.25
Butler et al. 1994	NFK2	3.10	4.90	1.58

Common carp (*Cyprinus carpio*)

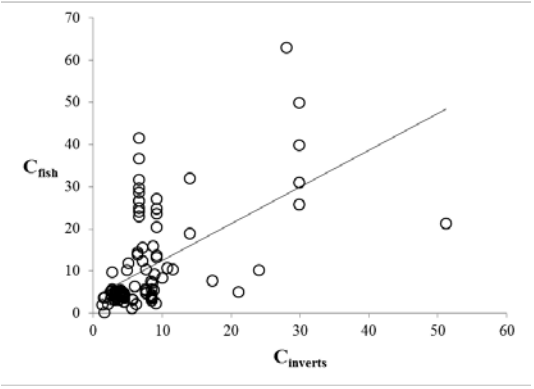
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	BSW1	5.00	12.00	2.40
Butler et al. 1994	RB1	21.00	5.10	0.24
Butler et al. 1995	ME4	1.55	3.90	2.52
Butler et al. 1995	ME4	1.55	3.70	2.39
Butler et al. 1995	ME4	1.55	3.80	2.45
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	ME3	2.55	5.20	2.04
Butler et al. 1995	SJ1	2.50	5.30	2.12
Butler et al. 1995	SJ1	2.50	3.40	1.36
Butler et al. 1995	MN1	2.70	5.80	2.15
Butler et al. 1995	MN1	2.70	9.80	3.63
Butler et al. 1995	MN1	2.70	5.40	2.00
Butler et al. 1997	MN5	8.60	16.00	1.86
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	3.30	1.10
GEI 2013	SWB	7.06	12.50	1.77
GEI 2013	SWB	7.06	15.61	2.21
GEI 2013	SW11	8.41	3.14	0.37
GEI 2013	SW11	8.41	3.52	0.42
GEI 2013	SW11	8.41	3.66	0.44
GEI 2013	SW11	8.41	3.85	0.46
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	3.60	0.43
GEI 2013	SW11	8.41	3.79	0.45
GEI 2013	SW11	8.41	3.95	0.47
GEI 2013	SW11	8.41	4.14	0.49
GEI 2013	SW11	8.41	4.34	0.52
GEI 2013	SW11	8.41	3.56	0.42
GEI 2013	SW2-1	6.60	26.73	4.05
GEI 2013	SW2-1	6.60	26.74	4.05
GEI 2013	SW2-1	6.60	28.74	4.36
GEI 2013	SW2-1	6.60	29.73	4.51
GEI 2013	SW2-1	6.60	41.57	6.30
GEI 2013	SW2-1	6.60	22.96	3.48
GEI 2013	SW2-1	6.60	24.27	3.68
GEI 2013	SW2-1	6.60	25.09	3.80
GEI 2013	SW2-1	6.60	31.74	4.81
GEI 2013	SW2-1	6.60	36.81	5.58
GEI 2013	SW2-1	9.14	13.29	1.45
GEI 2013	SW2-1	9.14	13.77	1.51
GEI 2013	SW2-1	9.14	20.49	2.24
GEI 2013	SW2-1	9.14	24.84	2.72

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW2-1	9.14	23.65	2.59
GEI 2013	SW2-1	9.14	27.27	2.99
GEI 2013	SW4-1	3.33	3.55	1.07
GEI 2013	SW4-1	3.33	4.68	1.41
GEI 2013	SW4-1	3.33	3.91	1.18
GEI 2013	SW4-1	3.33	4.36	1.31
GEI 2013	SW4-1	3.33	4.48	1.35
GEI 2013	SW4-1	3.33	4.60	1.38
GEI 2013	SW4-1	3.33	4.78	1.44
GEI 2013	SW9	4.45	2.73	0.61
GEI 2013	SW9	4.45	2.99	0.67
GEI 2013	SW9	4.45	3.64	0.82
GEI 2013	SW9	4.45	3.80	0.85
GEI 2013	SW9	4.45	3.90	0.88
GEI 2013	SW9	4.45	4.26	0.96
GEI 2013	SW9	4.45	4.53	1.02
GEI 2013	SW9	4.45	3.70	0.83
GEI 2013	SW9	4.45	3.77	0.85
GEI 2013	SW9	4.45	4.14	0.93
GEI 2013	SW9	4.45	4.41	0.99
GEI 2013	SW9	4.45	4.50	1.01
GEI 2013	SW9	4.45	4.69	1.05
GEI 2013	SW88	3.96	3.88	0.98
GEI 2013	SW88	3.96	5.33	1.35
GEI 2013	SW88	3.96	5.49	1.39
GEI 2013	SW88	3.96	5.66	1.43
Grasso et al. 1995	9	7.59	4.70	0.62
Grasso et al. 1995	9	7.59	4.93	0.65
Grasso et al. 1995	9	7.59	5.51	0.73
Lambing et al. 1994	S34	14.00	19.00	1.36
Lambing et al. 1994	S34	14.00	32.00	2.29
Low and Mullins 1990	5	5.60	1.20	0.21
Low and Mullins 1990	7	1.60	0.30	0.19
May et al. 2008	KR	17.20	7.78	0.45
May et al. 2008	NSCL	10.70	10.80	1.01
May et al. 2008	NSK	8.81	9.33	1.06
May et al. 2008	NSP	24.00	10.30	0.43
May et al. 2008	SSAL	11.50	10.50	0.91
May et al. 2008	SSAU	8.35	7.59	0.91
May et al. 2008	SSO	10.00	8.48	0.85
May et al. 2008	SSW	7.60	10.40	1.37
Mueller et al. 1991	R2	6.40	14.40	2.25

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.00	2.19
Mueller et al. 1991	R1	8.70	5.60	0.64
Mueller et al. 1991	A3	6.00	6.50	1.08
Mueller et al. 1991	A6	5.60	3.40	0.61
Mueller et al. 1991	A2	8.50	7.30	0.86
Peterson et al. 1991	7	3.83	4.24	1.11
Peterson et al. 1991	7	3.83	4.41	1.15
Peterson et al. 1991	7	3.83	4.73	1.23
Peterson et al. 1991	7	3.83	5.16	1.35
Peterson et al. 1991	7	3.83	5.21	1.36
Roddy et al. 1991	18	3.10	3.20	1.03
Roddy et al. 1991	18	3.10	3.90	1.26
Roddy et al. 1991	18	3.10	4.60	1.48
Roddy et al. 1991	18	3.10	4.70	1.52
Roddy et al. 1991	18	3.10	4.80	1.55
Roddy et al. 1991	18	3.10	5.30	1.71
Lemly 1985	Badin Lake	5.70	3.17	0.56
Lemly 1985	Belews Lake	51.15	21.29	0.42
Lemly 1985	High Rock Lake	9.05	2.45	0.27
Rinella and Schuler 1992	Harney Lake	2.05	2.20	1.07
Rinella and Schuler 1992	S. Malheur Lake	1.20	2.00	1.67



Median ratio: 1.20

R^2: 0.32
F: 54.27
df: 116
P: < 0.001

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	HCRT	2.81	0.49	0.18
Mason et al. 2000	HCRT	2.81	1.18	0.42
Mason et al. 2000	HCRT	2.81	1.97	0.70
GEI 2013	SW4-1	3.33	4.65	1.40
GEI 2013	SW4-1	3.33	4.96	1.49

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW4-1	3.33	5.52	1.66
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.31	1.90
GEI 2013	SW4-1	3.33	6.53	1.96
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	LG1	3.37	3.41	1.01
GEI 2013	LG1	3.37	3.58	1.06
GEI 2013	LG1	3.37	3.75	1.11
GEI 2013	LG1	3.37	3.78	1.12
GEI 2013	LG1	3.37	4.10	1.22
GEI 2013	LG1	3.39	3.23	0.95
GEI 2013	LG1	3.39	3.72	1.10
GEI 2013	LG1	3.39	3.74	1.10
GEI 2013	LG1	3.39	3.78	1.12
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.03	1.19
GEI 2013	LG1	3.39	4.12	1.22
GEI 2013	LG1	3.39	5.11	1.51
GEI 2013	LG1	3.39	5.21	1.54
GEI 2013	LG1	3.39	5.34	1.58
GEI 2013	LG1	3.56	3.28	0.92
GEI 2013	LG1	3.56	3.37	0.95
GEI 2013	LG1	3.56	3.82	1.07
GEI 2013	LG1	3.56	3.86	1.09
GEI 2013	LG1	3.56	4.02	1.13
GEI 2013	LG1	3.56	4.16	1.17
GEI 2013	LG1	3.56	4.49	1.26
GEI 2013	LG1	3.56	4.53	1.27
GEI 2013	LG1	3.56	4.63	1.30
GEI 2013	LG1	3.56	4.77	1.34
GEI 2013	CC1	3.76	5.43	1.44
GEI 2013	CC1	3.76	5.57	1.48
GEI 2013	CC1	3.76	6.51	1.73
GEI 2013	CC1	3.76	6.71	1.78
GEI 2013	CC1	3.76	7.12	1.89
GEI 2013	CC1	4.69	3.99	0.85
GEI 2013	CC1	4.69	4.06	0.87
GEI 2013	CC1	4.69	4.08	0.87
GEI 2013	CC1	4.69	4.25	0.91
GEI 2013	CC1	4.69	4.44	0.95
GEI 2013	CC1	4.69	4.48	0.96
GEI 2013	CC1	4.69	4.50	0.96

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	4.72	1.01
GEI 2013	CC1	4.69	5.24	1.12
GEI 2013	CC1	4.69	5.44	1.16
GEI 2013	CC1	5.86	4.98	0.85
GEI 2013	CC1	5.86	5.39	0.92
GEI 2013	CC1	5.86	5.77	0.99
GEI 2013	CC1	5.86	6.39	1.09
GEI 2013	CC1	5.86	6.43	1.10
GEI 2013	CC1	5.86	6.50	1.11
GEI 2013	CC1	5.86	6.57	1.12
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.47	1.28
GEI 2014	Bond Creek, BC-2	2.96	2.46	0.83
GEI 2014	Bond Creek, BC-2	2.96	3.22	1.09
GEI 2014	Bond Creek, BC-2	2.96	2.64	0.89
GEI 2014	Bond Creek, BC-2	2.96	2.96	1.00
GEI 2014	Bond Creek, BC-2	2.96	4.47	1.51
GEI 2014	Bond Creek, BC-3	3.02	3.09	1.02
GEI 2014	Bond Creek, BC-3	3.02	2.91	0.96
GEI 2014	Bond Creek, BC-3	3.02	3.45	1.14
GEI 2014	Bond Creek, BC-3	3.02	2.69	0.89
GEI 2014	Bond Creek, BC-3	3.02	3.30	1.09
GEI 2014	Bond Creek, BC-3	5.87	3.44	0.59
GEI 2014	Bond Creek, BC-3	5.87	2.62	0.45
GEI 2014	Bond Creek, BC-3	5.87	3.23	0.55
GEI 2014	Cow Camp Creek, CC-2	5.65	3.05	0.54
GEI 2014	Cow Camp Creek, CC-2	5.65	3.69	0.65
GEI 2014	Cow Camp Creek, CC-2	5.65	3.84	0.68
GEI 2014	Cow Camp Creek, CC-2	5.65	4.44	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	3.98	0.70
GEI 2014	Hazy Creek, C-HC1	8.03	6.84	0.85
GEI 2014	Hazy Creek, C-HC1	8.03	3.78	0.47
GEI 2014	Hazy Creek, C-HC1	8.03	5.81	0.72
GEI 2014	Hazy Creek, C-HC1	8.03	4.29	0.53
GEI 2014	Hazy Creek, C-HC1	8.03	3.59	0.45
GEI 2014	Laurel Fork, C-LF1	12.73	6.52	0.51
GEI 2014	Laurel Fork, C-LF1	12.73	6.81	0.54
GEI 2014	Laurel Fork, C-LF1	12.73	5.11	0.40
GEI 2014	Laurel Fork, C-LF1	12.73	5.16	0.41
GEI 2014	Laurel Fork, C-LF1	12.73	5.46	0.43
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.81	0.63

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.89	0.81
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.58	0.60
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.81	0.80
GEI 2014	Little Marsh Fork, C-LMF1	6.02	5.82	0.97
GEI 2014	Dry Creek, DC-1	3.37	4.62	1.37
GEI 2014	Dry Creek, DC-1	3.37	5.15	1.53
GEI 2014	Dry Creek, DC-1	3.37	6.46	1.92
GEI 2014	Dry Creek, DC-1	3.37	5.73	1.70
GEI 2014	Dry Creek, DC-1	3.37	4.59	1.36
GEI 2014	Dry Creek, DC-1	2.29	4.94	2.16
GEI 2014	Dry Creek, DC-1	2.29	4.04	1.76
GEI 2014	Dry Creek, DC-1	2.29	3.62	1.58
GEI 2014	Dry Creek, DC-1	2.29	3.84	1.68
GEI 2014	Dry Creek, DC-1	2.15	4.02	1.87
GEI 2014	Dry Creek, DC-1	2.15	3.60	1.67
GEI 2014	Dry Creek, DC-1	2.15	3.28	1.52
GEI 2014	Dry Creek, DC-1	2.15	3.03	1.41
GEI 2014	Dry Creek, DC-1	2.15	4.01	1.86
GEI 2014	Dry Creek, DC-1	1.97	3.51	1.78
GEI 2014	Dry Creek, DC-2	3.42	5.32	1.56
GEI 2014	Dry Creek, DC-2	3.42	4.62	1.35
GEI 2014	Dry Creek, DC-2	3.42	4.43	1.30
GEI 2014	Dry Creek, DC-2	3.42	4.56	1.34
GEI 2014	Dry Creek, DC-2	3.42	6.38	1.87
GEI 2014	Dry Creek, DC-2	3.42	2.96	0.87
GEI 2014	Dry Creek, DC-2	3.42	3.58	1.05
GEI 2014	Dry Creek, DC-2	3.42	3.22	0.94
GEI 2014	Dry Creek, DC-2	3.42	4.07	1.19
GEI 2014	Dry Creek, DC-2	3.42	3.28	0.96
GEI 2014	Dry Creek, DC-2	3.16	6.52	2.07
GEI 2014	Dry Creek, DC-2	3.16	4.92	1.56
GEI 2014	Dry Creek, DC-2	3.16	3.10	0.98
GEI 2014	Dry Creek, DC-2	3.16	3.14	1.00
GEI 2014	Dry Creek, DC-2	3.16	4.36	1.38
GEI 2014	Dry Creek, DC-2	3.16	3.12	0.99
GEI 2014	Dry Creek, DC-2	3.16	5.40	1.71
GEI 2014	Dry Creek, DC-2	2.93	2.85	0.97
GEI 2014	Dry Creek, DC-2	2.93	4.94	1.69
GEI 2014	Dry Creek, DC-2	2.93	5.17	1.76
GEI 2014	Dry Creek, DC-2	2.93	3.47	1.18
GEI 2014	Dry Creek, DC-2	2.93	2.49	0.85
GEI 2014	Dry Creek, DC-3	7.18	23.79	3.31

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7.18	16.06	2.24
GEI 2014	Dry Creek, DC-3	7.18	24.43	3.40
GEI 2014	Dry Creek, DC-3	7.18	22.20	3.09
GEI 2014	Dry Creek, DC-3	7.18	26.28	3.66
GEI 2014	Dry Creek, DC-3	9.23	21.48	2.33
GEI 2014	Dry Creek, DC-3	9.23	21.24	2.30
GEI 2014	Dry Creek, DC-3	9.23	21.46	2.33
GEI 2014	Dry Creek, DC-3	9.23	22.48	2.44
GEI 2014	Dry Creek, DC-3	9.23	18.80	2.04
GEI 2014	Dry Creek, DC-3	9.23	16.87	1.83
GEI 2014	Dry Creek, DC-4	19.42	15.86	0.82
GEI 2014	Dry Creek, DC-4	19.42	12.76	0.66
GEI 2014	Dry Creek, DC-4	19.42	28.50	1.47
GEI 2014	Dry Creek, DC-4	19.42	18.14	0.93
GEI 2014	Dry Creek, DC-4	19.42	17.55	0.90
GEI 2014	Dry Creek, DC-4	18.10	34.60	1.91
GEI 2014	Dry Creek, DC-4	18.10	23.70	1.31
GEI 2014	Foidel Creek, FOC-1	3.06	8.00	2.61
GEI 2014	Foidel Creek, FOC-1	3.06	9.68	3.16
GEI 2014	Foidel Creek, FOC-1	3.06	8.86	2.89
GEI 2014	Foidel Creek, FOC-1	3.06	2.51	0.82
GEI 2014	Foidel Creek, FOC-1	3.06	2.86	0.93
GEI 2014	Foidel Creek, FOC-1	3.06	4.24	1.39
GEI 2014	Foidel Creek, FOC-1	3.06	3.27	1.07
GEI 2014	Foidel Creek, FOC-1	3.06	5.03	1.64
GEI 2014	Foidel Creek, FOC-2	2.18	2.07	0.95
GEI 2014	Foidel Creek, FOC-2	2.18	3.06	1.41
GEI 2014	Foidel Creek, FOC-2	2.18	3.82	1.76
GEI 2014	Foidel Creek, FOC-2	2.18	2.26	1.04
GEI 2014	Foidel Creek, FOC-2	2.18	2.02	0.93
GEI 2014	Foidel Creek, FOC-2	2.18	2.28	1.05
GEI 2014	Foidel Creek, FOC-2	2.18	2.44	1.12
GEI 2014	Foidel Creek, FOC-2	2.18	2.62	1.21
GEI 2014	Grassy Creek, GC-2	4.20	5.28	1.26
GEI 2014	Grassy Creek, GC-2	4.20	6.13	1.46
GEI 2014	Grassy Creek, GC-2	4.20	6.29	1.50
GEI 2014	Grassy Creek, GC-2	4.20	4.80	1.15
GEI 2014	Grassy Creek, GC-2	4.20	4.59	1.09
GEI 2014	Grassy Creek, GC-2	4.58	3.27	0.71
GEI 2014	Grassy Creek, GC-2	4.58	5.50	1.20
GEI 2014	Grassy Creek, GC-2	4.58	3.64	0.80
GEI 2014	Grassy Creek, GC-2	4.58	4.29	0.94

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-2	4.58	3.01	0.66
GEI 2014	Grassy Creek, GC-2	3.96	7.48	1.89
GEI 2014	Grassy Creek, GC-2	3.96	6.12	1.55
GEI 2014	Grassy Creek, GC-2	3.96	8.61	2.18
GEI 2014	Grassy Creek, GC-2	3.96	7.09	1.79
GEI 2014	Grassy Creek, GC-2	3.96	5.06	1.28
GEI 2014	Grassy Creek, GC-2	3.96	4.95	1.25
GEI 2014	Grassy Creek, GC-2	3.96	3.85	0.97
GEI 2014	Grassy Creek, GC-2	3.96	5.32	1.34
GEI 2014	Grassy Creek, GC-2	3.96	4.04	1.02
GEI 2014	Grassy Creek, GC-2	3.96	4.25	1.08
GEI 2014	Grassy Creek, GC-2	3.97	3.48	0.88
GEI 2014	Grassy Creek, GC-2	3.97	3.86	0.97
GEI 2014	Grassy Creek, GC-2	3.97	4.08	1.03
GEI 2014	Grassy Creek, GC-2	3.97	4.58	1.15
GEI 2014	Grassy Creek, GC-2	3.97	3.53	0.89
GEI 2014	Grassy Creek, GC-3	4.54	6.07	1.34
GEI 2014	Grassy Creek, GC-3	4.54	7.25	1.60
GEI 2014	Grassy Creek, GC-3	4.54	5.25	1.16
GEI 2014	Grassy Creek, GC-3	4.54	5.65	1.25
GEI 2014	Grassy Creek, GC-3	4.54	10.75	2.37
GEI 2014	Grassy Creek, GC-3	4.55	3.30	0.73
GEI 2014	Grassy Creek, GC-3	4.55	3.98	0.88
GEI 2014	Grassy Creek, GC-3	4.55	3.46	0.76
GEI 2014	Grassy Creek, GC-3	4.55	4.14	0.91
GEI 2014	Grassy Creek, GC-3	4.55	3.81	0.84
GEI 2014	Grassy Creek, GC-3	4.34	11.05	2.55
GEI 2014	Grassy Creek, GC-3	4.34	7.22	1.66
GEI 2014	Grassy Creek, GC-3	4.34	9.61	2.22
GEI 2014	Grassy Creek, GC-3	4.34	6.03	1.39
GEI 2014	Grassy Creek, GC-3	4.34	3.95	0.91
GEI 2014	Grassy Creek, GC-3	4.34	4.82	1.11
GEI 2014	Grassy Creek, GC-3	4.34	5.18	1.19
GEI 2014	Grassy Creek, GC-3	4.34	4.46	1.03
GEI 2014	Grassy Creek, GC-3	4.34	4.21	0.97
GEI 2014	Grassy Creek, GC-3	4.35	2.97	0.68
GEI 2014	Grassy Creek, GC-3	4.35	4.16	0.96
GEI 2014	Grassy Creek, GC-3	4.35	4.29	0.99
GEI 2014	Grassy Creek, GC-3	4.35	3.69	0.85
GEI 2014	Grassy Creek, GC-3	4.35	4.73	1.09
GEI 2014	Grassy Creek, GC-4	5.10	4.30	0.84
GEI 2014	Grassy Creek, GC-4	5.10	5.17	1.01

Creek chub (*Semotilus atromaculatus*)

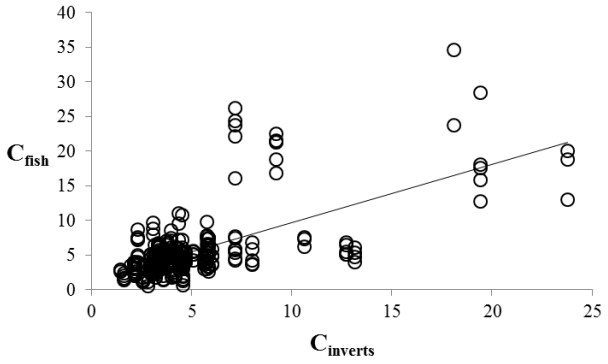
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-4	5.10	5.46	1.07
GEI 2014	Grassy Creek, GC-4	5.10	5.63	1.10
GEI 2014	Grassy Creek, GC-4	5.10	5.15	1.01
GEI 2014	Grassy Creek, GC-4	5.76	7.72	1.34
GEI 2014	Grassy Creek, GC-4	5.76	6.04	1.05
GEI 2014	Grassy Creek, GC-4	5.76	7.88	1.37
GEI 2014	Grassy Creek, GC-4	5.76	9.77	1.70
GEI 2014	Grassy Creek, GC-4	5.76	7.35	1.28
GEI 2014	Grassy Creek, GC-4	5.76	4.17	0.72
GEI 2014	Grassy Creek, GC-4	5.76	4.86	0.84
GEI 2014	Grassy Creek, GC-4	5.76	5.02	0.87
GEI 2014	Grassy Creek, GC-4	5.76	4.79	0.83
GEI 2014	Grassy Creek, GC-4	5.76	6.56	1.14
GEI 2014	Grassy Creek, GC-4	5.76	5.31	0.92
GEI 2014	Grassy Creek, GC-4 US	13.16	4.05	0.31
GEI 2014	Grassy Creek, GC-4 US	13.16	4.80	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	5.41	0.41
GEI 2014	Grassy Creek, GC-4 US	13.16	6.05	0.46
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.96	0.69
GEI 2014	Big Horse Creek, H-BHC3	5.78	2.97	0.51
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.84	0.66
GEI 2014	Sally Fork, H-BLB2	1.64	2.42	1.48
GEI 2014	Sally Fork, H-BLB2	1.64	1.65	1.01
GEI 2014	Sally Fork, H-BLB2	1.64	1.68	1.03
GEI 2014	Sally Fork, H-BLB2	1.64	2.02	1.23
GEI 2014	Sally Fork, H-BLB2	1.64	1.46	0.89
GEI 2014	Hubberson Gulch, HG-2	3.44	4.41	1.28
GEI 2014	Hubberson Gulch, HG-2	3.44	3.56	1.04
GEI 2014	Hubberson Gulch, HG-2	3.44	4.48	1.30
GEI 2014	Hubberson Gulch, HG-2	1.46	2.95	2.03
GEI 2014	Hubberson Gulch, HG-2	1.46	2.66	1.83
GEI 2014	Hubberson Gulch, HG-2	1.46	2.87	1.97
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	3.04	0.75
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.81	0.45
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.35	0.58
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.91	0.47
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.83	0.70
GEI 2014	Laurel Creek, H-LC1	4.57	1.29	0.28

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Laurel Creek, H-LC1	4.57	2.04	0.45
GEI 2014	Laurel Creek, H-LC1	4.57	1.49	0.33
GEI 2014	Laurel Creek, H-LC1	4.57	1.85	0.41
GEI 2014	Laurel Creek, H-LC1	4.57	0.67	0.15
GEI 2014	Lick Creek, H-LKC1	2.59	1.83	0.71
GEI 2014	Lick Creek, H-LKC1	2.59	1.40	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.41	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.19	0.46
GEI 2014	Lick Creek, H-LKC1	2.59	1.22	0.47
GEI 2014	Mud River, H-MR3	3.86	4.75	1.23
GEI 2014	Mud River, H-MR3	3.86	4.60	1.19
GEI 2014	Mud River, H-MR3	3.86	5.06	1.31
GEI 2014	Mud River, H-MR3	3.86	3.32	0.86
GEI 2014	Mud River, H-MR3	3.86	4.19	1.08
GEI 2014	Mud River, H-MR5	3.58	1.51	0.42
GEI 2014	Mud River, H-MR5	3.58	1.43	0.40
GEI 2014	Mud River, H-MR5	3.58	1.98	0.55
GEI 2014	Mud River, H-MR5	3.58	3.80	1.06
GEI 2014	Mud River, H-MR5	3.58	3.44	0.96
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	7.56	0.71
GEI 2014	Sugartree Branch, H-SB1	10.62	6.20	0.58
GEI 2014	Middle Creek, MC-1	3.21	2.75	0.86
GEI 2014	Middle Creek, MC-1	3.21	4.74	1.48
GEI 2014	Middle Creek, MC-1	3.21	4.01	1.25
GEI 2014	Middle Creek, MC-1	3.21	3.94	1.23
GEI 2014	Middle Creek, MC-1	3.21	3.63	1.13
GEI 2014	Middle Creek, MC-1	3.21	1.83	0.57
GEI 2014	Middle Creek, MC-1	3.21	1.85	0.57
GEI 2014	Middle Creek, MC-1	3.21	2.50	0.78
GEI 2014	Middle Creek, MC-1	3.21	2.33	0.73
GEI 2014	Middle Creek, MC-1	3.21	2.56	0.80
GEI 2014	Middle Creek, MC-2	4.19	1.93	0.46
GEI 2014	Middle Creek, MC-2	4.19	1.89	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.51	0.60
GEI 2014	Middle Creek, MC-2	4.19	1.87	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.13	0.51
GEI 2014	Sage Creek, SC-3	2.29	7.33	3.20
GEI 2014	Sage Creek, SC-3	2.29	7.23	3.16
GEI 2014	Sage Creek, SC-3	2.29	7.60	3.32
GEI 2014	Sage Creek, SC-3	2.29	4.77	2.08
GEI 2014	Sage Creek, SC-3	2.29	8.70	3.80

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Sage Creek, SC-4	23.79	18.77	0.79
GEI 2014	Sage Creek, SC-4	23.79	20.08	0.84
GEI 2014	Sage Creek, SC-4	23.79	13.05	0.55
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.41	1.04
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.65	1.07
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.47	0.62
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.83	0.81
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.37	0.75
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.69	0.80
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.31	0.60



Median ratio: 1.06

R^2: 0.41
F: 214.7
df: 303
P: < 0.001

Cutthroat trout (*Oncorhynchus clarkii*)

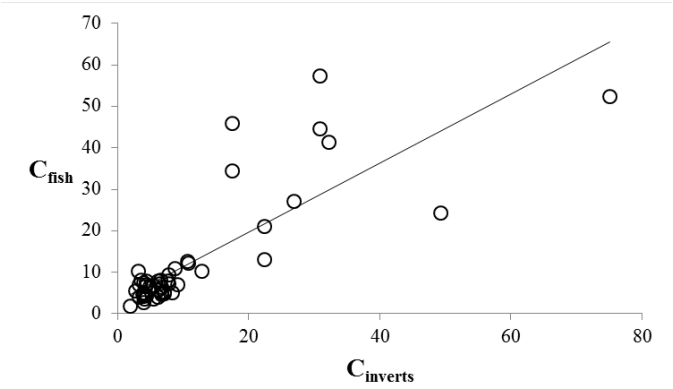
Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ShpC	1.90	1.80	0.95
McDonald and Strosher 1998	ER 745	2.74	5.40	1.97
Hamilton and Buhl 2005	SC	4.10	3.50	0.85
McDonald and Strosher 1998	ER 747	4.29	6.57	1.53
Hamilton and Buhl 2005	UAC	5.00	6.60	1.32
Hamilton and Buhl 2004	ACM	6.70	6.30	0.94
Hamilton and Buhl 2005	DC	8.70	11.00	1.26
McDonald and Strosher 1998	ER 746	10.70	12.71	1.19
Hamilton and Buhl 2005	BGS	10.80	12.20	1.13
Hamilton and Buhl 2004	DVC	12.80	10.20	0.80
Hamilton and Buhl 2004	UEMC	26.90	27.00	1.00
Hamilton and Buhl 2004	LEMC	75.20	52.30	0.70
Minnow 2007	BA6	3.27	6.98	2.13
Minnow 2007	AL4	3.92	4.44	1.13
Minnow 2007	MI5	4.00	5.12	1.28

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	EL12	4.01	7.42	1.85
Minnow 2007	EL14	4.41	4.52	1.02
Minnow 2007	FO9	4.44	7.80	1.76
Minnow 2007	MI3	6.21	5.65	0.91
Minnow 2007	MI2	6.69	5.16	0.77
Minnow 2007	EL1	7.08	4.82	0.68
Minnow 2007	LI8	7.81	9.36	1.20
Minnow 2007	FO10	17.51	45.94	2.62
Minnow 2007	HA7	22.41	21.10	0.94
Minnow 2007	CL11	30.87	57.27	1.86
Orr et al. 2012	Alexander Creek	3.92	2.71	0.69
Orr et al. 2012	Elk River 1	6.23	5.61	0.90
Orr et al. 2012	Elk River 1	6.23	7.94	1.27
Orr et al. 2012	Elk River 1	7.08	5.35	0.76
Orr et al. 2012	Elk River 12	3.81	4.58	1.20
Orr et al. 2012	Elk River 12	4.01	4.89	1.22
Orr et al. 2012	Fording River 22	3.10	10.28	3.32
Orr et al. 2012	Fording River 23	7.72	7.92	1.03
Orr et al. 2012	Fording River 9	4.44	6.92	1.56
Orr et al. 2012	Line Creek 8	6.61	7.99	1.21
Orr et al. 2012	Line Creek 8	7.81	7.13	0.91
Orr et al. 2012	Michel Creek 2	8.38	5.13	0.61
Orr et al. 2012	Michel Creek 2	6.69	4.63	0.69
Orr et al. 2012	Michel Creek 3	5.42	3.51	0.65
Orr et al. 2012	Michel Creek 3	6.21	4.02	0.65
Orr et al. 2012	Michel Creek 5	4.00	4.12	1.03
Orr et al. 2012	Fording River MP1	5.49	6.84	1.25
Orr et al. 2012	Barnes Lake Wetland 6	3.27	3.92	1.20
Orr et al. 2012	Clode Pond 11	32.22	41.27	1.28
Orr et al. 2012	Clode Pond 11	30.87	44.70	1.45
Orr et al. 2012	Elk Lakes 14	6.40	7.14	1.12
Orr et al. 2012	Elk Lakes 14	4.41	4.35	0.99
Orr et al. 2012	Fording River Oxbow 10	49.26	24.34	0.49
Orr et al. 2012	Fording River Oxbow 10	17.51	34.41	1.97
Orr et al. 2012	Henretta Lake 27	9.16	6.90	0.75
Orr et al. 2012	O'Rourke Lake 1	3.63	8.05	2.22
Orr et al. 2012	Harmer Pond 7	22.41	13.08	0.58

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.12

R^2: 0.66
F: 95.81
df: 50
P: < 0.001

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	4	1.80	2.10	1.17
Birkner 1978	22	11.30	11.00	0.97
Birkner 1978	27	34.60	79.00	2.28
Birkner 1978	23	15.50	34.50	2.23
Birkner 1978	1	1.75	2.10	1.20
Butler et al. 1991	10	4.80	8.10	1.69
Butler et al. 1991	3	6.20	9.50	1.53
Butler et al. 1993	SP2	3.40	6.00	1.76
Butler et al. 1993	SP2	3.15	8.20	2.60
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.80	3.17
Butler et al. 1993	U1	2.45	6.40	2.61
Butler et al. 1993	R2	3.90	6.60	1.69
Butler et al. 1993	R2	3.70	6.60	1.78
Butler et al. 1993	ST2	4.50	12.80	2.84
Butler et al. 1993	ST2	4.10	7.60	1.85
Butler et al. 1993	ST2	4.10	16.00	3.90
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	SB2	3.75	5.70	1.52
Butler et al. 1993	SB2	3.75	8.60	2.29
Butler et al. 1993	SB2	3.65	9.90	2.71
Butler et al. 1993	WSB2	4.75	17.10	3.60
Butler et al. 1993	WSB2	3.60	4.20	1.17
Butler et al. 1993	WSB2	3.60	10.00	2.78

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	WSB2	3.00	8.10	2.70
Butler et al. 1994	CRC	7.50	20.40	2.72
Butler et al. 1994	CF1	3.60	7.90	2.19
Butler et al. 1994	GUN2	28.00	7.50	0.27
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	TGC	4.90	11.00	2.24
Butler et al. 1994	AD	2.70	9.60	3.56
Butler et al. 1994	LSW1	3.90	73.00	18.72
Butler et al. 1994	OMD	73.00	13.00	0.18
Butler et al. 1994	PSW1	3.70	22.00	5.95
Butler et al. 1994	MKP	32.00	51.00	1.59
Butler et al. 1995	AK	0.78	2.60	3.35
Butler et al. 1995	AK	0.78	2.90	3.74
Butler et al. 1995	AK	0.78	2.80	3.61
Butler et al. 1995	DD	0.86	3.40	3.95
Butler et al. 1995	DD	0.86	3.90	4.53
Butler et al. 1995	DD	0.86	3.60	4.19
Butler et al. 1995	HD1	0.83	3.90	4.73
Butler et al. 1995	HD1	0.83	2.50	3.03
Butler et al. 1995	HD1	0.83	2.60	3.15
Butler et al. 1995	HD2	0.98	1.50	1.53
Butler et al. 1995	HD2	0.98	1.60	1.63
Butler et al. 1995	ME1	3.40	5.60	1.65
Butler et al. 1995	ME2	1.25	4.80	3.84
Butler et al. 1995	ME4	1.55	1.40	0.90
Butler et al. 1995	ME4	1.55	5.90	3.81
Butler et al. 1995	ME3	2.55	4.30	1.69
Butler et al. 1995	ME3	2.55	5.30	2.08
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	SD	1.40	4.90	3.50
Butler et al. 1995	SD	1.40	3.00	2.14
Butler et al. 1995	SD	1.40	4.00	2.86
Butler et al. 1995	WC	6.75	18.40	2.73
Butler et al. 1995	WC	6.75	22.90	3.39
Butler et al. 1995	WC	6.75	26.40	3.91
Butler et al. 1995	YJ2	1.65	11.00	6.67
Butler et al. 1995	YJ2	1.65	4.00	2.42
Butler et al. 1997	MNP2	4.40	11.00	2.50
Butler et al. 1997	MUD2	3.45	7.70	2.23
Butler et al. 1997	MUD2	3.45	12.00	3.48
Butler et al. 1997	MUD2	3.45	6.50	1.88
Butler et al. 1997	WCP	9.70	10.00	1.03

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	WCP	9.70	15.00	1.55
Butler et al. 1997	TR25	1.80	4.00	2.22
Butler et al. 1997	TR25	1.80	5.20	2.89
Butler et al. 1997	TR25	1.80	6.00	3.33
Butler et al. 1997	TRH	1.60	4.20	2.63
Butler et al. 1997	TRH	1.60	4.30	2.69
Butler et al. 1997	TRH	1.60	2.20	1.38
Butler et al. 1997	TRH	1.60	3.00	1.88
Butler et al. 1997	MN5	8.60	7.30	0.85
Butler et al. 1997	MNP1	0.70	1.70	2.43
Butler et al. 1997	MNP1	0.70	1.80	2.57
GEI 2013	SWA1	3.64	4.07	1.12
GEI 2013	SWA1	3.64	4.68	1.29
GEI 2013	SWA1	3.64	4.76	1.31
GEI 2013	SWA1	3.64	5.45	1.50
GEI 2013	SWA1	3.64	5.71	1.57
GEI 2013	SWA1	3.64	3.62	1.00
GEI 2013	SWA1	3.64	3.72	1.02
GEI 2013	SWA1	3.64	4.43	1.22
GEI 2013	SWA1	3.64	4.52	1.24
GEI 2013	SWA1	3.64	4.66	1.28
GEI 2013	SWA1	2.81	4.48	1.60
GEI 2013	SWA1	2.81	4.53	1.61
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.24	1.87
GEI 2013	SWA1	2.81	5.76	2.05
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	3.98	1.42
GEI 2013	SWA1	2.81	4.04	1.44
GEI 2013	SWA1	2.81	4.33	1.54
GEI 2013	SWA1	2.81	4.81	1.71
GEI 2013	SWB	7.06	7.38	1.05
GEI 2013	SWB	7.06	8.49	1.20
GEI 2013	SWB	7.06	8.72	1.24
GEI 2013	SWB	7.06	9.80	1.39
GEI 2013	SWB	7.06	8.61	1.22
GEI 2013	SWB	7.06	9.02	1.28
GEI 2013	SWB	7.06	9.11	1.29
GEI 2013	SWB	7.06	9.30	1.32
GEI 2013	SWB	7.06	9.53	1.35
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	11.22	1.51

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWB	7.44	12.25	1.65
GEI 2013	SWB	7.44	12.43	1.67
GEI 2013	SWB	7.44	12.46	1.68
GEI 2013	SWB	7.44	9.36	1.26
GEI 2013	SWB	7.44	9.46	1.27
GEI 2013	SWB	7.44	9.78	1.32
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.66	1.43
GEI 2013	SW11	8.41	5.70	0.68
GEI 2013	SW11	8.41	7.05	0.84
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW11	8.41	4.68	0.56
GEI 2013	SW11	8.41	5.29	0.63
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW2-1	6.60	12.83	1.95
GEI 2013	SW2-1	6.60	14.80	2.24
GEI 2013	SW2-1	6.60	20.13	3.05
GEI 2013	SW2-1	6.60	26.75	4.06
GEI 2013	SW2-1	6.60	30.48	4.62
GEI 2013	SW2-1	6.60	12.51	1.90
GEI 2013	SW2-1	6.60	16.70	2.53
GEI 2013	SW2-1	6.60	17.21	2.61
GEI 2013	SW2-1	6.60	18.27	2.77
GEI 2013	SW2-1	6.60	20.66	3.13
GEI 2013	SW2-1	9.14	13.31	1.46
GEI 2013	SW2-1	9.14	15.63	1.71
GEI 2013	SW2-1	9.14	15.77	1.73
GEI 2013	SW2-1	9.14	16.79	1.84
GEI 2013	SW2-1	9.14	17.00	1.86
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	19.39	2.12
GEI 2013	SW2-1	9.14	22.50	2.46
GEI 2013	SW1	7.82	9.11	1.16
GEI 2013	SW1	7.82	9.15	1.17
GEI 2013	SW1	7.82	11.15	1.43
GEI 2013	SW1	7.82	11.23	1.44
GEI 2013	SW1	7.82	13.76	1.76
GEI 2013	SW1	7.82	9.82	1.26
GEI 2013	SW1	7.82	8.45	1.08
GEI 2013	SW1	7.82	8.88	1.14
GEI 2013	SW1	7.82	9.41	1.20

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	11.07	1.42
GEI 2013	SW1	6.54	7.01	1.07
GEI 2013	SW1	6.54	7.86	1.20
GEI 2013	SW1	6.54	7.98	1.22
GEI 2013	SW1	6.54	8.23	1.26
GEI 2013	SW1	6.54	8.50	1.30
GEI 2013	SW1	6.54	9.48	1.45
GEI 2013	SW1	6.54	9.95	1.52
GEI 2013	SW1	6.54	10.09	1.54
GEI 2013	SW1	6.54	10.19	1.56
GEI 2013	SW4-1	3.33	5.88	1.77
GEI 2013	SW4-1	3.33	5.89	1.77
GEI 2013	SW4-1	3.33	6.07	1.83
GEI 2013	SW4-1	3.33	6.61	1.99
GEI 2013	SW4-1	3.33	6.87	2.07
GEI 2013	SW4-1	3.33	4.85	1.46
GEI 2013	SW4-1	3.33	5.25	1.58
GEI 2013	SW4-1	3.33	5.39	1.62
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	SW9	4.45	5.57	1.25
GEI 2013	SW9	4.45	5.93	1.33
GEI 2013	SW9	4.45	6.14	1.38
GEI 2013	SW9	4.45	6.20	1.39
GEI 2013	SW9	4.45	6.56	1.47
GEI 2013	SW9	4.45	7.57	1.70
GEI 2013	SW88	3.96	4.73	1.20
GEI 2013	SW88	3.96	4.96	1.25
GEI 2013	SW88	3.96	5.55	1.40
GEI 2013	SW88	3.96	5.56	1.41
GEI 2013	SW88	3.96	6.32	1.60
GEI 2013	SW88	3.96	5.13	1.30
GEI 2013	SW88	3.96	5.86	1.48
GEI 2013	SW88	3.96	6.07	1.53
GEI 2013	CC1	3.76	3.79	1.01
GEI 2013	CC1	3.76	5.23	1.39
GEI 2013	CC1	3.76	7.36	1.96
GEI 2013	CC1	3.76	8.69	2.31
GEI 2013	CC1	3.76	9.07	2.41
GEI 2013	CC1	4.69	5.92	1.26
GEI 2013	CC1	4.69	7.68	1.64
GEI 2013	CC1	4.69	7.59	1.62

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	6.49	1.39
GEI 2013	CC1	4.69	7.14	1.52
GEI 2013	CC1	5.86	6.68	1.14
GEI 2013	CC1	5.86	7.73	1.32
GEI 2013	CC1	5.86	7.88	1.35
GEI 2013	CC1	5.86	8.45	1.44
GEI 2013	CC1	5.86	11.69	2.00
GEI 2013	CC1	5.86	9.21	1.57
GEI 2013	CC1	5.86	9.70	1.66
GEI 2013	LG1	3.56	4.81	1.35
GEI 2013	LG1	3.56	4.86	1.37
GEI 2013	LG1	3.56	5.05	1.42
GEI 2013	LG1	3.56	5.47	1.54
GEI 2013	LG1	3.56	5.56	1.56
GEI 2013	LG1	3.56	3.72	1.05
GEI 2013	LG1	3.56	4.09	1.15
GEI 2013	LG1	3.56	3.26	0.92
GEI 2013	LG1	3.56	3.35	0.94
GEI 2013	LG1	3.56	4.20	1.18
GEI 2013	LG1	3.39	3.60	1.06
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.27	1.26
GEI 2013	LG1	3.39	4.45	1.31
GEI 2013	LG1	3.39	5.18	1.53
GEI 2013	LG1	3.39	5.51	1.63
Grasso et al. 1995	17	1.91	6.59	3.45
Grasso et al. 1995	17	1.91	6.60	3.46
Grasso et al. 1995	17	1.91	7.30	3.82
Grasso et al. 1995	10	1.85	2.74	1.48
Grasso et al. 1995	10	1.85	2.79	1.51
Grasso et al. 1995	10	1.85	2.90	1.57
Lambing et al. 1994	S46	6.20	5.10	0.82
Lambing et al. 1994	S48	3.05	2.50	0.82
Lambing et al. 1994	S11	14.50	11.00	0.76
Lambing et al. 1994	S11	14.50	33.00	2.28
Lambing et al. 1994	S34	14.00	25.00	1.79
Lambing et al. 1994	S39	5.85	7.90	1.35
Lambing et al. 1994	S39	5.85	21.00	3.59
Lemly 1985	Badin Lake	5.70	1.50	0.26
Lemly 1985	Belews Lake	51.15	13.60	0.27
Lemly 1985	High Rock Lake	9.05	1.89	0.21
GEI 2014	DC-4	19.42	27.69	1.43

Fathead minnow (*Pimephales promelas*)

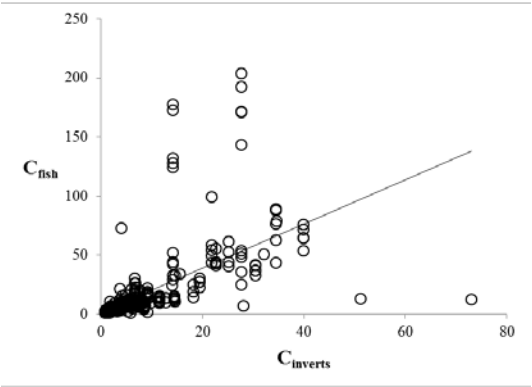
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	DC-4	19.42	27.88	1.44
GEI 2014	DC-4	19.42	23.05	1.19
GEI 2014	DC-4	19.42	30.61	1.58
GEI 2014	DC-4	18.10	19.32	1.07
GEI 2014	DC-4	18.10	14.48	0.80
GEI 2014	DC-4	18.10	25.42	1.40
GEI 2014	FOC-2	2.18	4.13	1.90
GEI 2014	FOC-2	2.18	4.10	1.89
GEI 2014	FOC-2	2.18	5.50	2.53
GEI 2014	FOC-2	2.18	4.85	2.23
GEI 2014	FOC-2	2.18	4.65	2.14
GEI 2014	SC-1	5.85	7.95	1.36
GEI 2014	SC-1	5.85	7.62	1.30
GEI 2014	SC-1	5.85	7.88	1.35
GEI 2014	SC-1	5.85	8.46	1.45
GEI 2014	SC-1	5.85	7.29	1.25
GEI 2014	SC-1	5.85	8.94	1.53
GEI 2014	SC-1	5.85	8.28	1.42
GEI 2014	SC-1	5.85	8.62	1.47
GEI 2014	SC-1	5.85	6.17	1.05
GEI 2014	SC-1	5.85	6.09	1.04
GEI 2014	SC-1	5.85	9.23	1.58
GEI 2014	SC-1	5.85	10.00	1.71
GEI 2014	SC-1	5.85	8.12	1.39
GEI 2014	SC-1	5.85	6.71	1.15
GEI 2014	SC-1	5.85	8.34	1.43
GEI 2014	SC-1	4.94	10.22	2.07
GEI 2014	SC-1	4.94	10.86	2.20
GEI 2014	SC-1	4.94	9.82	1.99
GEI 2014	SC-1	4.94	9.45	1.91
GEI 2014	SC-1	4.94	10.30	2.09
GEI 2014	SC-2	14.33	15.86	1.11
GEI 2014	SC-2	14.33	15.08	1.05
GEI 2014	SC-2	14.33	13.33	0.93
GEI 2014	SC-2	14.33	12.04	0.84
GEI 2014	SC-2	14.33	13.82	0.96
GEI 2014	SC-2	11.44	9.64	0.84
GEI 2014	SC-2	11.44	14.94	1.31
GEI 2014	SC-2	11.44	10.91	0.95
GEI 2014	SC-2	11.44	16.06	1.40
GEI 2014	SC-2	11.44	14.60	1.28
GEI 2014	SC-2	12.75	13.81	1.08

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-2	12.75	14.10	1.11
GEI 2014	SC-2	12.75	10.68	0.84
GEI 2014	SC-3	11.41	11.65	1.02
GEI 2014	SC-3	11.41	10.95	0.96
GEI 2014	SC-3	11.41	10.84	0.95
GEI 2014	SC-3	11.41	13.48	1.18
GEI 2014	SC-3	8.58	7.70	0.90
GEI 2014	SC-3	8.58	6.46	0.75
GEI 2014	SC-3	8.58	6.97	0.81
GEI 2014	SC-3	8.58	10.64	1.24
GEI 2014	SC-3	8.58	7.85	0.91
GEI 2014	SC-3	5.75	13.75	2.39
GEI 2014	SC-3	5.75	11.19	1.95
GEI 2014	SC-3	5.75	12.68	2.20
GEI 2014	SC-4	7.39	6.33	0.86
GEI 2014	SC-4	7.39	14.39	1.95
GEI 2014	SC-4	5.18	2.72	0.53
GEI 2014	SC-4	5.18	11.95	2.31
GEI 2014	SC-4	5.18	7.98	1.54
GEI 2014	SC-4	5.18	6.75	1.30
GEI 2014	SC-6	39.87	72.33	1.81
GEI 2014	SC-6	39.87	76.00	1.91
GEI 2014	SC-6	39.87	64.73	1.62
GEI 2014	SC-6	39.87	54.09	1.36
GEI 2014	SC-6	39.87	64.64	1.62
GEI 2014	SC-6	34.35	76.89	2.24
GEI 2014	SC-6	34.35	89.67	2.61
GEI 2014	SC-6	34.35	63.32	1.84
GEI 2014	SC-6	34.35	88.44	2.57
GEI 2014	SC-6	34.35	44.15	1.29
GEI 2014	SC-6	27.54	51.65	1.88
GEI 2014	SC-6	27.54	35.92	1.30
GEI 2014	SC-6	27.54	25.55	0.93
GEI 2014	SC-6	27.54	54.25	1.97
GEI 2014	SC-6	27.54	48.94	1.78
GEI 2014	SC-6	27.54	204.26	7.42
GEI 2014	SC-6	27.54	143.62	5.22
GEI 2014	SC-6	27.54	192.93	7.01
GEI 2014	SC-6	27.54	171.89	6.24
GEI 2014	SC-6	27.54	171.36	6.22
GEI 2014	SC-8	22.62	43.12	1.91
GEI 2014	SC-8	22.62	43.36	1.92

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-8	22.62	55.81	2.47
GEI 2014	SC-8	22.62	44.60	1.97
GEI 2014	SC-8	22.62	41.67	1.84
GEI 2014	SC-9	30.36	37.18	1.22
GEI 2014	SC-9	30.36	41.32	1.36
GEI 2014	SC-9	30.36	37.20	1.23
GEI 2014	SC-9	30.36	33.25	1.10
GEI 2014	SC-9	30.36	41.94	1.38
GEI 2014	SC-8	21.77	99.40	4.57
GEI 2014	SC-8	21.77	54.47	2.50
GEI 2014	SC-8	21.77	59.07	2.71
GEI 2014	SC-8	21.77	43.70	2.01
GEI 2014	SC-8	21.77	50.18	2.31
GEI 2014	SC-9	25.06	40.82	1.63
GEI 2014	SC-9	25.06	61.80	2.47
GEI 2014	SC-9	25.06	44.74	1.79
GEI 2014	SC-9	25.06	52.97	2.11
GEI 2014	SC-8	14.15	52.46	3.71
GEI 2014	SC-8	14.15	29.43	2.08
GEI 2014	SC-8	14.15	44.58	3.15
GEI 2014	SC-8	14.15	33.44	2.36
GEI 2014	SC-8	14.15	42.86	3.03
GEI 2014	SC-8	14.15	128.33	9.07
GEI 2014	SC-8	14.15	173.33	12.25
GEI 2014	SC-8	14.15	132.34	9.35
GEI 2014	SC-8	14.15	124.90	8.83
GEI 2014	SC-8	14.15	177.97	12.58



Median ratio: 1.57

R^2: 0.35
F: 185.7
df: 344
P: < 0.001

Flannelmouth sucker (*Catostomus latipinnis*)

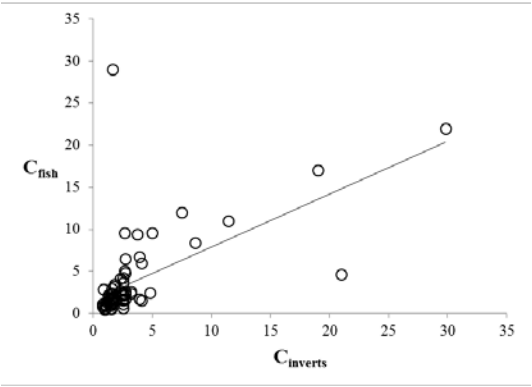
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.50	0.52
Butler et al. 1991	7	29.80	22.00	0.74
Butler et al. 1991	4	3.90	1.70	0.44
Butler et al. 1991	9	4.10	1.50	0.37
Butler et al. 1991	9	4.10	6.00	1.46
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	P1	1.50	2.40	1.60
Butler et al. 1993	LP3	1.12	0.92	0.83
Butler et al. 1993	LP3	1.12	1.40	1.26
Butler et al. 1993	LP4	3.20	2.40	0.75
Butler et al. 1993	LP4	3.20	2.60	0.81
Butler et al. 1994	CRC	7.50	12.00	1.60
Butler et al. 1994	LZA1	19.00	17.00	0.89
Butler et al. 1994	BSW1	5.00	9.60	1.92
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	COL1	1.50	0.50	0.33
Butler et al. 1994	COL1	1.50	0.60	0.40
Butler et al. 1994	COL1	1.50	0.63	0.42
Butler et al. 1994	COL1	1.50	0.92	0.61
Butler et al. 1994	COL1	1.50	1.00	0.67
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	COL1	1.50	1.70	1.13
Butler et al. 1994	COL1	1.50	1.80	1.20
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	RB3	1.60	29.00	18.13
Butler et al. 1994	RB1	21.00	4.60	0.22
Butler et al. 1994	LSW1	3.90	6.70	1.72
Butler et al. 1994	PSW1	3.70	9.40	2.54
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	AK	0.78	0.90	1.16
Butler et al. 1995	AK	0.78	0.82	1.06
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	HD1	0.83	2.90	3.52
Butler et al. 1995	HD2	0.98	0.49	0.50
Butler et al. 1995	HD2	0.98	0.54	0.55
Butler et al. 1995	HD2	0.98	0.62	0.63
Butler et al. 1995	HD2	0.98	0.96	0.98
Butler et al. 1995	ME2	1.25	1.60	1.28
Butler et al. 1995	ME2	1.25	1.40	1.12
Butler et al. 1995	ME2	1.25	2.00	1.60
Butler et al. 1995	ME2	1.25	2.20	1.76
Butler et al. 1995	ME4	1.55	1.50	0.97

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME4	1.55	1.30	0.84
Butler et al. 1995	ME4	1.55	1.90	1.23
Butler et al. 1995	ME4	1.55	2.40	1.55
Butler et al. 1995	ME4	1.55	3.00	1.94
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	2.10	0.82
Butler et al. 1995	ME3	2.55	2.40	0.94
Butler et al. 1995	ME3	2.55	3.60	1.41
Butler et al. 1995	SJ1	2.50	1.71	0.68
Butler et al. 1995	SJ1	2.50	1.50	0.60
Butler et al. 1995	SJ1	2.50	2.20	0.88
Butler et al. 1995	SJ1	2.50	0.61	0.24
Butler et al. 1995	SJ1	2.50	1.10	0.44
Butler et al. 1995	SJ1	2.50	4.20	1.68
Butler et al. 1995	YJ2	1.65	1.60	0.97
Butler et al. 1995	YJ2	1.65	2.40	1.45
Butler et al. 1995	MN1	2.70	6.50	2.41
Butler et al. 1995	MN1	2.70	1.70	0.63
Butler et al. 1995	MN1	2.70	4.80	1.78
Butler et al. 1995	MP	1.60	1.20	0.75
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1997	MN3	2.70	2.30	0.85
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MUD	2.30	4.10	1.78
Butler et al. 1997	MUD	2.30	2.70	1.17
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	MN4	2.65	5.10	1.92
Butler et al. 1997	MN4	2.65	9.60	3.62
Butler et al. 1997	MN5	8.60	8.40	0.98
Butler et al. 1997	MNQ	1.80	2.10	1.17
Butler et al. 1997	MNQ	1.80	3.20	1.78
Butler et al. 1997	MNQ	1.80	3.50	1.94

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio

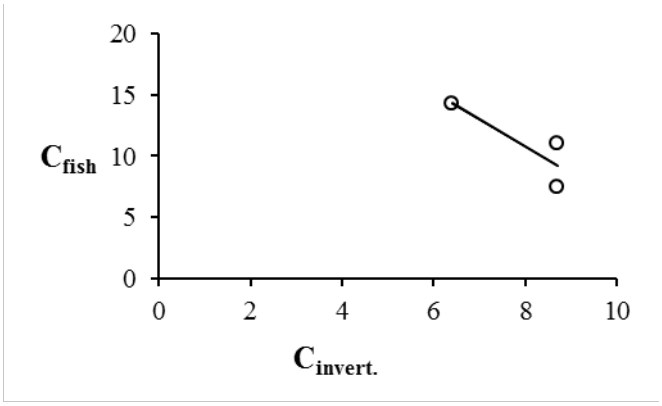


Median ratio: 0.98

R^2: 0.36
F: 41.6
df: 73
P: < 0.001

Gizzard shad (*Dorosoma cepedianum*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.30	2.23
Mueller et al. 1991	R1	8.70	7.50	0.86
Mueller et al. 1991	R1	8.70	11.00	1.26



Median ratio: 1.26

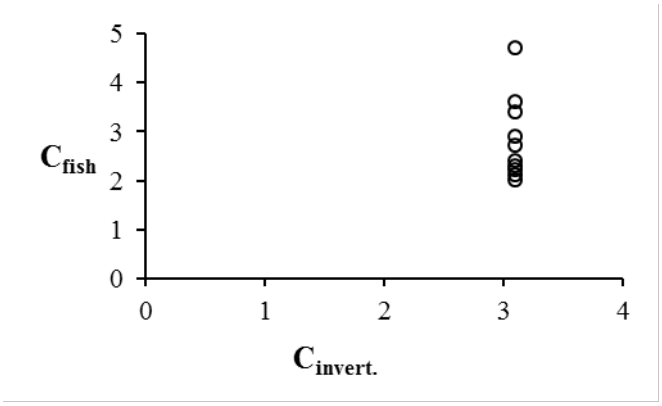
R^2: 0.74
F: 2.78
df: 1
P: 0.39
Not used because P > 0.05 and negative slope.

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	2.70	0.87

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	4.70	1.52



Median ratio: 0.82

R^2: 0.0
F: 0.0
df: 8
P: 1.0

Not used because no slope and P>0.05.

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	7.90	1.65
Butler et al. 1991	7	29.80	15.20	0.51
Butler et al. 1991	7	29.80	25.10	0.84
Butler et al. 1991	3	6.20	6.40	1.03
Butler et al. 1994	LZA1	19.00	37.00	1.95
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	ME3	2.55	5.00	1.96
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	CH1	7.50	9.50	1.27
Butler et al. 1997	MUD2	3.45	7.60	2.20
Butler et al. 1997	MUD2	3.45	7.00	2.03
Butler et al. 1997	TR25	1.80	4.40	2.44
Butler et al. 1997	TRH	1.60	3.30	2.06
GEI 2013	SWA1	2.81	2.96	1.06
GEI 2013	SWA1	2.81	3.21	1.14
GEI 2013	SWA1	2.81	3.24	1.16
GEI 2013	SWA1	2.81	3.69	1.32
GEI 2013	SWA1	2.81	3.88	1.38
GEI 2013	SWB	7.44	11.94	1.61
GEI 2013	SW11	8.41	4.54	0.54

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW11	8.41	4.84	0.58
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	7.00	0.83
GEI 2013	SW11	8.41	7.13	0.85
GEI 2013	SW9	4.45	4.38	0.98
GEI 2013	SW9	4.45	5.06	1.14
GEI 2013	SW9	4.45	5.53	1.24
GEI 2013	SW9	4.45	5.80	1.30
GEI 2013	SW9	4.45	7.29	1.64
GEI 2013	SW88	3.96	7.14	1.81
GEI 2013	SW88	3.96	7.41	1.87
GEI 2013	LG1	3.39	4.11	1.21
GEI 2013	LG1	3.39	4.33	1.28
GEI 2013	LG1	3.39	5.71	1.68
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	4.00	1.29
Roddy et al. 1991	18	3.10	5.20	1.68
Roddy et al. 1991	18	3.10	5.70	1.84
Lemly 1985	Badin Lake	5.70	2.18	0.38
Lemly 1985	Belews Lake	51.15	13.99	0.27
Lemly 1985	High Rock Lake	9.05	2.10	0.23
GEI 2014	C-BCR2	6.81	9.47	1.39
GEI 2014	C-BCR2	6.81	9.29	1.37
GEI 2014	C-BCR2	6.81	8.04	1.18
GEI 2014	C-CC1	4.40	7.23	1.64
GEI 2014	C-CC1	4.40	11.76	2.67
GEI 2014	C-CC2	5.56	4.59	0.83
GEI 2014	C-CC2	5.56	3.04	0.55
GEI 2014	C-CC2	5.56	5.34	0.96
GEI 2014	C-CF1	3.39	3.62	1.07
GEI 2014	C-CF1	3.39	2.95	0.87
GEI 2014	C-CF1	3.39	3.23	0.95
GEI 2014	C-CF1	3.39	5.55	1.64
GEI 2014	C-CLF1	9.30	3.99	0.43
GEI 2014	C-CLF1	9.30	5.23	0.56
GEI 2014	C-CLF1	9.30	4.75	0.51
GEI 2014	C-CLF1	9.30	4.87	0.52
GEI 2014	C-CLF1	9.30	3.58	0.39
GEI 2014	C-CLF2	6.85	5.76	0.84
GEI 2014	C-CLF2	6.85	5.89	0.86
GEI 2014	C-CLF2	6.85	4.78	0.70

Green sunfish (*Lepomis cyanellus*)

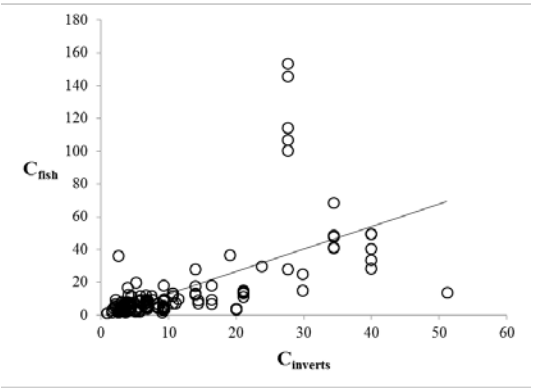
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	C-CLF2	6.85	5.11	0.75
GEI 2014	C-CLF2	6.85	4.10	0.60
GEI 2014	C-LFWOC1	9.32	10.10	1.08
GEI 2014	C-TF1	20.00	4.15	0.21
GEI 2014	C-TF1	20.00	3.47	0.17
GEI 2014	C-TF1	20.00	4.14	0.21
GEI 2014	C-TF1	20.00	4.11	0.21
GEI 2014	C-TF1	20.00	3.41	0.17
GEI 2014	C-WOC1	6.65	12.05	1.81
GEI 2014	C-WOC1	6.65	9.60	1.44
GEI 2014	C-WOC1	6.65	8.66	1.30
GEI 2014	C-WOC1	6.65	5.81	0.87
GEI 2014	C-WOC1	6.65	7.54	1.13
GEI 2014	H-BB1	16.29	9.55	0.59
GEI 2014	H-BB1	16.29	18.27	1.12
GEI 2014	H-BB1	16.29	7.08	0.43
GEI 2014	H-BHC1	5.08	3.69	0.73
GEI 2014	H-BHC1	5.08	2.48	0.49
GEI 2014	H-BHC1	5.08	3.29	0.65
GEI 2014	H-BHC1	5.08	3.49	0.69
GEI 2014	H-BHC1	5.08	3.70	0.73
GEI 2014	H-JSB1	4.03	4.83	1.20
GEI 2014	H-JSB1	4.03	2.57	0.64
GEI 2014	H-JSB1	4.03	3.73	0.93
GEI 2014	H-LF1	9.09	3.12	0.34
GEI 2014	H-LF1	9.09	5.96	0.66
GEI 2014	H-LF1	9.09	4.30	0.47
GEI 2014	H-LF1	9.09	4.02	0.44
GEI 2014	H-LF1	9.09	5.29	0.58
GEI 2014	H-MR2	2.14	9.57	4.47
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	5.80	2.71
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	6.88	3.22
GEI 2014	H-MR3	3.86	8.09	2.10
GEI 2014	H-MR3	3.86	16.98	4.40
GEI 2014	H-MR3	3.86	6.80	1.76
GEI 2014	H-MR3	3.86	8.52	2.21
GEI 2014	H-MR3	3.86	6.62	1.72
GEI 2014	H-MR4	9.26	9.01	0.97
GEI 2014	H-MR4	9.26	8.78	0.95
GEI 2014	H-MR4	9.26	18.33	1.98

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	H-MR4	9.26	9.84	1.06
GEI 2014	H-MR4	9.26	5.94	0.64
GEI 2014	H-MR5	3.58	5.50	1.54
GEI 2014	H-MR5	3.58	3.52	0.98
GEI 2014	H-MR5	3.58	2.41	0.67
GEI 2014	H-MR5	3.58	3.09	0.86
GEI 2014	H-MR5	3.58	1.94	0.54
GEI 2014	H-MR6	2.49	36.20	14.54
GEI 2014	H-MR6	2.49	2.58	1.04
GEI 2014	H-MR6	2.49	1.94	0.78
GEI 2014	H-MR6	2.49	1.74	0.70
GEI 2014	H-MR6	2.49	2.69	1.08
GEI 2014	H-SB1	10.62	11.90	1.12
GEI 2014	H-SB1	10.62	13.39	1.26
GEI 2014	H-SB1	10.62	7.64	0.72
GEI 2014	H-SB1	10.62	13.45	1.27
GEI 2014	H-SF1	21.05	13.59	0.65
GEI 2014	H-SF1	21.05	14.22	0.68
GEI 2014	H-SF1	21.05	15.27	0.73
GEI 2014	H-SF1	21.05	14.58	0.69
GEI 2014	H-SF1	21.05	11.25	0.53
GEI 2014	H-SF2	13.95	17.74	1.27
GEI 2014	H-SF2	13.95	12.86	0.92
GEI 2014	H-SF2	13.95	12.76	0.91
GEI 2014	H-SF2	13.95	13.41	0.96
GEI 2014	H-SF2	13.95	28.23	2.02
GEI 2014	H-UB1	3.02	4.43	1.47
GEI 2014	H-UB1	3.02	4.91	1.63
GEI 2014	H-UB1	3.02	3.73	1.23
GEI 2014	H-UB1	3.02	8.00	2.65
GEI 2014	H-UB1	3.02	8.36	2.77
GEI 2014	SC-1-25	4.00	12.78	3.19
GEI 2014	SC-2	14.33	9.31	0.65
GEI 2014	SC-2	14.33	7.59	0.53
GEI 2014	SC-2	11.44	10.23	0.89
GEI 2014	SC-2-27	23.76	30.00	1.26
GEI 2014	SC-3	5.75	8.66	1.51
GEI 2014	SC-3	5.75	11.72	2.04
GEI 2014	SC-3	5.75	9.59	1.67
GEI 2014	SC-4	5.18	19.86	3.83
GEI 2014	SC-6	39.87	49.55	1.24
GEI 2014	SC-6	39.87	49.56	1.24

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-6	39.87	33.71	0.85
GEI 2014	SC-6	39.87	28.73	0.72
GEI 2014	SC-6	39.87	40.64	1.02
GEI 2014	SC-6	34.35	41.51	1.21
GEI 2014	SC-6	34.35	48.02	1.40
GEI 2014	SC-6	34.35	68.66	2.00
GEI 2014	SC-6	34.35	41.27	1.20
GEI 2014	SC-6	34.35	48.76	1.42
GEI 2014	SC-6	27.54	28.17	1.02
GEI 2014	SC-6	27.54	106.88	3.88
GEI 2014	SC-6	27.54	114.55	4.16
GEI 2014	SC-6	27.54	100.41	3.65
GEI 2014	SC-6	27.54	153.64	5.58
GEI 2014	SC-6	27.54	145.62	5.29
GEI 2014	S-SC1	11.01	7.47	0.68



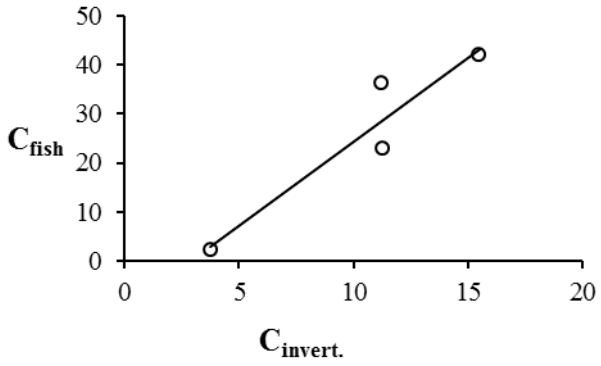
Median ratio: 1.12

R^2: 0.37
F: 93.17
df: 160
P: < 0.001

Iowa darter (*Etheostoma exile*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	7	3.75	2.10	0.56
Birkner 1978	20	11.20	36.30	3.24
Birkner 1978	22	11.30	23.00	2.04
Birkner 1978	23	15.50	41.90	2.70

Iowa darter (*Etheostoma exile*)



Median ratio: 2.37

R^2: 0.90
F: 17.3
df: 2
P: 0.055
Not used because P > 0.05

Largemouth bass (*Micropterus salmoides*)

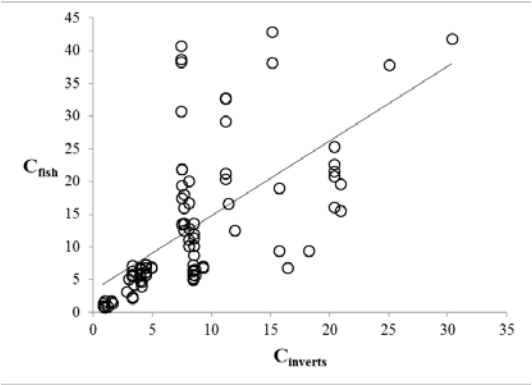
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	MP	1.60	1.40	0.88
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	5.10	1.70
GEI 2013	SWA1	2.81	3.17	1.13
GEI 2013	SW11	8.41	5.02	0.60
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	5.19	0.62
GEI 2013	SW11	8.41	6.26	0.74
GEI 2013	SW11	8.41	6.48	0.77
GEI 2013	SW11	8.41	7.22	0.86
GEI 2013	SW4-1	3.33	5.53	1.66
GEI 2013	SW4-1	3.33	5.65	1.70
GEI 2013	SW4-1	3.33	5.72	1.72
GEI 2013	SW4-1	3.33	5.80	1.74
GEI 2013	SW4-1	3.33	6.34	1.91
GEI 2013	SW4-1	3.33	7.14	2.15
GEI 2013	SW9	4.45	5.78	1.30
GEI 2013	SW9	4.45	5.79	1.30
GEI 2013	SW9	4.45	6.19	1.39
GEI 2013	SW9	4.45	6.87	1.54
GEI 2013	SW9	4.45	7.27	1.63
GEI 2013	SW9	4.45	7.36	1.65
GEI 2013	SW88	3.96	4.87	1.23
GEI 2013	SW88	3.96	5.73	1.45
GEI 2013	SW88	3.96	5.77	1.46
GEI 2013	SW88	3.96	5.93	1.50
GEI 2013	SW88	3.96	6.62	1.67
GEI 2013	SW88	3.96	6.84	1.73

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	LG1	3.39	4.29	1.27
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.80	1.39
Saiki et al. 1993	GT5	4.90	6.90	1.41
Saiki et al. 1993	GT4	4.05	4.70	1.16
Saiki et al. 1993	GT4	4.05	4.00	0.99
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR2	3.30	2.40	0.73
Saiki et al. 1993	SJR3	1.50	1.80	1.20
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR1	0.95	0.80	0.85
Saiki et al. 1993	SJR1	0.95	1.80	1.90
Saiki et al. 1993	ET7	0.86	0.86	1.00
Saiki et al. 1993	ET7	0.86	1.00	1.16
Rinella and Schuler 1992	S. Malheur Lake	1.20	0.92	0.77
Crutchfield 2000	transect 3	11.95	12.52	1.05
Crutchfield 2000	transect 3	11.40	16.67	1.46
Crutchfield 2000	transect 3	9.25	6.83	0.74
Crutchfield 2000	transect 3	9.25	6.99	0.76
Crutchfield 2000	transect 3	8.60	6.59	0.77
Crutchfield 2000	transect 3	8.60	5.69	0.66
Crutchfield 2000	transect 4	20.90	15.53	0.74
Crutchfield 2000	transect 4	20.90	19.68	0.94
Crutchfield 2000	transect 4	15.70	18.95	1.21
Crutchfield 2000	transect 4	15.70	9.43	0.60
Crutchfield 2000	transect 4	16.45	6.83	0.42
Crutchfield 2000	transect 4	18.25	9.43	0.52
GEI 2014	ARB	11.21	20.41	1.82
GEI 2014	ARB	11.21	32.75	2.92
GEI 2014	ARB	11.21	32.73	2.92
GEI 2014	ARB	11.21	29.23	2.61
GEI 2014	ARB	11.21	21.26	1.90
GEI 2014	ARE	20.40	25.35	1.24
GEI 2014	ARE	20.40	20.80	1.02
GEI 2014	ARE	20.40	22.67	1.11
GEI 2014	ARE	20.40	21.57	1.06
GEI 2014	ARE	20.40	16.05	0.79
GEI 2014	ARM	8.51	13.62	1.60
GEI 2014	ARM	8.51	10.13	1.19
GEI 2014	ARM	8.51	12.00	1.41
GEI 2014	ARM	8.51	11.40	1.34

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARM	8.51	8.71	1.02
GEI 2014	ARM	7.68	12.52	1.63
GEI 2014	ARM	7.68	13.59	1.77
GEI 2014	ARM	7.68	17.99	2.34
GEI 2014	ARM	7.68	13.49	1.76
GEI 2014	ARM	7.68	15.98	2.08
GEI 2014	ARN	8.06	11.16	1.39
GEI 2014	ARN	8.06	12.89	1.60
GEI 2014	ARN	8.06	16.74	2.08
GEI 2014	ARN	8.06	10.12	1.26
GEI 2014	ARN	8.06	20.08	2.49
GEI 2014	ARN	7.49	17.47	2.33
GEI 2014	ARN	7.49	13.48	1.80
GEI 2014	ARN	7.49	19.44	2.60
GEI 2014	ARN	7.49	21.87	2.92
GEI 2014	ARN	7.49	21.91	2.92
GEI 2014	ARN	7.44	30.73	4.13
GEI 2014	ARN	7.44	40.71	5.47
GEI 2014	ARN	7.44	38.75	5.21
GEI 2014	ARN	7.44	38.24	5.14
GEI 2014	SC-5	15.13	38.13	2.52
GEI 2014	SC-5	15.13	42.86	2.83
GEI 2014	SC-9	30.36	41.87	1.38
GEI 2014	SC-9	25.06	37.84	1.51

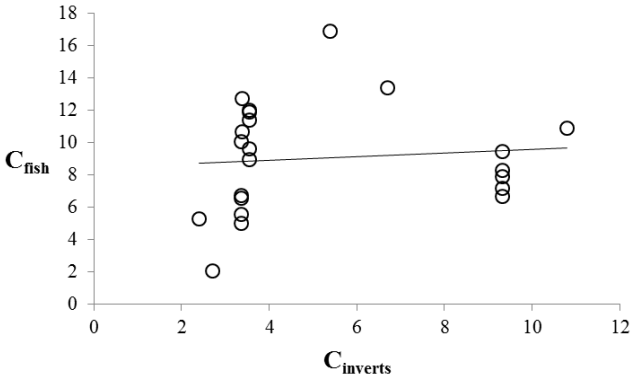


Median ratio: 1.39

R^2: 0.40
F: 61.45
df: 91
P: < 0.001

Longnose dace (*Rhinichthys cataractae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Lambing et al. 1994	S33	2.40	5.30	2.21
Mueller et al. 1991	A1	2.70	2.10	0.78
GEI 2013	LG1	3.37	5.05	1.50
GEI 2013	LG1	3.37	5.57	1.65
GEI 2013	LG1	3.37	6.57	1.95
GEI 2013	LG1	3.37	6.75	2.00
GEI 2013	LG1	3.37	10.08	2.99
GEI 2013	LG1	3.39	10.69	3.15
GEI 2013	LG1	3.39	12.77	3.77
GEI 2013	LG1	3.56	8.95	2.52
GEI 2013	LG1	3.56	9.63	2.71
GEI 2013	LG1	3.56	11.41	3.21
GEI 2013	LG1	3.56	11.94	3.36
GEI 2013	LG1	3.56	12.04	3.39
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	8.32	0.89
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	9.47	1.02
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.94	0.85
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	6.67	0.72
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.19	0.77
Mueller et al. 1991	T1	5.40	16.90	3.13
Hamilton and Buhl 2005	CC	6.70	13.40	2.00
Hamilton and Buhl 2005	BGS	10.80	10.90	1.01

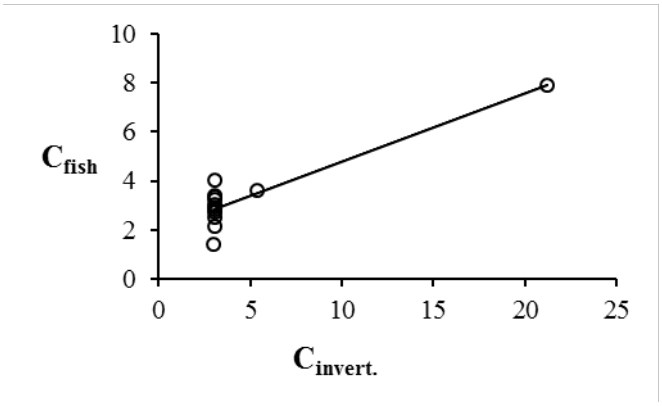


Median ratio: 2.00

R^2: 0.01
F: 0.19
df: 20
P: 0.83
Not used because P > 0.05

Longnose sucker (*Catostomus catostomus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	FL17	3.03	1.40	0.46
Butler et al. 1994	NFK2	3.10	2.10	0.68
Butler et al. 1994	NFK2	3.10	2.50	0.81
Butler et al. 1994	NFK2	3.10	2.70	0.87
Butler et al. 1994	NFK2	3.10	2.80	0.90
Butler et al. 1994	NFK2	3.10	2.90	0.94
Butler et al. 1994	NFK2	3.10	3.00	0.97
Butler et al. 1994	NFK2	3.10	3.20	1.03
Butler et al. 1994	NFK2	3.10	3.30	1.06
Butler et al. 1994	NFK2	3.10	3.40	1.10
Butler et al. 1994	NFK2	3.10	4.00	1.29
Mueller et al. 1991	T1	5.40	3.60	0.67
Minnow 2007	FL17	21.22	7.90	0.37



Median ratio: 0.90

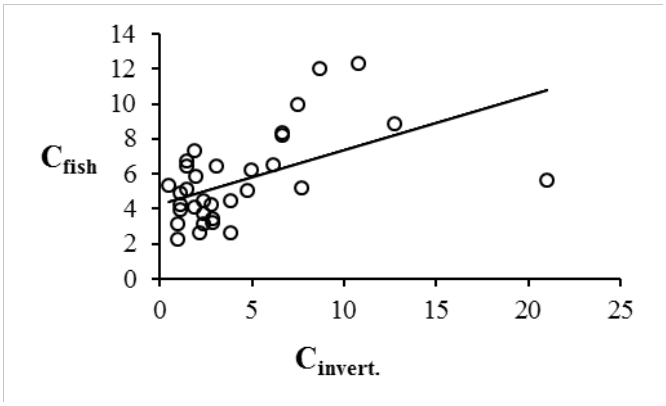
R^2: 0.83
F: 54.66
df: 11
P: <0.001

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	5.30	10.60
Butler et al. 1993	LP2	1.00	2.20	2.20
Butler et al. 1993	LP2	1.00	3.10	3.10
Butler et al. 1993	LP3	1.12	3.90	3.50
Butler et al. 1993	LP3	1.12	4.20	3.77
Butler et al. 1993	LP3	1.12	4.90	4.39
Butler et al. 1993	P1	1.50	5.10	3.40
Butler et al. 1993	P1	1.50	6.40	4.27
Butler et al. 1993	P1	1.50	6.70	4.47
Hamilton and Buhl 2004	ShpC	1.90	4.10	2.16
Butler et al. 1993	P1	1.95	7.30	3.74
Butler et al. 1994	NFK3	2.00	5.80	2.90

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	MN2	2.20	2.60	1.18
Butler et al. 1997	CHK	2.40	3.10	1.29
Butler et al. 1997	CHK	2.40	4.40	1.83
Lambing et al. 1994	S33	2.40	3.70	1.54
Butler et al. 1991	12	2.80	4.20	1.50
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	MN1	2.90	3.40	1.17
Butler et al. 1994	NFK2	3.10	6.40	2.06
Butler et al. 1991	4	3.90	2.60	0.67
Butler et al. 1991	4	3.90	4.40	1.13
Butler et al. 1991	10	4.80	5.00	1.04
Hamilton and Buhl 2005	UAC	5.00	6.20	1.24
Butler et al. 1991	3	6.20	6.50	1.05
Hamilton and Buhl 2004	ACM	6.70	8.30	1.24
Hamilton and Buhl 2005	CC	6.70	8.20	1.22
Butler et al. 1993	F2	7.50	9.90	1.32
Hamilton and Buhl 2004	LBR	7.70	5.20	0.68
Hamilton and Buhl 2005	DC	8.70	12.00	1.38
Hamilton and Buhl 2005	BGS	10.80	12.30	1.14
Hamilton and Buhl 2004	DVC	12.80	8.80	0.69
Butler et al. 1994	HCC1	21.00	5.60	0.27



Median ratio: 1.38

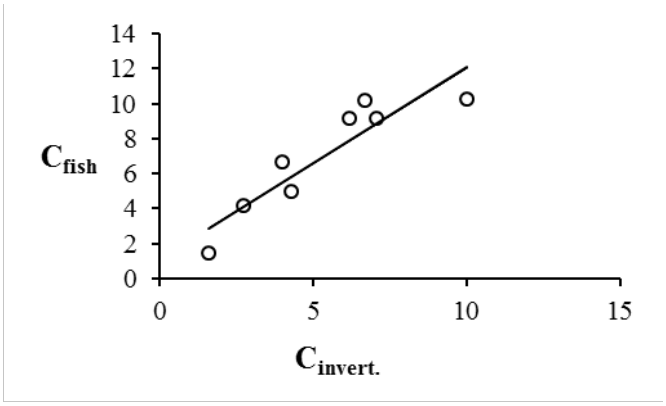
R^2: 0.27
F: 11.62
df: 31
P: < 0.001

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Low and Mullins 1990	7	1.60	1.40	0.88
McDonald and Strosher 1998	ER 745	2.74	4.17	1.52
Minnow 2007	EL12	4.01	6.60	1.65
McDonald and Strosher 1998	ER 747	4.29	4.93	1.15

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	MI3	6.21	9.12	1.47
Minnow 2007	MI2	6.69	10.16	1.52
Minnow 2007	EL1	7.08	9.12	1.29
Minnow 2007	FO23	10.00	10.20	1.02



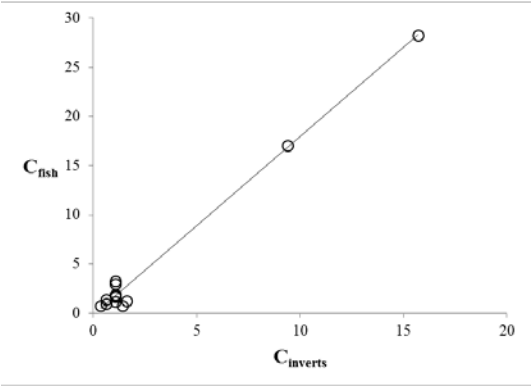
Median ratio: 1.38

R^2: 0.83
F: 30.27
df: 6
P: <0.001

Northern pike (*Esox lucius*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.96	2.78
Butler et al. 1995	TT	1.07	3.24	3.04
Butler et al. 1995	TT	1.07	1.65	1.55
Butler et al. 1995	TT	1.07	1.18	1.11
Butler et al. 1995	TT	1.07	1.90	1.78
Butler et al. 1995	TT	1.07	1.80	1.69
Butler et al. 1995	PU	0.61	0.93	1.52
Butler et al. 1995	PU	0.61	1.40	2.30
Muscatello et al. 2008	David Lake	1.39	0.78	0.56
Muscatello et al. 2008	Delta Lake	9.38	17.02	1.81
Muscatello et al. 2008	Unknown Lake	15.71	28.28	1.80
Muscatello and Janz 2009	Indigo Lake	0.36	0.75	2.08
Muscatello and Janz 2009	Vulture Lake	1.62	1.26	0.78

Northern pike (*Esox lucius*)

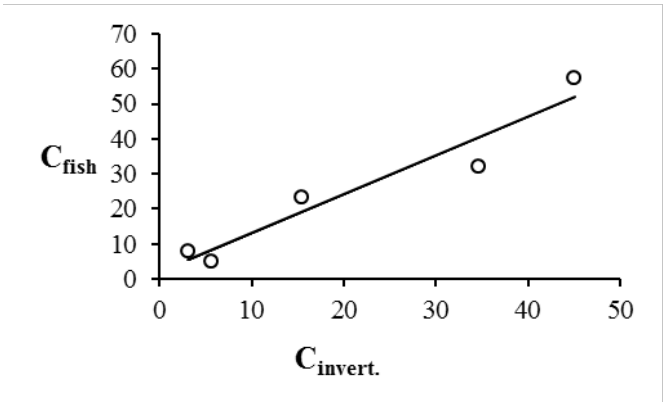


Median ratio: 1.78

R^2: 0.99
F: 982.9
df: 11
P: <0.001

Northern plains killfish (*Fundulus kansae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	3	3.10	7.70	2.48
Birkner 1978	11	5.65	5.00	0.88
Birkner 1978	23	15.50	23.10	1.49
Birkner 1978	27	34.60	31.90	0.92
Birkner 1978	30	45.05	57.40	1.27



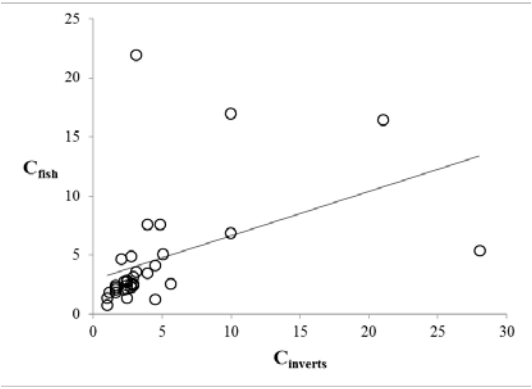
Median ratio: 1.27

R^2: 0.93
F: 37.8
df: 3
P: 0.008

Rainbow trout (*Oncorhynchus mykiss*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1993	F2	4.80	7.60	1.58
Butler et al. 1993	F2	3.90	7.60	1.95
Butler et al. 1993	LP2	1.00	0.78	0.78
Butler et al. 1993	LP2	1.00	1.40	1.40
Butler et al. 1993	LP3	1.12	1.90	1.70
Butler et al. 1994	GUN2	28.00	5.40	0.19
Butler et al. 1994	HCC1	21.00	16.48	0.78
Butler et al. 1994	NFK3	2.00	4.70	2.35
Butler et al. 1994	NFK2	3.10	21.98	7.09
Butler et al. 1994	NFK2	3.10	3.60	1.16
Butler et al. 1995	MP	1.60	1.88	1.18
Butler et al. 1995	MP	1.60	2.30	1.44
Butler et al. 1995	MP	1.60	2.50	1.56
Butler et al. 1995	MP	1.60	2.10	1.31
Butler et al. 1997	CHK	2.40	1.41	0.59
Butler et al. 1997	CHK	2.40	2.20	0.92
Butler et al. 1997	CHK	2.40	2.50	1.04
Butler et al. 1997	CHK	2.40	2.80	1.17
Butler et al. 1997	CHK	2.40	2.90	1.21
Butler et al. 1997	MN3	2.70	2.28	0.84
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MN3	2.70	4.90	1.81
Butler et al. 1997	MN2	2.20	2.10	0.95
Butler et al. 1997	MN2	2.20	2.80	1.27
Butler et al. 1997	MN1	2.90	2.50	0.86
Butler et al. 1997	MN1	2.90	2.60	0.90
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	WBR	5.05	5.10	1.01
Low and Mullins 1990	5	5.60	2.60	0.46
Casey 2005	Deerlick Creek	4.45	1.29	0.29
Casey 2005	Deerlick Creek	4.45	4.15	0.93
Casey 2005	Luscar Creek	9.95	6.88	0.69
Casey 2005	Luscar Creek	9.95	17.04	1.71

Rainbow trout (*Oncorhynchus mykiss*)



Median ratio: 1.07

R^2: 0.19
F: 7.52
df: 32
P: 0.002

Red shiner (*Cyprinella lutrensis*)

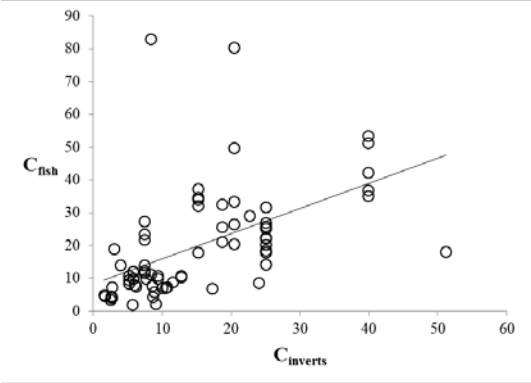
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	3	6.20	7.70	1.24
Butler et al. 1994	IW	8.35	83.00	9.94
Butler et al. 1994	AD	2.70	7.30	2.70
Butler et al. 1994	LW	3.00	19.00	6.33
Butler et al. 1994	LSW1	3.90	14.00	3.59
Butler et al. 1995	ME4	1.55	5.10	3.29
Butler et al. 1995	ME3	2.55	4.60	1.80
Butler et al. 1995	ME3	2.55	4.20	1.65
Butler et al. 1995	SJ1	2.50	3.50	1.40
Butler et al. 1995	YJ2	1.65	4.50	2.73
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	4.40	0.51
May et al. 2008	KR	17.20	7.03	0.41
May et al. 2008	NSCL	10.70	7.36	0.69
May et al. 2008	NSCU	10.50	7.24	0.69
May et al. 2008	NSK	8.81	5.81	0.66
May et al. 2008	NSP	24.00	8.62	0.36
May et al. 2008	SSAL	11.50	9.00	0.78
May et al. 2008	SSAU	8.35	11.20	1.34
May et al. 2008	SSO	10.00	7.16	0.72
May et al. 2008	SSW	7.60	10.00	1.32
Mueller et al. 1991	A3	6.00	8.10	1.35
Mueller et al. 1991	A2	8.50	7.90	0.93
Lemly 1985	Badin Lake	5.70	2.10	0.37
Lemly 1985	Belews Lake	51.15	18.25	0.36
Lemly 1985	High Rock Lake	9.05	2.18	0.24
GEI 2014	ARE	20.40	49.84	2.44

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARE	20.40	80.50	3.95
GEI 2014	ARE	20.40	20.57	1.01
GEI 2014	ARE	20.40	33.37	1.64
GEI 2014	ARE	20.40	26.50	1.30
GEI 2014	ARN	7.44	27.50	3.70
GEI 2014	ARN	7.44	23.58	3.17
GEI 2014	ARN	7.44	21.74	2.92
GEI 2014	FC-4	18.65	21.20	1.14
GEI 2014	FC-4	18.65	32.68	1.75
GEI 2014	FC-4	18.65	25.73	1.38
GEI 2014	GC-1	9.33	10.78	1.16
GEI 2014	GC-1	9.33	9.97	1.07
GEI 2014	SC-2	12.75	10.44	0.82
GEI 2014	SC-2	12.75	10.87	0.85
GEI 2014	SC-3	5.75	12.05	2.10
GEI 2014	SC-3	5.75	12.17	2.12
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-4	7.39	12.26	1.66
GEI 2014	SC-4	7.39	11.68	1.58
GEI 2014	SC-4	7.39	14.15	1.92
GEI 2014	SC-4	5.18	9.58	1.85
GEI 2014	SC-4	5.18	8.43	1.63
GEI 2014	SC-4	5.18	10.83	2.09
GEI 2014	SC-5	15.13	17.96	1.19
GEI 2014	SC-5	15.13	34.71	2.29
GEI 2014	SC-5	15.13	34.05	2.25
GEI 2014	SC-5	15.13	37.28	2.46
GEI 2014	SC-5	15.13	32.18	2.13
GEI 2014	SC-6	39.87	53.60	1.34
GEI 2014	SC-6	39.87	37.00	0.93
GEI 2014	SC-6	39.87	35.11	0.88
GEI 2014	SC-6	39.87	51.39	1.29
GEI 2014	SC-6	39.87	42.31	1.06
GEI 2014	SC-8	22.62	29.20	1.29
GEI 2014	SC-9	25.06	22.55	0.90
GEI 2014	SC-9	25.06	18.02	0.72
GEI 2014	SC-9	25.06	25.94	1.04
GEI 2014	SC-9	25.06	18.68	0.75
GEI 2014	SC-9	25.06	14.28	0.57
GEI 2014	SC-9	25.06	31.67	1.26
GEI 2014	SC-9	25.06	20.43	0.82

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-9	25.06	22.27	0.89
GEI 2014	SC-9	25.06	27.05	1.08
GEI 2014	SC-9	25.06	25.28	1.01



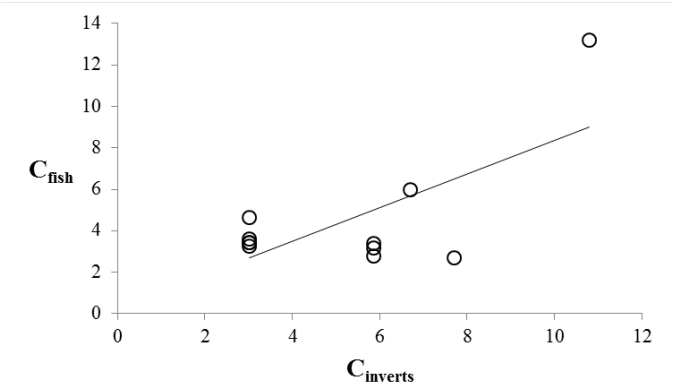
Median ratio: 1.31

R^2: 0.28
F: 26.57
df: 70
P: <0.001

Redside shiner (*Richardsonius balteatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ACM	6.70	6.00	0.90
Hamilton and Buhl 2004	LBR	7.70	2.70	0.35
Hamilton and Buhl 2005	BGS	10.80	13.20	1.22
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	4.64	1.54
GEI 2014	Bond Creek, BC-3	3.02	3.26	1.08
GEI 2014	Bond Creek, BC-3	5.87	3.18	0.54
GEI 2014	Bond Creek, BC-3	5.87	3.37	0.57
GEI 2014	Bond Creek, BC-3	5.87	2.79	0.47
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19

Redside shiner (*Richardsonius balteatus*)



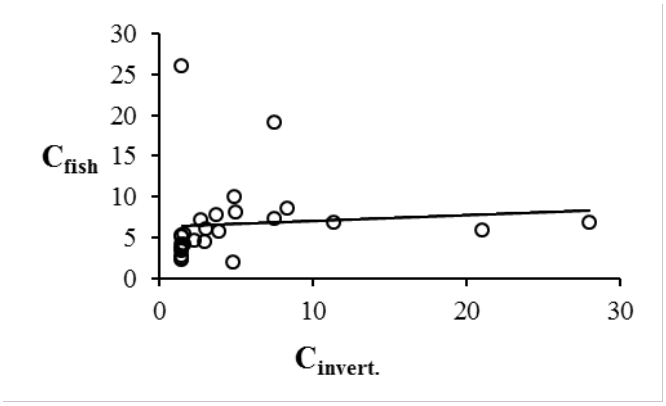
Median ratio: 1.08

R^2: 0.47
F: 7.84
df: 9
P: 0.011

Roundtail chub (*Gila robusta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	COL1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	2.50	1.67
Butler et al. 1994	COL1	1.50	2.70	1.80
Butler et al. 1994	COL1	1.50	3.30	2.20
Butler et al. 1994	COL1	1.50	3.70	2.47
Butler et al. 1994	COL1	1.50	4.10	2.73
Butler et al. 1994	COL1	1.50	5.10	3.40
Butler et al. 1994	COL1	1.50	5.30	3.53
Butler et al. 1994	COL1	1.50	26.00	17.33
Butler et al. 1994	RB3	1.60	5.40	3.38
Butler et al. 1995	MP	1.60	4.20	2.63
Butler et al. 1997	MUD	2.30	4.60	2.00
Butler et al. 1994	AD	2.70	7.10	2.63
Butler et al. 1994	LW	3.00	4.50	1.50
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	PSW1	3.70	7.70	2.08
Butler et al. 1994	LSW1	3.90	5.80	1.49
Butler et al. 1991	10	4.80	1.90	0.40
Butler et al. 1994	TGC	4.90	10.00	2.04
Butler et al. 1994	BSW1	5.00	8.10	1.62
Butler et al. 1993	F2	7.50	7.30	0.97
Butler et al. 1994	CRC	7.50	19.00	2.53
Butler et al. 1994	IW	8.35	8.50	1.02
Butler et al. 1997	NW2	11.40	6.90	0.61
Butler et al. 1994	RB1	21.00	5.90	0.28
Butler et al. 1994	GUN2	28.00	6.80	0.24

Roundtail chub (*Gila robusta*)



Median ratio: 1.98

R^2: 0.01
F: 0.18
df: 24
P: 0.834
Not used because P > 0.05

Sand shiner (*Notropis stramineus*)

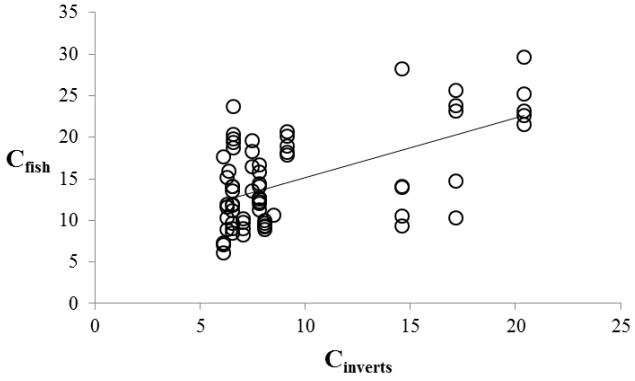
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	6.54	8.43	1.29
GEI 2013	SW1	6.54	9.02	1.38
GEI 2013	SW1	6.54	9.66	1.48
GEI 2013	SW1	6.54	11.21	1.71
GEI 2013	SW1	6.54	11.85	1.81
GEI 2013	SW1	6.54	11.94	1.83
GEI 2013	SW1	6.54	13.50	2.06
GEI 2013	SW1	6.54	14.05	2.15
GEI 2013	SW1	6.54	14.14	2.16
GEI 2013	SW2-1	6.60	18.70	2.84
GEI 2013	SW2-1	6.60	19.33	2.93
GEI 2013	SW2-1	6.60	19.77	3.00
GEI 2013	SW2-1	6.60	20.39	3.09
GEI 2013	SW2-1	6.60	23.70	3.59
GEI 2013	SWB	7.06	8.27	1.17
GEI 2013	SWB	7.06	9.01	1.28
GEI 2013	SWB	7.06	9.81	1.39
GEI 2013	SWB	7.06	10.22	1.45
GEI 2013	SW1	7.82	11.33	1.45
GEI 2013	SW1	7.82	12.05	1.54
GEI 2013	SW1	7.82	12.22	1.56
GEI 2013	SW1	7.82	12.55	1.60
GEI 2013	SW1	7.82	12.65	1.62
GEI 2013	SW1	7.82	12.68	1.62
GEI 2013	SW1	7.82	14.13	1.81
GEI 2013	SW1	7.82	14.43	1.85
GEI 2013	SW1	7.82	15.87	2.03

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	16.63	2.13
GEI 2013	SW2-1	9.14	17.84	1.95
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	18.98	2.08
GEI 2013	SW2-1	9.14	20.12	2.20
GEI 2013	SW2-1	9.14	20.73	2.27
GEI 2014	Arkansas River, ARE	20.40	21.50	1.05
GEI 2014	Arkansas River, ARE	20.40	23.20	1.14
GEI 2014	Arkansas River, ARE	20.40	22.64	1.11
GEI 2014	Arkansas River, ARE	20.40	25.24	1.24
GEI 2014	Arkansas River, ARE	20.40	29.70	1.46
GEI 2014	Arkansas River, ARM	8.51	10.67	1.25
GEI 2014	Arkansas River, ARN	8.06	9.69	1.20
GEI 2014	Arkansas River, ARN	8.06	9.27	1.15
GEI 2014	Arkansas River, ARN	8.06	9.96	1.24
GEI 2014	Arkansas River, ARN	8.06	9.29	1.15
GEI 2014	Arkansas River, ARN	8.06	8.86	1.10
GEI 2014	Arkansas River, ARN	7.49	13.60	1.82
GEI 2014	Arkansas River, ARN	7.49	18.34	2.45
GEI 2014	Arkansas River, ARN	7.49	16.46	2.20
GEI 2014	Arkansas River, ARN	7.49	19.64	2.62
GEI 2014	Fountain Creek, FC-4	14.59	13.95	0.96
GEI 2014	Fountain Creek, FC-4	14.59	9.34	0.64
GEI 2014	Fountain Creek, FC-4	14.59	14.06	0.96
GEI 2014	Fountain Creek, FC-4	14.59	28.26	1.94
GEI 2014	Fountain Creek, FC-4	14.59	10.53	0.72
GEI 2014	Fountain Creek, FC-4	17.15	10.28	0.60
GEI 2014	Fountain Creek, FC-4	17.15	23.76	1.39
GEI 2014	Fountain Creek, FC-4	17.15	14.77	0.86
GEI 2014	Fountain Creek, FC-4	17.15	23.13	1.35
GEI 2014	Fountain Creek, FC-4	17.15	25.62	1.49
GEI 2014	Fountain Creek, FCP	6.13	17.62	2.88
GEI 2014	Fountain Creek, FCP	6.13	7.32	1.19
GEI 2014	Fountain Creek, FCP	6.13	7.14	1.17
GEI 2014	Fountain Creek, FCP	6.13	6.05	0.99
GEI 2014	Fountain Creek, FCP	6.13	7.11	1.16
GEI 2014	Fountain Creek, FCP	6.35	15.93	2.51
GEI 2014	St. Charles River, SC-4	6.29	11.92	1.90
GEI 2014	St. Charles River, SC-4	6.29	15.14	2.41
GEI 2014	St. Charles River, SC-4	6.29	8.94	1.42
GEI 2014	St. Charles River, SC-4	6.29	10.33	1.64
GEI 2014	St. Charles River, SC-4	6.29	11.58	1.84

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.56

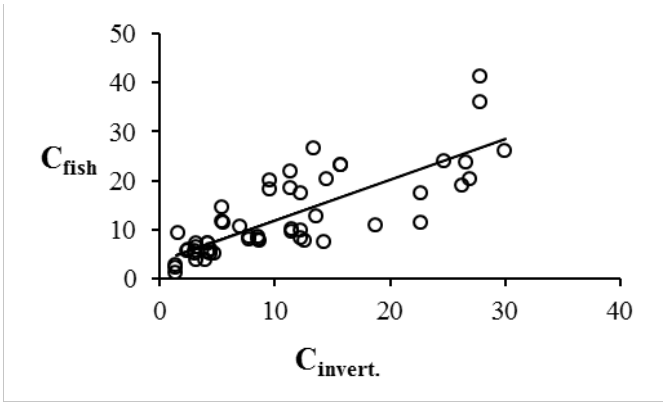
R^2: 0.32
F: 32.15
df: 67
P: <0.001

Sculpin (*Cottoidea*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	BK	1.43	1.16	0.81
Mason et al. 2000	BK	1.43	2.35	1.64
Mason et al. 2000	BK	1.43	2.64	1.84
Formation 2012	SFTC-1	1.63	9.31	5.71
Formation 2012	SFTC-1	2.42	5.68	2.35
Formation 2012	SFTC-1	2.49	5.87	2.36
Formation 2012	CC-75	3.11	5.03	1.62
Formation 2012	CC-75	3.11	5.58	1.79
Formation 2012	CC-350	3.16	6.47	2.05
Formation 2012	CC-350	3.16	7.12	2.26
Formation 2012	SFTC-1	3.21	3.75	1.17
Formation 2012	CC-75	3.97	3.77	0.95
Formation 2012	CC-75	4.16	7.08	1.70
Formation 2012	CC-75	4.16	7.19	1.73
Formation 2012	CC-350	4.20	5.28	1.26
Formation 2012	CC-150	4.46	5.04	1.13
Formation 2012	CC-150	4.46	6.01	1.35
Formation 2012	CC-150	4.70	5.14	1.09
Formation 2012	CC-3A	5.45	11.65	2.14
Formation 2012	CC-3A	5.45	14.45	2.65
Formation 2012	CC-3A	5.48	11.47	2.09
Formation 2012	CC-150	7.03	10.73	1.53
Formation 2012	DC-600	7.83	7.96	1.02
Formation 2012	DC-600	7.83	8.62	1.10

Sculpin (*Cottoidea*)

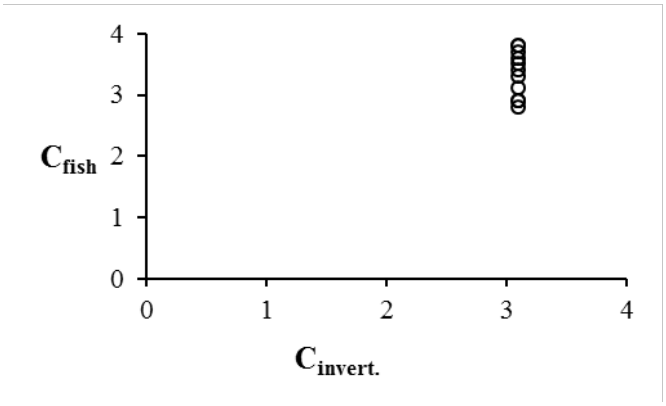
Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	8.53	7.87	0.92
Formation 2012	DC-600	8.53	8.50	1.00
Formation 2012	DC-600	8.65	7.63	0.88
Formation 2012	LSV-4	9.54	18.28	1.92
Formation 2012	LSV-4	9.54	20.01	2.10
Formation 2012	HS-3	11.40	18.57	1.63
Formation 2012	HS-3	11.40	21.85	1.92
Formation 2012	CC-350	11.45	9.53	0.83
Formation 2012	CC-350	11.45	10.03	0.88
Formation 2012	CC-1A	12.24	8.34	0.68
Formation 2012	CC-1A	12.24	9.94	0.81
Formation 2012	CC-1A	12.24	17.47	1.43
Formation 2012	CC-1A	12.57	7.78	0.62
Formation 2012	HS-3	13.41	26.63	1.99
Formation 2012	CC-1A	13.55	12.63	0.93
Formation 2012	CC-150	14.32	7.35	0.51
Formation 2012	CC-3A	14.50	20.20	1.39
Formation 2012	HS	15.70	23.23	1.48
Formation 2012	HS	15.70	23.25	1.48
Formation 2012	HS	18.70	10.95	0.59
Formation 2012	LSV-2C	22.62	11.38	0.50
Formation 2012	LSV-2C	22.62	17.47	0.77
Formation 2012	HS-3	24.70	23.93	0.97
Formation 2012	LSV-2C	26.31	18.85	0.72
Formation 2012	HS-3	26.55	23.68	0.89
Formation 2012	LSV-2C	26.95	20.32	0.75
Formation 2012	HS	27.80	35.93	1.29
Formation 2012	HS	27.80	41.30	1.49
Formation 2012	LSV-2C	30.00	25.95	0.87



Median ratio: 1.29

R^2: 0.63
F: 87.0
df: 51
P: <0.001

Shorthead redhorse (*Moxostoma macrolepidotum*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.10	1.00
Roddy et al. 1991	18	3.10	3.30	1.06
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.50	1.13
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	3.70	1.19
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	3.80	1.23

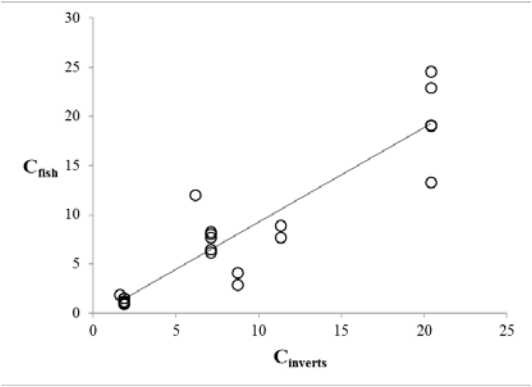


Median ratio: 1.10

R^2: 0.00
F: 0.00
df: 9
P: 1.0
Not used because P > 0.05

Smallmouth bass (*Micropterus dolomieu*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	SU	1.85	1.55	0.84
Butler et al. 1995	SU	1.85	1.22	0.66
Butler et al. 1995	SU	1.85	0.98	0.53
Butler et al. 1995	SU	1.85	1.14	0.62
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	MNP3	6.15	12.00	1.95
Mueller et al. 1991	R1	8.70	2.90	0.33
Mueller et al. 1991	R1	8.70	4.10	0.47
GEI 2014	ARE	20.40	24.61	1.21
GEI 2014	ARE	20.40	22.97	1.13
GEI 2014	ARE	20.40	13.28	0.65

Smallmouth bass (*Micropterus dolomieu*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	ARE	20.40	19.06	0.93
GEI 2014	ARE	20.40	19.11	0.94
GEI 2014	ARM	7.10	8.25	1.16
GEI 2014	ARM	7.10	8.04	1.13
GEI 2014	ARM	7.10	7.72	1.09
GEI 2014	ARM	7.10	6.21	0.87
GEI 2014	ARM	7.10	6.51	0.92
GEI 2014	C-SC1	11.30	8.94	0.79
GEI 2014	C-SC1	11.30	7.68	0.68



Median ratio: 0.86

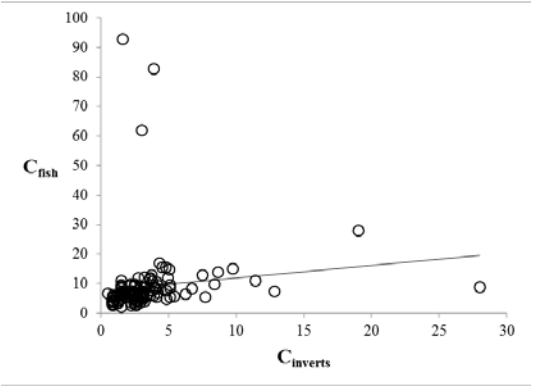
R^2: 0.84
F: 107.1
df: 20
P: <0.001

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1991	10	4.80	4.80	1.00
Butler et al. 1991	9	4.10	5.70	1.39
Butler et al. 1991	3	6.20	6.50	1.05
Butler et al. 1993	SP2	2.75	12.00	4.36
Butler et al. 1993	B2	1.35	5.80	4.30
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	D1	1.20	3.50	2.92
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.40	2.83
Butler et al. 1993	D2	1.45	4.90	3.38
Butler et al. 1993	D2	1.45	6.80	4.69
Butler et al. 1993	D2	1.45	6.50	4.48
Butler et al. 1993	F2	3.90	8.90	2.28
Butler et al. 1993	P1	1.95	5.50	2.82
Butler et al. 1993	SP1	2.95	7.30	2.47

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	SP1	2.95	8.90	3.02
Butler et al. 1993	SP1	2.95	7.00	2.37
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1993	U1	2.45	6.90	2.82
Butler et al. 1993	U1	2.45	7.30	2.98
Butler et al. 1993	U1	2.45	9.20	3.76
Butler et al. 1993	U1	2.45	9.40	3.84
Butler et al. 1993	U1	2.45	9.80	4.00
Butler et al. 1993	LP3	1.12	6.00	5.38
Butler et al. 1993	LP4	3.20	8.70	2.72
Butler et al. 1993	R2	4.30	17.10	3.98
Butler et al. 1993	R2	3.90	6.00	1.54
Butler et al. 1993	ST2	4.50	15.70	3.49
Butler et al. 1993	ST2	4.10	8.50	2.07
Butler et al. 1993	ST2	4.10	10.70	2.61
Butler et al. 1993	ST2	3.35	9.30	2.78
Butler et al. 1993	R1	4.00	8.50	2.13
Butler et al. 1993	ST1	2.25	6.80	3.02
Butler et al. 1993	SB2	3.60	12.10	3.36
Butler et al. 1993	SB2	3.75	7.80	2.08
Butler et al. 1993	SB2	3.75	10.80	2.88
Butler et al. 1993	SB1	2.15	10.00	4.65
Butler et al. 1993	SB1	2.15	9.50	4.42
Butler et al. 1993	SB1	2.15	7.80	3.63
Butler et al. 1993	WSB2	4.75	15.60	3.28
Butler et al. 1993	WSB2	3.60	11.70	3.25
Butler et al. 1993	WSB2	3.00	6.20	2.07
Butler et al. 1993	WSB2	3.00	7.60	2.53
Butler et al. 1994	CRC	7.50	13.00	1.73
Butler et al. 1994	CF1	3.60	6.10	1.69
Butler et al. 1994	GUN2	28.00	8.90	0.32
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	LZA1	19.00	28.00	1.47
Butler et al. 1994	NFK3	2.00	7.10	3.55
Butler et al. 1994	NFK2	3.10	6.90	2.23
Butler et al. 1994	NFK2	3.10	4.80	1.55
Butler et al. 1994	NFK2	3.10	5.40	1.74
Butler et al. 1994	NFK2	3.10	5.70	1.84
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	NFK2	3.10	6.20	2.00
Butler et al. 1994	NFK2	3.10	6.30	2.03
Butler et al. 1994	NFK2	3.10	6.40	2.06

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1994	NFK2	3.10	6.70	2.16
Butler et al. 1994	NFK2	3.10	7.40	2.39
Butler et al. 1994	NFK2	3.10	8.70	2.81
Butler et al. 1994	TGC	4.90	12.00	2.45
Butler et al. 1994	BSW1	5.00	15.00	3.00
Butler et al. 1994	COL1	1.50	2.30	1.53
Butler et al. 1994	COL1	1.50	5.00	3.33
Butler et al. 1994	COL1	1.50	7.30	4.87
Butler et al. 1994	COL1	1.50	7.40	4.93
Butler et al. 1994	COL1	1.50	8.40	5.60
Butler et al. 1994	COL1	1.50	8.60	5.73
Butler et al. 1994	COL1	1.50	9.30	6.20
Butler et al. 1994	COL1	1.50	9.60	6.40
Butler et al. 1994	COL1	1.50	11.00	7.33
Butler et al. 1994	RB3	1.60	93.00	58.13
Butler et al. 1994	SMF	4.80	7.80	1.63
Butler et al. 1994	LW	3.00	62.00	20.67
Butler et al. 1994	LSW1	3.90	83.00	21.28
Butler et al. 1994	PSW1	3.70	13.00	3.51
Butler et al. 1995	AK	0.78	4.30	5.55
Butler et al. 1995	AK	0.78	3.10	4.00
Butler et al. 1995	AK	0.78	4.00	5.16
Butler et al. 1995	DD	0.86	5.60	6.51
Butler et al. 1995	DD	0.86	4.40	5.12
Butler et al. 1995	DD	0.86	6.00	6.98
Butler et al. 1995	HD1	0.83	2.80	3.39
Butler et al. 1995	HD1	0.83	3.20	3.88
Butler et al. 1995	HD1	0.83	5.30	6.42
Butler et al. 1995	ME1	3.40	6.40	1.88
Butler et al. 1995	ME2	1.25	6.10	4.88
Butler et al. 1995	ME3	2.55	2.80	1.10
Butler et al. 1995	ME3	2.55	7.00	2.75
Butler et al. 1995	ME3	2.55	5.50	2.16
Butler et al. 1995	NW	5.10	8.70	1.71
Butler et al. 1995	SJ1	2.50	4.30	1.72
Butler et al. 1995	SJ1	2.50	5.10	2.04
Butler et al. 1995	SJ1	2.50	2.90	1.16
Butler et al. 1995	YJ2	1.65	6.50	3.94
Butler et al. 1995	YJ2	1.65	6.30	3.82
Butler et al. 1995	YJ2	1.65	7.10	4.30
Butler et al. 1995	MN1	2.70	5.50	2.04
Butler et al. 1997	CHK	2.40	5.20	2.17

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1997	CHK	2.40	3.80	1.58
Butler et al. 1997	MN3	2.70	6.00	2.22
Butler et al. 1997	MN3	2.70	4.30	1.59
Butler et al. 1997	MN2	2.20	2.70	1.23
Butler et al. 1997	MN2	2.20	3.60	1.64
Butler et al. 1997	MN1	2.90	3.70	1.28
Butler et al. 1997	MUD	2.30	7.20	3.13
Butler et al. 1997	MUD	2.30	6.10	2.65
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	WBR	5.05	9.70	1.92
Butler et al. 1997	WBR	5.05	5.50	1.09
Butler et al. 1997	MN4	2.65	7.90	2.98
Butler et al. 1997	MN5	8.60	14.00	1.63
Butler et al. 1997	MNQ	1.80	5.90	3.28
Hamilton and Buhl 2004	DVC	12.80	7.50	0.59
Hamilton and Buhl 2004	USC	0.50	6.90	13.80
Hamilton and Buhl 2004	ACM	6.70	8.50	1.27
Hamilton and Buhl 2004	LBR	7.70	5.60	0.73
Hamilton and Buhl 2005	LiB	5.40	5.80	1.07
Hamilton and Buhl 2005	SLC	9.70	15.20	1.57

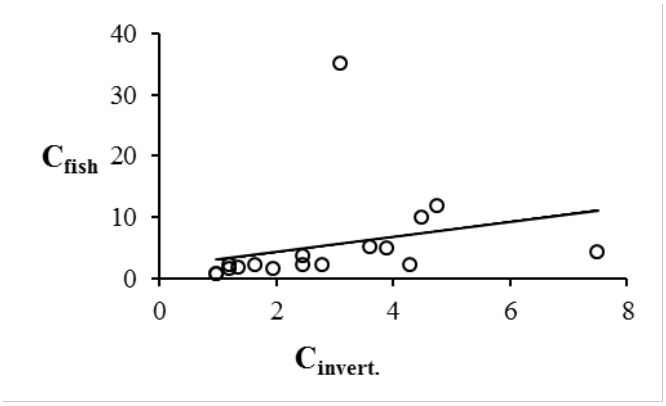


Median ratio: 2.76

R^2: 0.01
F: 1.76
df: 118
P: 0.177
Not used because P > 0.05

Sucker (Catostomidae)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	HD2	0.98	0.68	0.69
Butler et al. 1995	HD2	0.98	0.76	0.78
Butler et al. 1993	D1	1.20	2.30	1.92
Rinella and Schuler 1992	Malheur Lake	1.20	1.60	1.33

Sucker (Catostomidae)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	YJ2	1.65	2.20	1.33
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	U1	2.45	2.30	0.94
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1991	12	2.80	2.10	0.75
Butler et al. 1994	NFK2	3.10	35.00	11.29
Butler et al. 1993	SB2	3.60	5.10	1.42
Butler et al. 1993	R2	3.90	5.00	1.28
Butler et al. 1993	R2	4.30	2.20	0.51
Butler et al. 1993	ST2	4.50	10.00	2.22
Butler et al. 1993	WSB2	4.75	11.80	2.48
Butler et al. 1993	F2	7.50	4.20	0.56

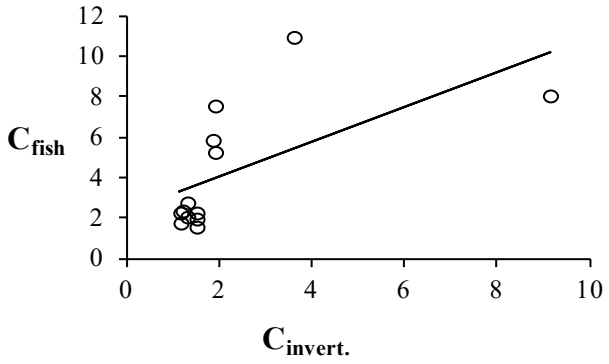


Median ratio: 1.33

R^2: 0.07
F: 1.10
df: 15
P: 0.360
Not used because P > 0.05

Sunfish (Centrarchidae)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Welsh and Maughan 1994	outfall drain	1.15	2.30	2.00
Welsh and Maughan 1994	Pretty Water	1.16	1.80	1.56
Welsh and Maughan 1994	Hart Mine Marsh	1.20	2.40	2.00
Welsh and Maughan 1994	outfall drain	1.30	2.10	1.62
Welsh and Maughan 1994	outfall drain	1.30	2.80	2.15
Welsh and Maughan 1994	Pretty Water	1.50	1.60	1.07
Welsh and Maughan 1994	Old Channel	1.50	2.00	1.33
Welsh and Maughan 1994	Pretty Water	1.50	2.30	1.53
Welsh and Maughan 1994	Cibola Lake	1.85	5.90	3.19
Welsh and Maughan 1994	Cibola Lake	1.90	5.30	2.79
Welsh and Maughan 1994	Cibola Lake	1.90	7.60	4.00
Welsh and Maughan 1994	Oxbow Lake	3.60	11.00	3.06

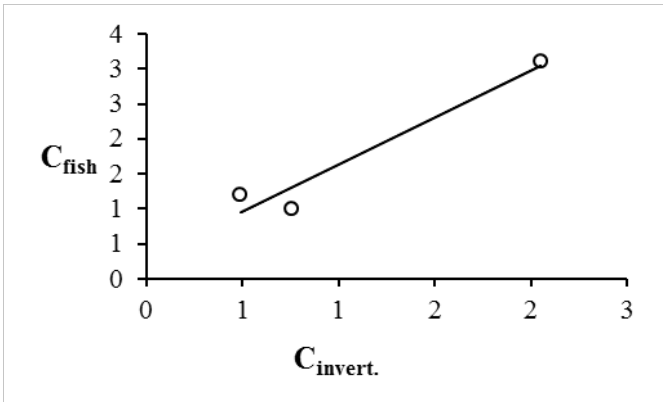
Sunfish (Centrarchidae)				
GEI 2013	SW2-1	9.14	8.10	0.89



Median ratio: 2.00

R^2: 0.38
F: 6.66
df: 11
P: 0.013

Tui chub (*Gila bicolor*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Sorenson & Schwarzbach 1991	5	0.49	1.20	2.45
Sorenson & Schwarzbach 1991	4	0.76	1.00	1.32
Rinella and Schuler 1992	Harney Lake	2.05	3.10	1.51

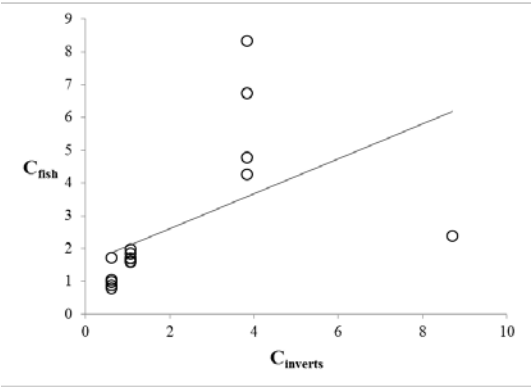


Median ratio: 1.51

R^2: 0.94
F: 15.9
df: 1
P: 0.175
Not used because P > 0.05

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.86	1.75
Butler et al. 1995	TT	1.07	1.62	1.52
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	2.00	1.88

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	PU	0.61	1.72	2.82
Butler et al. 1995	PU	0.61	1.05	1.73
Butler et al. 1995	PU	0.61	0.81	1.33
Butler et al. 1995	PU	0.61	1.00	1.64
Butler et al. 1995	PU	0.61	0.89	1.46
Mueller et al. 1991	R1	8.70	2.40	0.28
Peterson et al. 1991	7	3.83	4.27	1.11
Peterson et al. 1991	7	3.83	4.79	1.25
Peterson et al. 1991	7	3.83	6.76	1.77
Peterson et al. 1991	7	3.83	8.35	2.18



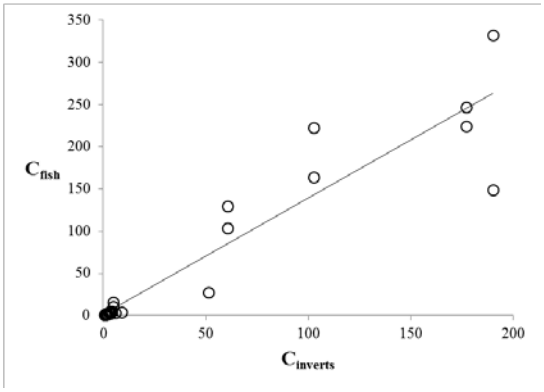
Median ratio: 1.60

R^2: 0.28
F: 5.46
df: 14
P: 0.018

Western mosquitofish (*Gambusia affinis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	SWA1	3.64	2.91	0.80
GEI 2013	SWA1	2.81	3.01	1.07
GEI 2013	SWA1	2.81	3.49	1.24
GEI 2013	SWA1	2.81	3.66	1.30
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.27	1.52
Lemly 1985	Badin Lake	5.70	3.35	0.59
Lemly 1985	Belews Lake	51.15	27.20	0.53
Lemly 1985	High Rock Lake	9.05	3.54	0.39
Saiki and Lowe 1987	Kesterson Pond 11	60.65	130.00	2.14
Saiki and Lowe 1987	Kesterson Pond 11	60.65	104.00	1.71
Saiki and Lowe 1987	Kesterson Pond 2	177.00	224.00	1.27
Saiki and Lowe 1987	Kesterson Pond 2	177.00	247.00	1.40
Saiki and Lowe 1987	Kesterson Pond 8	102.50	164.00	1.60

Western mosquitofish (*Gambusia affinis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Saiki and Lowe 1987	Kesterson Pond 8	102.50	223.00	2.18
Saiki and Lowe 1987	San Luis Drain	190.00	149.00	0.78
Saiki and Lowe 1987	San Luis Drain	190.00	332.00	1.75
Saiki and Lowe 1987	Volta Pond 26	1.42	1.28	0.90
Saiki and Lowe 1987	Volta Pond 26	1.42	1.24	0.87
Saiki and Lowe 1987	Volta Wasteway	2.23	1.35	0.61
Saiki and Lowe 1987	Volta Wasteway	2.23	1.36	0.61
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.30	1.54
Saiki et al. 1993	GT5	4.90	16.00	3.27
Saiki et al. 1993	GT5	4.90	11.00	2.24
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.90	1.21
Saiki et al. 1993	SJR2	3.30	4.50	1.36
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR1	0.95	0.95	1.01
Saiki et al. 1993	SJR1	0.95	1.30	1.38
Saiki et al. 1993	ET7	0.86	0.90	1.05
Saiki et al. 1993	ET7	0.86	1.00	1.16



Median ratio: 1.21

R^2: 0.89
F: 263.3
df: 33
P: <0.001

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	LP3	1.12	2.50	2.24
Butler et al. 1993	B1	1.25	2.60	2.08
Butler et al. 1993	D2	1.45	1.90	1.31
Butler et al. 1993	D2	1.45	2.50	1.72

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	P1	1.50	1.70	1.13
Butler et al. 1993	P1	1.50	1.80	1.20
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1995	SU	1.85	1.20	0.65
GEI 2014	Arkansas River, ARB	11.21	14.90	1.33
GEI 2014	Arkansas River, ARB	11.21	20.39	1.82
GEI 2014	Arkansas River, ARB	11.21	13.82	1.23
GEI 2014	Arkansas River, ARB	11.21	8.36	0.75
GEI 2014	Arkansas River, ARB	11.21	10.88	0.97
GEI 2014	Arkansas River, ARB	11.21	21.55	1.92
GEI 2014	Arkansas River, ARB	11.21	18.70	1.67
GEI 2014	Arkansas River, ARB	11.21	24.53	2.19
GEI 2014	Arkansas River, ARB	11.21	15.02	1.34
GEI 2014	Arkansas River, ARB	11.21	28.29	2.52
GEI 2014	Arkansas River, ARE	20.4	18.21	0.89
GEI 2014	Arkansas River, ARE	20.4	19.54	0.96
GEI 2014	Arkansas River, ARE	20.4	15.27	0.75
GEI 2014	Arkansas River, ARE	20.4	11.37	0.56
GEI 2014	Arkansas River, ARE	20.4	17.86	0.88
GEI 2014	Arkansas River, ARE	20.4	10.62	0.52
GEI 2014	Arkansas River, ARE	20.4	17.51	0.86
GEI 2014	Arkansas River, ARE	20.4	24.66	1.21
GEI 2014	Arkansas River, ARE	20.4	18.92	0.93
GEI 2014	Arkansas River, ARE	20.4	21.70	1.06
GEI 2014	Arkansas River, ARM	8.51	10.13	1.19
GEI 2014	Arkansas River, ARM	8.51	9.24	1.09
GEI 2014	Arkansas River, ARM	8.51	8.30	0.97
GEI 2014	Arkansas River, ARM	8.51	10.09	1.19
GEI 2014	Arkansas River, ARM	7.68	16.18	2.11
GEI 2014	Arkansas River, ARM	7.68	13.21	1.72
GEI 2014	Arkansas River, ARM	7.68	11.96	1.56
GEI 2014	Arkansas River, ARM	7.68	8.58	1.12
GEI 2014	Arkansas River, ARM	7.68	9.73	1.27
GEI 2014	Arkansas River, ARM	7.1	8.19	1.15
GEI 2014	Arkansas River, ARM	7.1	7.96	1.12
GEI 2014	Arkansas River, ARN	7.49	9.15	1.22
GEI 2014	Arkansas River, ARN	7.49	8.61	1.15
GEI 2014	Arkansas River, ARN	7.49	7.06	0.94
GEI 2014	Arkansas River, ARN	7.49	11.57	1.54
GEI 2014	Arkansas River, ARN	7.49	11.56	1.54
GEI 2014	Arkansas River, ARN	7.44	21.20	2.85
GEI 2014	Arkansas River, ARN	7.44	23.28	3.13

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Arkansas River, ARN	7.44	20.85	2.80
GEI 2014	Arkansas River, ARN	7.44	25.91	3.48
GEI 2014	Bond Creek, BC-2	2.96	3.06	1.03
GEI 2014	Bond Creek, BC-2	2.96	3.54	1.19
GEI 2014	Bond Creek, BC-2	2.96	3.04	1.03
GEI 2014	Bond Creek, BC-3	3.02	2.76	0.91
GEI 2014	Bond Creek, BC-3	3.02	2.85	0.94
GEI 2014	Bond Creek, BC-3	3.02	2.47	0.82
GEI 2014	Bond Creek, BC-3	3.02	2.03	0.67
GEI 2014	Bond Creek, BC-3	3.02	2.23	0.74
GEI 2014	Cow Camp Creek, CC-2	5.65	4.46	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	4.45	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	6.19	1.09
GEI 2014	Cow Camp Creek, CC-2	5.65	4.71	0.83
GEI 2014	Cow Camp Creek, CC-2	5.65	5.38	0.95
GEI 2014	Seng Creek, C-SC1	11.302	20.32	1.80
GEI 2014	Dry Creek, DC-4	19.42	26.07	1.34
GEI 2014	Dry Creek, DC-4	19.42	22.55	1.16
GEI 2014	Dry Creek, DC-4	19.42	14.29	0.74
GEI 2014	Dry Creek, DC-4	19.42	14.25	0.73
GEI 2014	Dry Creek, DC-4	19.42	14.67	0.76
GEI 2014	Dry Creek, DC-4	18.1	29.83	1.65
GEI 2014	Dry Creek, DC-4	18.1	30.65	1.69
GEI 2014	Dry Creek, DC-4	18.1	20.87	1.15
GEI 2014	Dry Creek, DC-4	18.1	12.06	0.67
GEI 2014	Fountain Creek, FC-4	18.65	24.54	1.32
GEI 2014	Fountain Creek, FCP	6.13	5.33	0.87
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.75	0.94
GEI 2014	Fountain Creek, FCP	6.13	4.37	0.71
GEI 2014	Fountain Creek, FCP	5.38	8.50	1.58
GEI 2014	Fountain Creek, FCP	5.38	5.94	1.10
GEI 2014	Fountain Creek, FCP	5.38	5.97	1.11
GEI 2014	Fountain Creek, FCP	5.38	5.76	1.07
GEI 2014	Fountain Creek, FCP	5.38	5.61	1.04
GEI 2014	Fountain Creek, FCP	6.35	15.82	2.49
GEI 2014	Fountain Creek, FCP	6.35	5.68	0.90
GEI 2014	Fountain Creek, FCP	6.35	10.17	1.60
GEI 2014	Fountain Creek, FCP	6.35	12.34	1.94
GEI 2014	Fountain Creek, FCP	6.35	10.64	1.68
GEI 2014	Foidel Creek, FOC-2	2.175	1.74	0.80

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Foidel Creek, FOC-2	2.175	1.25	0.57
GEI 2014	Foidel Creek, FOC-2	2.175	1.76	0.81
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	1.64	0.76
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	2.29	1.05
GEI 2014	Grassy Creek, GC-2	4.195	4.45	1.06
GEI 2014	Grassy Creek, GC-2	4.195	4.42	1.05
GEI 2014	Grassy Creek, GC-2	4.195	2.51	0.60
GEI 2014	Grassy Creek, GC-3	4.535	2.78	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.84	0.63
GEI 2014	Grassy Creek, GC-3	4.535	4.37	0.96
GEI 2014	Grassy Creek, GC-3	4.535	2.89	0.64
GEI 2014	Grassy Creek, GC-3	4.545	4.29	0.94
GEI 2014	Grassy Creek, GC-3	4.545	3.16	0.69
GEI 2014	Grassy Creek, GC-3	4.545	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.34	4.12	0.95
GEI 2014	Grassy Creek, GC-3	4.35	3.96	0.91
GEI 2014	Grassy Creek, GC-4	5.1	0.93	0.18
GEI 2014	Grassy Creek, GC-4	5.1	1.40	0.27
GEI 2014	Grassy Creek, GC-4	5.1	4.12	0.81
GEI 2014	Grassy Creek, GC-4	5.76	7.04	1.22
GEI 2014	Grassy Creek, GC-4	5.76	7.42	1.29
GEI 2014	Grassy Creek, GC-4	5.76	4.22	0.73
GEI 2014	Grassy Creek, GC-4	5.76	4.75	0.82
GEI 2014	Grassy Creek, GC-4 US	13.16	6.32	0.48
GEI 2014	Grassy Creek, GC-4 US	13.16	4.74	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.98	0.38
GEI 2014	Grassy Creek, GC-4 US	13.16	4.75	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.88	0.37
GEI 2014	Middle Creek, MC-1	3.21	2.03	0.63
GEI 2014	Middle Creek, MC-1	3.21	2.18	0.68
GEI 2014	Middle Creek, MC-1	3.21	1.79	0.56
GEI 2014	Middle Creek, MC-1	3.21	2.28	0.71
GEI 2014	Middle Creek, MC-1	3.21	2.54	0.79
GEI 2014	Middle Creek, MC-2	4.19	2.58	0.62
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	1.86	0.44
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.10	0.50
GEI 2014	St. Charles River, SC-3	5.75	7.74	1.35

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	St. Charles River, SC-3	5.75	12.34	2.15
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.76	3.09
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	38.23	2.53
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.59	3.08
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	60.66	4.01
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	43.70	2.89
GEI 2014	St. Charles River, SC-9	25.06	35.97	1.44
GEI 2014	St. Charles River, SC-9	25.06	38.53	1.54
GEI 2014	St. Charles River at I-25, SC-I	4.335	7.62	1.76
GEI 2014	St. Charles River at I-25, SC-I	4.335	8.20	1.89
GEI 2014	St. Charles River at I-25, SC-I	4.335	6.10	1.41
GEI 2014	St. Charles River at I-25, SC-I	4.335	22.44	5.18
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.29	0.99
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.79	1.11
GEI 2014	St. Charles River at I-25, SC-I	4.335	47.18	10.88
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.88	1.12
GEI 2014	Wildhorse Creek, WHC	56.14	5.43	0.10
GEI 2014	Wildhorse Creek, WHC	34.24	36.23	1.06
GEI 2014	Wildhorse Creek, WHC	34.24	10.22	0.30
GEI 2014	Wildhorse Creek, WHC	34.24	52.16	1.52
GEI 2014	Wildhorse Creek, WHC	34.24	40.81	1.19
GEI 2014	Wildhorse Creek, WHC	34.24	26.45	0.77
GEI 2014	Wildhorse Creek, WHC	62.34	61.90	0.99
GEI 2014	Wildhorse Creek, WHC	62.34	15.88	0.25
GEI 2014	Wildhorse Creek, WHC	62.34	27.40	0.44
GEI 2014	Wildhorse Creek, WHC	62.34	23.10	0.37
Muscatello and Janz 2009	Indigo Lake	0.36	0.99	2.75
Muscatello and Janz 2009	Vulture Lake	1.62	3.37	2.08
Grasso et al. 1995	17	1.91	2.84	1.49
Grasso et al. 1995	17	1.91	3.19	1.67
Grasso et al. 1995	17	1.91	3.44	1.80
Grasso et al. 1995	17	1.91	3.64	1.91

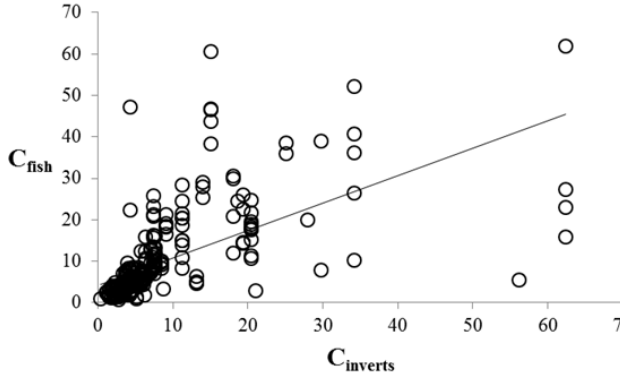
White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Grasso et al. 1995	17	1.91	4.00	2.09
Grasso et al. 1995	17	1.91	4.01	2.10
Butler et al. 1994	NFK3	2.00	3.90	1.95
Butler et al. 1993	ST1	2.25	4.90	2.18
Lambing et al. 1994	S33	2.40	3.50	1.46
Mueller et al. 1991	A1	2.70	4.20	1.56
GEI 2013	SWA1	2.81	2.83	1.01
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.18	1.49
Mason et al. 2000	HCRT	2.81	0.81	0.29
Mason et al. 2000	HCRT	2.81	1.43	0.51
Mason et al. 2000	HCRT	2.81	1.43	0.51
Butler et al. 1993	WSB2	3.00	3.90	1.30
Butler et al. 1993	SP2	3.15	3.50	1.11
Butler et al. 1993	LP4	3.20	2.80	0.88
GEI 2013	SW4-1	3.33	3.01	0.91
GEI 2013	SW4-1	3.33	3.45	1.04
GEI 2013	SW4-1	3.33	3.50	1.05
GEI 2013	SW4-1	3.33	3.62	1.09
GEI 2013	SW4-1	3.33	4.04	1.22
GEI 2013	SW4-1	3.33	4.08	1.23
GEI 2013	SW4-1	3.33	4.13	1.24
GEI 2013	SW4-1	3.33	4.17	1.25
GEI 2013	SW4-1	3.33	4.34	1.31
GEI 2013	SW4-1	3.33	4.78	1.44
Butler et al. 1993	ST2	3.35	7.00	2.09
GEI 2013	LG1	3.37	3.54	1.05
GEI 2013	LG1	3.37	3.55	1.05
GEI 2013	LG1	3.37	3.90	1.16
GEI 2013	LG1	3.37	3.95	1.17
GEI 2013	LG1	3.37	4.48	1.33
GEI 2013	LG1	3.39	3.00	0.88
GEI 2013	LG1	3.56	2.72	0.77
GEI 2013	LG1	3.56	2.80	0.79
GEI 2013	LG1	3.56	2.89	0.81
GEI 2013	LG1	3.56	2.99	0.84
GEI 2013	LG1	3.56	3.04	0.86
GEI 2013	LG1	3.56	3.08	0.87
GEI 2013	LG1	3.56	3.13	0.88
GEI 2013	LG1	3.56	3.18	0.89
GEI 2013	LG1	3.56	3.25	0.91
GEI 2013	LG1	3.56	3.27	0.92

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	WSB2	3.60	4.30	1.19
Butler et al. 1993	WSB2	3.60	6.30	1.75
GEI 2013	SWA1	3.64	2.83	0.78
GEI 2013	SWA1	3.64	3.39	0.93
GEI 2013	SWA1	3.64	3.47	0.95
GEI 2013	SWA1	3.64	3.55	0.98
GEI 2013	SWA1	3.64	3.63	1.00
GEI 2013	SWA1	3.64	3.75	1.03
Butler et al. 1993	SB2	3.65	4.30	1.18
Butler et al. 1993	R2	3.70	4.20	1.14
Butler et al. 1993	SB2	3.75	4.80	1.28
GEI 2013	CC1	3.76	5.99	1.59
GEI 2013	CC1	3.76	6.56	1.74
GEI 2013	CC1	3.76	7.21	1.92
GEI 2013	CC1	3.76	7.42	1.97
GEI 2013	CC1	3.76	7.62	2.03
Peterson et al. 1991	7	3.83	3.30	0.86
Peterson et al. 1991	7	3.83	4.64	1.21
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1991	4	3.90	5.30	1.36
GEI 2013	SW88	3.96	4.63	1.17
GEI 2013	SW88	3.96	4.75	1.20
Butler et al. 1993	R1	4.00	9.50	2.38
Butler et al. 1993	ST2	4.10	8.30	2.02
GEI 2013	SW9	4.45	4.07	0.91
GEI 2013	SW9	4.45	4.18	0.94
GEI 2013	SW9	4.45	4.19	0.94
GEI 2013	SW9	4.45	4.20	0.94
GEI 2013	SW9	4.45	4.40	0.99
GEI 2013	SW9	4.45	5.18	1.16
GEI 2013	CC1	4.69	4.51	0.96
GEI 2013	CC1	4.69	4.57	0.98
GEI 2013	CC1	4.69	4.94	1.05
GEI 2013	CC1	4.69	5.02	1.07
GEI 2013	CC1	4.69	5.81	1.24
GEI 2013	CC1	4.69	6.01	1.28
GEI 2013	CC1	4.69	6.43	1.37
GEI 2013	CC1	4.69	7.25	1.55
GEI 2013	CC1	4.69	8.00	1.71
GEI 2013	CC1	4.69	8.52	1.82
Butler et al. 1993	F2	4.80	5.20	1.08
GEI 2013	CC1	5.86	5.00	0.85

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	CC1	5.86	5.37	0.92
GEI 2013	CC1	5.86	5.59	0.95
GEI 2013	CC1	5.86	5.71	0.98
GEI 2013	CC1	5.86	5.90	1.01
GEI 2013	CC1	5.86	6.61	1.13
GEI 2013	CC1	5.86	6.79	1.16
GEI 2013	CC1	5.86	6.82	1.16
GEI 2013	CC1	5.86	7.29	1.25
GEI 2013	CC1	5.86	7.48	1.28
Butler et al. 1991	3	6.20	1.80	0.29
GEI 2013	SWB	7.06	7.18	1.02
GEI 2013	SWB	7.06	7.36	1.04
GEI 2013	SWB	7.06	7.98	1.13
GEI 2013	SWB	7.06	8.03	1.14
GEI 2013	SWB	7.06	9.65	1.37
GEI 2013	SWB	7.06	12.76	1.81
GEI 2013	SWB	7.06	12.85	1.82
GEI 2013	SWB	7.06	13.16	1.86
GEI 2013	SWB	7.44	8.21	1.10
GEI 2013	SWB	7.44	8.77	1.18
GEI 2013	SWB	7.44	8.85	1.19
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	13.59	1.83
GEI 2013	SWB	7.44	15.75	2.12
GEI 2013	SWB	7.44	16.40	2.21
Butler et al. 1994	IW	8.35	9.70	1.16
Mueller et al. 1991	R1	8.70	3.40	0.39
GEI 2013	SW2-1	9.14	16.54	1.81
GEI 2013	SW2-1	9.14	18.14	1.99
GEI 2013	SW2-1	9.14	18.54	2.03
GEI 2013	SW2-1	9.14	19.16	2.10
GEI 2013	SW2-1	9.14	21.29	2.33
Lambing et al. 1994	S34	14.00	25.30	1.81
Lambing et al. 1994	S34	14.00	28.00	2.00
Lambing et al. 1994	S34	14.00	29.00	2.07
Butler et al. 1994	HCC1	21.00	3.00	0.14
Butler et al. 1994	GUN2	28.00	20.00	0.71
Butler et al. 1991	7	29.80	7.90	0.27

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio



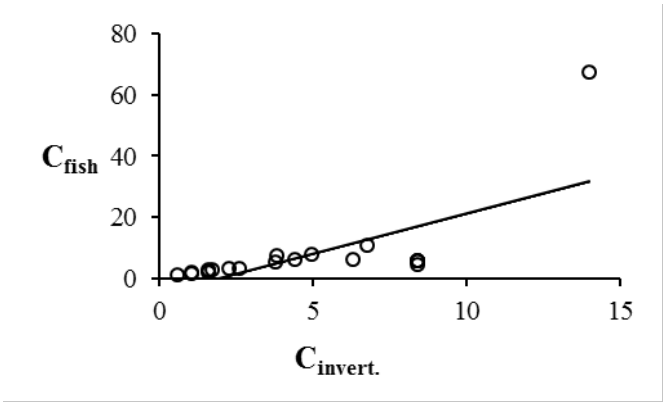
Median ratio: 1.11

R^2: 0.38
F: 174.4
df: 284
P: <0.001

Yellow perch (*Perca flavescens*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	PU	0.61	1.10	1.80
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	MP	1.60	2.00	1.25
Butler et al. 1995	MP	1.60	2.20	1.38
Butler et al. 1995	MP	1.60	2.70	1.69
Belize et al. 2006	Halfway	1.74	2.72	1.56
Belize et al. 2006	Geneva	2.29	3.30	1.44
Belize et al. 2006	Bethel	2.61	3.09	1.19
Belize et al. 2006	McFarlane	3.79	5.40	1.42
Peterson et al. 1991	7	3.83	7.33	1.91
Belize et al. 2006	Long	4.42	6.28	1.42
Belize et al. 2006	Ramsey	4.97	7.64	1.54
Belize et al. 2006	Windy	6.32	6.06	0.96
Belize et al. 2006	Nelson	6.79	10.68	1.57
GEI 2013	SW11	8.41	4.54	0.54
GEI 2013	SW11	8.41	5.49	0.65
GEI 2013	SW11	8.41	5.50	0.65
GEI 2013	SW11	8.41	5.58	0.66
GEI 2013	SW11	8.41	5.68	0.68
Lambing et al. 1994	S34	14.00	67.00	4.79

Yellow perch (*Perca flavescens*)



Median ratio: 1.42

R^2: 0.46
F: 16.24
df: 19
P: <0.001

Table B-7. Final vertebrate Trophic Transfer Factor (TTF) values, including estimated values using taxonomic classification.

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
alligator gar	*Atractosteus spatula*	Lepistosteiformes	Lepisosteidae	Atractosteus	1.21	All fish
black bullhead	*Ameiurus melas*	Siluriformes	Ictaluridae	Ameiurus	0.85	Exact match
black crappie	*Pomoxis nigromaculatus*	Perciformes	Centrarchidae	Pomoxis	2.67	Exact match
black redhorse	*Moxostoma duquesnei*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
blacknose dace	*Rhinichthys atratulus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Exact match
blue catfish	*Ictalurus furcatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Genus Ictalurus
bluegill	*Lepomis macrochirus*	Perciformes	Centrarchidae	Lepomis	1.03	Exact match
bluehead sucker	*Catostomus discobolus*	Cypriniformes	Catostomidae	Catostomus	1.04	Exact match
brassy minnow	*Hybognathus hankinsoni*	Cypriniformes	Cyprinidae	Hybognathus	1.20	Family Cyprinidae
brook stickleback	*Culaea inconstans*	Gasterosteiformes	Gasterosteidae	Culaea	1.79	Exact match
brook trout	*Salvelinus fontinalis*	Salmoniformes	Salmonidae	Salvelinus	0.88	Exact match
brown bullhead	*Ameiurus nebulosus*	Siluriformes	Ictaluridae	Ameiurus	0.85	Genus Ameiurus
brown trout	*Salmo trutta*	Salmoniformes	Salmonidae	Salmo	1.38	Exact match
bullhead		Siluriformes	Ictaluridae		0.77	Family Ictaluridae
burbot	*Lota lota*	lota	Gadiformes	Lotidae	1.21	All fish
chain pickerel	*Esox niger*	Esociformes	Esocidae	Esox	1.78	Genus Esox
channel catfish	*Ictalurus punctatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Exact match
common carp	*Cyprinus carpio*	Cypriniformes	Cyprinidae	Cyprinus	1.20	Exact match
common snook	*Centropomus undecimalis*	Perciformes	Centropomidae	Centropomus	1.41	Order Perciformes
crappie	*Pomoxis sp.*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
creek chub	*Semotilus atromaculatus*	Cypriniformes	Cyprinidae	Semotilus	1.06	Exact match
cutthroat trout	*Oncorhynchus clarkii*	Salmoniformes	Salmonidae	Oncorhynchus	1.12	Exact match
desert pupfish	*Cyprinodon macularius*	Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.24	Order Cyprinodontiformes
dolly varden	*Salvelinus malma*	Salmoniformes	Salmonidae	Salvelinus	0.88	Genus Salvelinus
fathead minnow	*Pimephales promelas*	Cypriniformes	Cyprinidae	Pimephales	1.57	Exact match
flannelmouth sucker	*Catostomus latipinnis*	Cypriniformes	Catostomidae	Catostomus	0.98	Exact match
flathead catfish	*Pylodictis olivaris*	Siluriformes	Ictaluridae	Pylodictus	0.77	Family Ictaluridae
flathead chub	*Platygobio gracilis*	Cypriniformes	Cyprinidae	Platygobio	1.20	Family Cyprinidae
freshwater drum	*Aplodinotus grunniens*	Perciformes	Sciaenidae	Aplodinotus	1.41	Order Perciformes
gizzard shad	*Dorosoma cepedianum*	Clupeiformes	Clupeidae	Dorosoma	1.21	All fish
goldeye	*Hiodon alosoides*	Hiodontiformes	Hiodontidae	Hiodon	1.21	All fish
green sunfish	*Lepomis cyanellus*	Perciformes	Centrarchidae	Lepomis	1.12	Exact match
iowa darter	*Etheostoma exile*	Perciformes	Percidae	Etheostoma	1.51	Family Percidae
kokanee salmon	*Oncorhynchus nerka*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
largemouth bass	*Micropterus salmoides*	Perciformes	Centrarchidae	Micropterus	1.39	Exact match
largescale sucker	*Catostomus macrocheilus*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
longnose dace	*Rhinichthys cataractae*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
longnose sucker	*Catostomus catostomus*	Cypriniformes	Catostomidae	Catostomus	0.90	Exact match
mottled sculpin	*Cottus bairdi*	Scorpaeniformes	Cottidae	Cottus	1.38	Exact match
mountain whitefish	*Prosopium williamsoni*	Salmoniformes	Salmonidae	Prosopium	1.38	Exact match
ninespine stickleback	Pungitius pungitius	pungitius	Gasterosteiformes	Gasterosteidae	1.79	Family Gasterosteidae
northern pike	*Esox lucius*	Esociformes	Esocidae	Esox	1.78	Exact match
northern pikeminnow	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
northern plains killifish	*Fundulus kansae*	Cyprinodontiformes	Fundulidae	Fundulus	1.27	Exact match
northern redbelly dace	*Chrosomus eos*	Cypriniformes	Cyprinidae	Chrosomus	1.20	Family Cyprinidae
northern squawfish	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
quillback	*Carpiodes cyprinus*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
rainbow trout	*Oncorhynchus mykiss*	Salmoniformes	Salmonidae	Oncorhynchus	1.07	Exact match
razorback sucker	*Xyrauchen texanus*	Cypriniformes	Catostomidae	Xyrauchen	1.01	Family Catostomidae
red shiner	*Cyprinella lutrensis*	Cypriniformes	Cyprinidae	Cyprinella	1.31	Family Cyprinidae
redbreast sunfish	*Lepomis auritus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redear sunfish	*Lepomis microlophus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redside shiner	*Richardsonius balteatus*	Cypriniformes	Cyprinidae	Richardsonius	1.08	Exact match
river carpsucker	*Carpiodes carpio*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
river redhorse	*Moxostoma carinatum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
rock bass	*Ambloplites rupestris*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
roundtail chub	*Gila robusta*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
sacramento perch	*Archoplites interruptus*	Perciformes	Centrarchidae	Archoplites	1.12	Family Centrarchidae
sacramento pikeminnow	*Ptychocheilus grandis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
sailfin molly	*Poecilia latipinna*	Cyprinodontiformes	Poeciliidae	Poecilia	1.21	Family Poeciliidae
sand shiner	*Notropis stramineus*	Cypriniformes	Cyprinidae	Notropis	1.56	Exact match
sauger	*Sander canadensis*	Perciformes	Percidae	Sander	1.60	Genus Sander
sculpin	*Cottus sp.*	Scorpaeniformes	Cottidae	Cottus	1.29	Exact match
shadow bass	*Ambloplites ariommus*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
shorthead redhorse	*Moxostoma macrolepidotum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
silver carp	*Hypophthalmichthys molitrix*	Cypriniformes	Cyprinidae	Hypophthalmichthys	1.20	Family Cyprinidae
smallmouth bass	*Micropterus dolomieu*	Perciformes	Centrarchidae	Micropterus	0.86	Exact match
smallmouth buffalo	*Ictiobus bubalus*	Cypriniformes	Catostomidae	Ictiobus	1.01	Family Catostomidae
speckled dace	*Rhinichthys osculus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys
spottail shiner	Notropis hudsonius	hudsonius	Cypriniformes	Cyprinidae	1.56	Genus Notropis
spotted bass	*Micropterus punctulatus*	Perciformes	Centrarchidae	Micropterus	1.12	Genus Micropterus
spotted gar	*Lepisosteus oculatus*	Lepistosteiformes	Lepisosteidae	Lepisosteus	1.21	All fish
stonecat	*Noturus flavus*	Siluriformes	Ictaluridae	Noturus	0.77	Family Ictaluridae
striped bass	*Morone saxatilis*	Perciformes	Moronidae	Morone	1.48	Exact match
striped mullet	*Mugil cephalus*	Mugiliformes	Mugilidae	Mugil	1.21	All fish

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
sucker		Cypriniformes	Catostomidae		1.01	Family Catostomidae
tilapia		Perciformes	Cichlidae		1.41	Order Perciformes
trout species	*Oncorhynchus sp.*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
tui chub	*Gila bicolor*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
utah sucker	*Catostomus ardens*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
walleye	*Sander vitreus*	Perciformes	Percidae	Sander	1.60	Exact match
western mosquitofish	*Gambusia affinis*	Cyprinodontiformes	Poeciliidae	Gambusia	1.21	Exact match
white bass	*Morone chrysops*	Perciformes	Moronidae	Morone	1.48	Genus Morone
white crappie	*Pomoxis annularis*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
white sturgeon	*Acipenser transmontanus*	Acipenseriformes	Acipenseridae	Acipenser	1.21	All fish
white sucker	*Catostomus commersonii*	Cypriniformes	Catostomidae	Catostomus	1.11	Exact match
wiper	*Morone chrysops x Moron saxatilis*	Perciformes	Moronidae	Morone	1.48	Genus Morone
yellow perch	*Perca flavescens*	Perciformes	Percidae	Perca	1.42	Exact match

Table B-8. Food web models used to calculate composite TTFs to translate the egg-ovary FCV to a water-column value at aquatic sites where sufficient data was available to calculate an enrichment factor (EF).

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			black bullhead	0.85	Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion		Median of all insects	in	2.14	0.45	Median of all crustaceans	crs	1.41	0.45	Median of all bivalves	bvs	4.29	0.10											2.03	0.85	1.72
Default			black crappie	2.67	Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			blacknose dace	0.71	Eats immature aquatic insects, amphipods, and various other aquatic invertebrates; also eats algae and diatoms, which may be of little nutritional value (Smith 1979, Becker 1983).		Median of all insects	in	2.14	0.50	Median of all invertebrates except bivalves	all	1.48	0.50														1.81	0.71	1.29
Default			blue catfish	0.68	Bottom feeder. Eats mostly crustaceans and aquatic insects when young. Later, fishes and large invertebrates become most important (Moyle 1976). Also scavenges.		Median of all insects	in	2.14	0.36	Median insects and benthic crustaceans	in,bc	1.74	0.20	Median of all bivalves	bvs	4.29	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.36	2.28	0.68	1.56
Default			bluegill	1.03	Feeds opportunistically on aquatic insect larvae, planktonic crustaceans, flying insects, snails, and other small invertebrates; small fishes, fish eggs, crayfish, and algae sometimes are eaten. Larvae and juveniles often eat cladocerans and copepod nauplii. Adults eats mainly aquatic insects, crayfishes, and small fishes, or, in some bodies of water, mostly zooplankton. Feeds at all levels of water column.		Median of all insects	in	2.14	0.68	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.04	1.95	1.03	2.00
Default			bluehead sucker	1.04	Herbivore, Invertivore		TL1	TL1	1.00	0.60	Median of all invertebrates except bivalves	all	1.48	0.40														1.19	1.04	1.24
Default			brassy minnow	1.20	Eats algae, phyto- and zooplankton, benthic invertebrates, surface drift, bottom ooze (Becker 1983).		TL1	TL1	1.00	0.50	Median of planktonic crustacean	pc	1.41	0.40	Median of all insects	in	2.14	0.10										1.28	1.20	1.54

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			brook stickleback	1.79	Eats various aquatic invertebrates (including eggs and larvae), eggs and larvae of fishes, and algae. In a Manitoba lake, was opportunistic but heavily dependent on arthropods (Moodie 1986).		Median of all invertebrates except bivalves	all	1.48	0.80	TL1	TL1	1.00	0.20															1.38	1.79	2.47
Default			brook trout	0.88	Feeds opportunistically on various invertebrate and vertebrate animals, including primarily terrestrial and aquatic insects and planktonic crustaceans.		Median of all insects	in	2.14	0.60	crayfish	cr	1.46	0.10	Median of all bivalves	bvs	4.29	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.22	0.88	1.96
Default			brown bullhead	0.85	Bottom feeder. Young eat chironomid larvae and small crustaceans. Adults eat larger insect larvae and fishes, also fish eggs, mollusks, carrion, and plant material (Becker 1983, Moyle 1976).		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.85	1.79
Default			brown trout	1.38	Eats aquatic and terrestrial insects and their larvae, crustaceans (especially crayfish), molluscs, fishes, and other animals. In streams, young feed mainly on aquatic and terrestrial drift invertebrates; in lakes, they feed on zooplankton and benthic invertebrates (Sublette et al. 1990). Large adults feed on fishes, crayfish, and other benthic invertebrates.		Median of planktonic crustaceans	pc	1.41	0.20	Median of all insects	in	2.14	0.12	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.02	1.38	2.78
Default			bullhead	0.77	Black (not exotic to CO and NM): Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion. Stomach often contain substantial amounts of plant material of unknown nutritional value (Moyle 1976). Juveniles planktivorous; at about 27 mm TL, feed largely on crustaceans and midge larvae		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.77	1.62
Default			burbot	1.21	Young eat mainly immature aquatic insects, crayfish, molluscs, and other deepwater invertebrates. Larger individuals feed mostly on fishes (Becker 1983, Scott and Crossman 1973).		Median of all insects	in	2.14	0.25	Median of all crustaceans	crs	1.41	0.25									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.03	1.21	2.45
Default			channel catfish	0.68	Bottom feeder. Young eat mainly small invertebrates; as they grow, fishes and crayfish become increasingly important, though individuals of all sizes eat abundant aquatic insects. Large fish are mainly piscivorous (Moyle 1976).		Median of all insects	in	2.14	0.48	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	1.97	0.68	1.35

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			common carp	1.20	Omnivorous; adults eat mainly invertebrates, detritus, fish eggs, and plant material (Jester 1974, Becker 1983, Sublette et al. 1990).		Median of all invertebrates except bivalves	all	1.48	0.65	TL1	TL1	1.00	0.35														1.31	1.20	1.58
Default			crappie	2.67	Black: Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals. White: eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			creek chub	1.06	Feeds opportunistically on various plants and animals, from surface drift to benthos; mostly invertivorous but large individuals often picivorous (Becker 1983). Chironomid larvae and other larval insects important in diet of young.		Median of all invertebrates except bivalves	all	1.48	0.70	TL1	TL1	1.00	0.20										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.46	1.06	1.55
Default			cutthroat trout	1.12	Opportunistic. Inland cutthroats feed primarily on insects (aquatic and terrestrial); often feeds in and especially downstream from riffle areas; some large individuals feed mostly on fishes; also eats zooplankton and crustaceans.		Median of all insects	in	2.14	0.50	Median of all crustaceans	crs	1.41	0.20										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.3	2.04	1.12	2.29
Default			fathead minnow	1.57	Feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990).	expected diet of small invertebrates	Median of all insects	in	2.14	0.60	Median of all crustaceans	crs	1.41	0.20	TL1	TL1	1.00	0.20										1.77	1.57	2.78
Default			flannel mouth sucker	0.98	Herbivore, Invertivore Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25														1.55	0.98	1.52
Default			flathead chub	1.20	Opportunistic; eats aquatic and terrestrial insects and some algae (Olund and Cross 1961)		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20														1.91	1.20	2.30
Default			freshwater drum	1.41	Young feed mainly on minute crustaceans; adults mostly are bottom feeders, eat insect larvae, crustaceans, fishes, and (mostly in rivers) clams and		Median of all crustaceans	crs	1.41	0.44	Median of all insects	in	2.14	0.40	Median of all bivalves	bvs	4.29	0.04						Fish	Median all fish eating median all invertebrat	f+a	2.28	0.12	1.92	1.41	2.71

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					snails (Becker 1983, Scott and Crossman 1973, Lee et al. 1980).																			es						
Default			gizzard shad	1.21	Adults primarily bottom filter-feeding detritivores		TL1	TL1	1.00	1.00																		1.00	1.21	1.21
Default			goldeye	1.21	Young-of-year eat mainly microcrustaceans, also other invertebrates. Older individuals eat mainly aquatic insects obtained at surface but also various other animals, including frogs, fishes, and small mammals.		Median insects and benthic crustaceans	in,bc	1.74	1.00																		1.74	1.21	2.10
Default			green sunfish	1.12	Feeds opportunistically on the larger, more active invertebrates that occur with them, and on small fishes. Young feed mostly on crustaceans (zooplankton) and aquatic insect larvae. Adults eat more large aquatic and terrestrial insects, crayfish, and fishes		Median of all insects	in	2.14	0.58	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	2.05	1.12	2.29
Default			Iowa darter	1.51	Eats mainly various invertebrates; commonly ingested food items of adults are midge larvae, mayfly naiads, and amphipods, and of the young, copepods and cladocerans. Apparently feeds on swimming organisms and those on bottom.	expected diet of small invertebrates	Median of all insects	in	2.14	0.70	amphipods	am	1.22	0.16	crayfish	cr	1.46	0.08	Median of planktonic crustaceans	pc	1.41	0.06						1.90	1.51	2.87
Default			kokanee salmon	1.10	Zooplankton, insects.		Median of planktonic crustaceans	pc	1.41	0.80	Median of all insects	in	2.14	0.20														1.56	1.10	1.71
Default			largemouth bass	1.39	Fry feed mainly on zooplankton. Larger young eat insects, crustaceans, and fish fry. Adults eat mainly fishes, though sometimes prefer crayfish or amphibians (Moyle 1976, Smith 1979).		Median of all insects	in	2.14	0.10	crayfish	cr	1.46	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.18	1.39	3.04
Default			longnose dace	0.71	Eats mainly benthic insects, especially Diptera and mayflies (Becker 1983, Scott and Crossman 1973); also eats algae and plant material (Sublette et al. 1990). Terrestrial insects and fish eggs common in diet of adults from Lake Michigan (see Sublette et al. 1990).		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20														1.91	0.71	1.36
Default			longnose sucker	0.90	Eats mostly bottom invertebrates (Scott and Crossman 1973).		Median of all invertebrates except bivalves	all	1.48	1.00																		1.48	0.90	1.34

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			mottled sculpin	1.38	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	Median of all crustaceans	crs	1.41	0.10	TL1	TL1	1.00	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.97	1.38	2.72
Default			mountain whitefish	1.38	Feeds actively on aquatic and terrestrial insects. Also feeds on some fish eggs and occasionally on fishes. Bottom-oriented predator (Moyle 1976), occasionally feeds at surface (Sigler and Sigler 1987).		Median of all insects	in	2.14	0.90													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.16	1.38	2.97
Default			ninespine stickleback	1.79	Eats mainly small crustaceans and aquatic insects; sometimes also fish eggs and fry (Becker 1983).		Median of all crustaceans	crs	1.41	0.48	Median of all insects	in	2.14	0.44									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	1.80	1.79	3.22
Default			northern pike	1.78	Young initially eat large zooplankton and immature aquatic insects. After 7-10 days fishes begin to enter diet and eventually dominate. Adults feed opportunistically on vertebrates small enough to be engulfed. (Scott and Crossman 1973). Sight feeder.		Median insects and benthic crustaceans	in,bc	1.74	0.05													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.95	2.25	1.78	4.02
Default			northern plains killifish	1.27	Feed effectively at all levels and food habits are generalized. Prefer aquatic insects but also feed on plants.	Montana field guide (http://fieldguide.mt.gov/detail_AFCNB04600.aspx)	Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20														1.91	1.27	2.44
Default			northern redbelly dace	1.20	Eats mainly diatoms and filamentous algae, also zooplankton and aquatic insects.		TL1	TL1	1.00	0.70	Median of all insects	in	2.14	0.15	Median insects and benthic crustaceans	in,bc	1.74	0.15										1.28	1.20	1.54
Default			northern squawfish	1.20	Small individuals feed primarily on aquatic and terrestrial insects. Adults feed on fish, insects, insect larvae, crustaceans and some plankton during spring and summer. Fishes are the major component		Median of all insects	in	2.14	0.32	Median of all crustaceans	crs	1.41	0.08									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.17	1.20	2.61

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					of the diet in winter.																									
Default			rainbow trout	1.07	In lakes, feeds mostly on bottom-dwelling invertebrates (e.g., aquatic insects, amphipods, worms, fish eggs, sometimes small fish) and plankton. In streams, feeds primarily on drift organisms. May ingest aquatic vegetation (probably for attached invertebrates).		Median of all insects	in	2.14	0.75													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.18	1.07	2.33
Default			red shiner	1.31	Eats various small invertebrates (insects, crustaceans), plant material (digestibility may be low), and microorganisms (Becker 1983). In Virgin River, diet dominated by Ceratopongidae, Simuliidae, and Chironomidae (Greger and Deacon 1988).		Median insects and benthic crustaceans	in,bc	1.74	1.00																		1.74	1.31	2.27
Default			redside shiner	1.08	Feeds mainly on aquatic and terrestrial insects; also eats molluscs, plankton, and some small fish and fish eggs. Fry eat zooplankton and algae.		Median of all insects	in	2.14	0.70	Median of planktonic crustaceans	pc	1.41	0.10	Median of all bivalves	bvs	4.29	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.30	1.08	2.48
Default			river carpsucker	1.01	Mostly a bottom feeder, browses on periphyton associated with submerged rocks and debris, ingests various small planktonic plants and animals.		TL1	TL1	1.00	0.75	Median of planktonic crustaceans	pc	1.41	0.25														1.10	1.01	1.11
Default			roundtail chub	1.20	Opportunistic; eats available aquatic and terrestrial insects, gastropods, crustaceans, fishes, and sometimes filamentous algae (Sublette et al. 1990).		Median of all insects	in	2.14	0.55	Median of all crustaceans	crs	1.41	0.15	Median of all bivalves	bvs	4.29	0.15					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.38	1.20	2.86
Default			Sacramento perch	1.12	Opportunistic; diet mainly benthic insect larvae, snails, mid-water insects, zooplankton, and fishes (Moyle et al. 1989). Young feed mainly on small crustaceans, but as they grow Sacramento perch consume more aquatic insect larvae and pupae. Large adults feed mainly on other fishes when available.		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.12	1.44
Default			sailfin molly	1.21	Eats mainly algae, vascular plants, organic detritus, and mosquito larvae (and other small invertebrates).		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.21	1.56

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			sand shiner	1.56	Eats various aquatic and terrestrial invertebrates (especially chironomids), algae, and (mainly) bottom particulate matter (Becker 1983). Winter diet mostly chironomids larvae and mayfly and stonefly naiads (Ohio, see Sublette et al. 1990)		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25														1.55	1.56	2.43
Default			sauger	1.60	Larvae eat microcrustaceans. Young eat zooplankton, immature and adult aquatic insects, and fish fry; adults eat small fishes and various invertebrates (Scott and Crossman 1973), or are almost exclusively piscivorous (Burkhead and Jenkins 1991). Sight feeder, adapted to low light.		Median insects and benthic crustaceans	in,bc	1.74	0.36	Median of planktonic crustaceans	pc	1.41	0.10										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.54	2.00	1.60	3.20
Default			sculpin	1.29	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	crayfish	cr	1.46	0.15										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.06	1.29	2.66
Default			shorthead redhorse	1.01	Invertivore		Median of all invertebrates except bivalves	all	1.48	1.00																		1.48	1.01	1.49
Default			smallmouth bass	0.86	Adults almost entirely piscivorous if sufficient prey available		Median of all insects	in	2.14	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.25	0.86	1.93
Default			speckled dace	0.71	An omnivorous benthic feeder, at times feeding on drift in mid-water or rarely at the surface (Schreiber and Minckley 1981). The diet consists mostly of benthic insects, also includes other invertebrates, algae, and detritus (little or no plant material or detritus in some areas) (Sublette et al. 1990, Woodbury 1933, Greger and Deacon 1988). Young feed mainly on zooplankton.		Median of all insects	in	2.14	0.70	Median insects and benthic crustaceans	in,bc	1.74	0.15	TL1	TL1	1.00	0.15										1.91	0.71	1.36

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			stonecat	0.77	Eats mainly bottom invertebrates (insects, crayfish); sometimes also plant material and fishes (Becker 1983, Scott and Crossman 1973).		Median insects and benthic crustaceans	in,bc	1.74	0.70	TL1	TL1	1.00	0.20										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.65	0.77	1.26
Default			sucker	1.01	White: Larvae feed near surface on protozoans, diatoms, small crustaceans, and bloodworms. Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990). Bluehead: A bottom feeder. Scrapes algae and other organisms from rocks with chisel-like ridges inside each lip; ingests fine organism-laden sediments. May feed in stream riffles, or deeper rocky pools; in lakes it may feed over rocks near shore. May eat aquatic insect larvae. Flannelmouth: Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50															1.24	1.01	1.25
Default			tilapia	1.41	aureus: Eats mainly phytoplankton. mossambicus: Nonselective omnivore; eats planktonic algae, aquatic plants, invertebrates, and small fishes (Moyle 1976). zilli: Feeds on algae and higher plants, invertebrates, and occasionally eats dead or dying fish.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50															1.24	1.41	1.74
Default			tui chub	1.20	Adults opportunistic. They feed on plant material, plankton, insect larvae, crustaceans, fish fry and fish eggs, etc. Young feed on zooplankton. Coarse-rakered form eats more plant material, fine-rakered form more zooplankton.		TL1	TL1	1.00	0.40	Median of planktonic crustaceans	pc	1.41	0.28	Median of all insects	in	2.14	0.28	crayfish	cr	1.46	0.04							1.45	1.20	1.75
Default			Utah sucker	1.01	Bottom feeder. Varied diet; feeds freely on both animal and plant organisms, at all depths throughout the year. Grazes on filamentous algae.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50															1.24	1.01	1.25

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			walleye	1.60	Adults feed opportunistically on various fishes and larger invertebrates.		Median insects and benthic crustaceans	in,bc	1.74	0.50													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.01	1.60	3.21
Default			western mosquitofish	1.21	Opportunistic omnivore; eats mainly small invertebrates, often taken near water surface. Also eats small fishes and, in the absence of abundant animal food, algae and diatoms (Moyle 1976). Mosquitofish are principally carnivorous, and have strong, conical teeth and short guts (Meffe et al. 1983, Turner and Snelson 1984). They are reported to feed on rotifers, snails, spiders, insect larvae, crustaceans, algae, and fish fry, including their own progeny (Barnickol 1941, Minckley 1973, Meffe and Crump 1987). Cannibalism has been documented by several authors (Seale 1917, Krumholz 1948, Walters and Legner 1980, Harrington and Harrington 1982). Plant material is taken occasionally (Barnickol 1941) and may make up a significant portion of the diet during periods of scarcity of animal prey (Harrington and Harrington 1982). Grubb (1972) showed that anuran eggs from temporary ponds were preferentially selected over those breeding in permanent systems.		Median of all insects	in	2.14	0.75	Median of all crustaceans	crs	1.41	0.25															1.96	1.21	2.37
Default			white bass	1.48	Eats fishes, zooplankton, aquatic insects, oligochaetes, and crayfish; fishes often dominate diet of adults; diet may vary from place to place (Moyle 1976, Sublette et al. 1990).		Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.05	crayfish	cr	1.46	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.15	1.48	3.19
Default			white crappie	2.67	Eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			white sturgeon	1.21	A bottom feeder. Young feed mostly on the larvae of aquatic insects, crustaceans, and molluscs. A significant portion of the		Median insects and benthic	in,bc	1.74	0.31	Median of all bivalves	bvs	4.29	0.09									Fish	Median all fish eating median all	f+a	2.28	0.6	2.29	1.21	2.77

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					diet of larger sturgeon consists of fish.		crustaceans																	invertebrates						
Default			white sucker	1.11	Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990).	expected common spp in benthos	TL1	TL1	1.00	0.50	Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.10						1.43	1.11	1.58
Default			wiper	1.48	Adults are predatory on fishes and larger crustaceans (Hassler 1988).		crayfish	cr	1.46	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.11	1.48	3.13
Default			yellow perch	1.42	Larvae and young primarily zooplankton feeders; older young eat mostly invertebrates associated with bottom and with aquatic plants; adults feed among plants and along bottom on larger invertebrates and small fishes (Moyle 1976).		Median insects and benthic crustaceans	in,bc	1.74	0.64	Median of planktonic crustaceans	pc	1.41	0.13	TL1	TL1	1.00	0.07					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.16	1.73	1.42	2.47
Saiki et al. 1993			bluegill	1.03	site-specific: 0.23 chironomid; 0.3 microcrustacea; 0.47 amphipod	stomach analysis	amphipods	am	1.22	0.47	Median of planktonic crustaceans	pc	1.41	0.30	midges	mg	1.90	0.23										1.43	1.03	1.47
Saiki et al. 1993			largemouth bass	1.39	site-specific: 0.73 fish; 0.27 crayfish	stomach analysis	crayfish	cr	1.46	0.27	Saiki bluegill TTFcomp.	BG	1.47	0.73														1.47	1.39	2.04
Saiki et al. 1993			western mosquitofish	1.21	site-specific: 0.89 molluscs, and insects; 0.065 chironomid; 0.045 microcrustacea	stomach contents show a large terrestrial component	Median insects and benthic crustaceans	in,bc	1.74	0.89	midges	mg	1.90	0.07	Median of planktonic crustaceans	pc	1.41	0.05										1.74	1.21	2.10
Formation 2012	Crow Creek - CC150	CC-150	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.38	2.91
Formation 2012	Crow Creek - CC150	CC-150	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.29	2.74

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Crow Creek - 1A	CC-1A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.38	2.96
Formation 2012	Crow Creek - 1A	CC-1A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.29	2.78
Formation 2012	Crow Creek - CC350	CC-350	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.38	2.97
Formation 2012	Crow Creek - CC350	CC-350	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.29	2.79
Formation 2012	Crow Creek - 3A	CC-3A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.38	2.97
Formation 2012	Crow Creek - 3A	CC-3A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.29	2.78
Formation 2012	Crow Creek - CC75	CC-75	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.38	2.87
Formation 2012	Crow Creek - CC75	CC-75	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.29	2.69
Formation 2012	Deer Creek	DC-600	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.38	3.00
Formation 2012	Deer Creek	DC-600	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.29	2.81
Formation 2012	Hoopes Spring - HS	HS	brown trout	1.38	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.38	3.86
Formation 2012	Hoopes Spring - HS	HS	sculpin	1.29	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.29	3.63
Formation 2012	Hoopes Spring - HS3	HS-3	brown trout	1.38	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.38	2.63
Formation 2012	Hoopes Spring - HS3	HS-3	sculpin	1.29	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.29	2.47

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Sage Creek - LSV2C	LSV-2C	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.38	3.01
Formation 2012	Sage Creek - LSV2C	LSV-2C	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.29	2.83
Formation 2012	Sage Creek - LSV4	LSV-4	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.38	2.88
Formation 2012	Sage Creek - LSV4	LSV-4	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.29	2.70
Formation 2012	South Fork Tincup Cr.	SFTC-1	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.38	3.05
Formation 2012	South Fork Tincup Cr.	SFTC-1	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.29	2.86

Table B-9. Calculation of site-specific invertebrate proportions using invertebrate counts in Formation 2012

| Order | Genus | Habitat / Behavior | Functional Feeding | Tolerance | Stream | SF Tincup Creek | Crow Creek | | | | | | | | | | | | Deer Creek | | | | | | | | | | | | Hoopes Spring | | | | | | | | | | | | Sage Creek | | | | | | | | | | | | Crow Creek | | | | | | | | | | | | | Total |
|---|---|---

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek			Crow Creek								Deer Creek							Hoopes Spring		Sage Creek						Crow Creek									
					Location	SFTC1			CC75			CC150			CC350				DC600				HS		HS3			LSV2C		LSV4			CC1A				CC3A				Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
	sp.																																							
Trichoptera	Glossoma sp.	CN	SC	0														4																						4
Trichoptera	Helicopsyche sp.	CN	SC	3				3			5	4		5	93									2			2								19	81		214
Trichoptera	Hesperophylax sp.	CN	SH	5																			3	1	48		14	4										70
Trichoptera	Hydropsyche sp.	CN	F	4			5	47	50	23	29	17	11	74	97	41	1	14		2				2		8	9	11	53	63	91	29	105	79	151	1012
Trichoptera	Hydroptila sp.	CN	SC	6				8	9	1	1												1			16				1			2				39
Trichoptera	Lepidostoma spp.	SP/CB	SH	1		1	3		7		6	8			67	6		16				2									13	4		2	6	141
Trichoptera	Micrasema sp.	CN	SH	1				8		9	65	1	18	14			3			28			5			4			76	3	3		36			273
Trichoptera	Neothremma sp.	CN	SC	0														4																				4
Trichoptera	Oecetis disjuncta	CN	P	8		2								2	3																	3		7		17
Trichoptera	Onocosmoecus sp.	CB	SH	1		1																																1
Trichoptera	Oligophlebodes sp.	CN	SC	1				11																										2				13
Trichoptera	Parapsyche sp.	CN	P	1				16			5						7						1			2			1								32
Trichoptera	Psychoglypha sp.	SP/CB	CG	1					3																													3
Trichoptera	Rhyacophila spp.	CN	P	0		7	3	4	5	5	9	17	16	3	5	9	16	23	83					11	13		7										236
Trichoptera	Wormaldia spp.	CN	F	3			3				1	8	15	2	5	6	7							3	9				1			3	1	2	11	77
Coleoptera	Ametor sp.	SW	P	5																		1															1
Coleoptera	Brychius sp.	CB	SC	7		2			1	3																10											16
Coleoptera	Cleptelmis sp.	CN	CG/SC	4		3	26									4												5		1			1		6	46
Coleoptera	Dubiraphia sp.	CN	CG/SC	4		3																															3
Coleoptera	Heterlimnius corpulentus	CN/BU	CG/SC	4														30	32			5															67
Coleoptera	Optioservus quadrimaculatus	CN	CG/SC	4		97	267	43	109	68	40	205	153	78	162	167	7	2	5	12			5	21	33	18	132	151	27	153	74	246	69	83	129	2556
Coleoptera	Oreodytes sp.	SW/DV	P	5		6		1																													7
Coleoptera	Paracymus sp.	CN	P/OM	5								1																									1
Coleoptera	Zaitzevia parvula	CN/BU	CG/SC	4		170	57	5	4		1	1	7	23	5	18							3	3		2	2	7	2	6	16	11	8	2	13	366
Megaloptera	Sialis sp.	BU/CB	P	4		1			1	3		1																									6

Order	Genus	Habitat / Behavior	Functional Feeding Groups	Tolerance	Stream	SF Tincup Creek			Crow Creek							Deer Creek							Hoopes Spring							Sage Creek							Crow Creek									Total
					Location	SFTC1			CC75			CC150			CC350				DC600				HS				HS3				LSV2C			LSV4			CC1A				CC3A				
					Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Odonata	Ophiogomphus sp.	BU	P	1																																2		7		9
Hemiptera	Sigara sp.	SW	P	10		5																																		5
Diptera	Anopheles sp.	SW	F	8									1																												1
Diptera	Antocha sp.	BU	CG	3			5	1			4			6																			18		2		1			37
Diptera	Atherix sp.	BU	P	2																																26	22	24	3	44	119
Diptera	Chelifera sp.	SP/BU	CG	6				2			1						7			4													1				5			20
Diptera	Dixa sp.	BU	CG	1																	13																				13
Diptera	Empididae	SP/BU	P	6									1						5			2																			8
Diptera	Ephydridae	BU	CG	6							1								1			1																			3
Diptera	Glutops sp.	BU	P	3				1	2									1																							4
Diptera	Hexatoma	BU	P	2		19				9		1			5	4		1							9			4					5				16	1	74
Diptera	Limnophila sp.	BU	P	4							1			3			3					5								5	3			9				29
Diptera	Muscidae	BU	P	6																					1		3												4
Diptera	Pericoma sp.							2			1																													3
Diptera	Probezzia sp.	BU	P	6						3					2		1	1							2										2				11
Diptera	Ptychoptera sp.		CG	7																															1				1
Diptera	Simulium sp.	CN	F	6			18	78	5	30	26	49	17	17	5	102	9	15	8		5	4	13	21	38	24	25	24	12	114	35	8	1	26	31	760
Diptera	Tipula sp.	BU	SH	4					7	3				1					3					3			2			2		3	3			27
Chironomidae (family)	Chironomidae	BU/SP	CG/SH/P	6				188	195	173	143	99	143	68	10	30	33	88	151	92	124	25	23	83		20	43	91	149	36	56	35	41	21	8	2168
Hirudinea (class)	Helobdella sp.		PA/P	6								1																											1
Collembola	Collembola										2																													2
Oligochaeta (class)	Oligochaeta		CG	5			5	15	7	2	6	4	7	8	3	5	3	5	19	72	101	5	3		34		9	8	9			19	56		405
Bivalvia (class)	Pisidium sp.	BU	F	8		2		2	4		2						2	6		2	5	2	2		12					3	1	1	23		69
Gastropoda (class)	Fossaria sp.	CN	SC	8			2			1					2					52	57	27	4	4		8				15		1	1		174
Gastropoda (class)	Amnicola sp.	CN	SC	5										2	2	1	1												3		1			10
Gastropoda (class)	Gyraulus sp.	CN	SC																										1					1
Gastropoda (class)	Mentus sp.	CN	SC																	6														6
Gastropoda (class)	Physella sp.	CN	SC	8		19		3	2	1					3	1				114	55	7	2	6		14	32		1	2		3	23		288

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek		Crow Creek								Deer Creek						Hoopes Spring			Sage Creek						Crow Creek				
					Location	SFTC1		CC75			CC150			CC350			DC600			HS			HS3			LSV2C		LSV4			CC1A			CC3A			Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Gastropoda (class)	Valvata sp.	CN	SC																			1													1
Amphipoda	Gammarus sp.	SW/BU	OM	6																2			2	4	13	8	1	12				2				44
Ostracoda	Ostracoda	SW	CG	8						1													460	2	9	30	13	8	1	1						525
Tricladida	Polycelis coronata		OM	1										4																						4
Acari (subclass)	Acari		P	8				2		2	3	4		2	6	7															2	2	5			35
					% Subsampled	50	50	12.5	12.5	66.6	12.5	25	50	25	12.5	50	100	33.3	75	50	33.3	100	12.5	33.3	100	25	25	75	12.5	25	25	50	25	25	50	13872
					Total abundance	394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
					Total taxa	24	19	27	25	22	26	24	16	23	24	21	23	23	16	15	13	14	21	22	14	23	21	17	21	20	18	22	30	20	15	
					Total Counts	788	972	4128	4048	741.7417	3720	1928	1068	1908	4288	984	420	1435.435	545.3333	996	1246.246	91	4768	1411.411	280	2164	2128	593.3333	3560	1948	1808	926	1860	2012	1000	
					Density (#/1m2)	2835	3496	14849	14561	2668	13381	6935	3842	6863	15424	3540	1511	5163	1962	3583	4483	327	17151	5077	1007	7784	7655	2134	12806	7007	6504	3331	6691	7237	3597	
						394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
							880			1516			1481			1505			1307			1004			1346			1518	445			1402			1468	13872

Functional Feeding Groups (FFG): CG = Collector-Gatherer, SC = Scraper, F = Filterer, P = Predator, SH = Shredder, OM = Omnivore, Habitat/Behavior (Hab/Beh): BU = Burrower, SW = Swimmer, CN = Clinger, CB = Climber, SP = Sprawler, DV = Diver

Table B-10. Summary of Formation 2012 invertebrate data.

Phylum	Subphylum	Class	Subclass	Infraclass	Superorder	Order	Lookup ID	Common name	SFTC1		CC75		CC150		CC350		DC600		HS		HS3		LSV2C		LSV4		CC1A		CC3A	
									Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion
Arthropoda		Insecta	Pterygota		Ephemeropteroidea	Ephemeroptera	Ephemeroptera	Mayflies	36	0.04	185	0.12	231	0.16	192	0.13	444	0.34	115	0.11	325	0.24	421	0.28	56	0.13	168	0.12	136	0.09
Arthropoda		Insecta	Pterygota		Exopterygota	Plecoptera	Plecoptera	Stoneflies	107	0.12	85	0.06	40	0.03	18	0.01	195	0.15	59	0.06	20	0.01	26	0.02	30	0.07	11	0.01	22	0.01
Arthropoda		Insecta			Amphiesmenoptera	Trichoptera	Trichoptera	Caddisflies	30	0.03	268	0.18	283	0.19	539	0.36	229	0.18	34	0.03	230	0.17	324	0.21	135	0.30	325	0.23	623	0.42
Arthropoda		Insecta	Pterygota	Neoptera	Endopterygota	Coleoptera	Coleoptera	Beetles	631	0.72	234	0.15	408	0.28	457	0.30	76	0.06	18	0.02	65	0.05	327	0.22	29	0.07	507	0.36	311	0.21
Arthropoda		Insecta		Neoptera		Megaloptera	Megaloptera	Alderflies, dobsonflies and fishflies	1	0.00	4	0.00	1	0.00																
Arthropoda		Insecta	Pterygota		Odonatoptera	Odonata	Odonata	Dragonflies and damselflies																			2	0.00	7	0.00
Arthropoda		Insecta		Neoptera	Paraneoptera	Hemiptera	Hemiptera	True bugs (cicadas, aphids, planthoppers, leafhoppers, shield bugs)	5	0.01																				
Arthropoda		Insecta			Panorpida	Diptera	Diptera	True flies	42	0.05	143	0.09	103	0.07	145	0.10	55	0.04	29	0.03	89	0.07	85	0.06	36	0.08	221	0.16	166	0.11
Arthropoda		Insecta				Chironomidae (family)	Chironomidae (family)	Midges			556	0.37	385	0.26	108	0.07	272	0.21	241	0.24	106	0.08	154	0.10	149	0.33	127	0.09	70	0.05
Annelida		Clitellata	Hirudinea				Hirudinea (class)	Leeches					1	0.00																
Arthropoda		Entognatha				Collembola	Collembola	Springtails (not insects!)					2	0.00																
Annelida		Clitellata	Oligochaeta				Oligochaeta (class)	Worms	5	0.01	24	0.02	17	0.01	16	0.01	27	0.02	178	0.18	3	0.00	43	0.03	8	0.02	9	0.01	75	0.05
Mollusca		Bivalvia					Bivalvia (class)	Clams	2	0.00	6	0.00	2	0.00			8	0.01	9	0.01	2	0.00	12	0.01			4	0.00	24	0.02
Mollusca		Gastropoda					Gastropoda (class)	Snails and slugs	21	0.02	7	0.00			11	0.01	1	0.00	319	0.32	16	0.01	54	0.04	1	0.00	21	0.01	29	0.02
Arthropoda	Crustacea	Malacostraca				Amphipoda	Amphipoda	Crustaceans											2	0.00	19	0.01	21	0.01			2	0.00		
Arthropoda	Crustacea	Ostracoda					Ostracoda	Sea shrimp					1	0.00							471	0.35	51	0.03	1	0.00	1	0.00		
Platyhelminthes		Turbellaria				Tricladida	Tricladida	Flatworms							4	0.00														
Arthropoda	Chelicerata	Arachnida	Acari				Acari (subclass)	Mites and ticks			4	0.00	7	0.00	15	0.01											4	0.00	5	0.00
							Total		880		1516		1481		1505		1307		1004		1346		1518		445		1402		1468	
								Midge		0.00		0.37		0.26		0.07		0.21		0.24		0.08		0.10		0.33		0.09		0.05
								Mayfly		0.04		0.12		0.16		0.13		0.34		0.11		0.24		0.28		0.13		0.12		0.09
								Other insects		0.93		0.48		0.56		0.77		0.42		0.14		0.30		0.50		0.52		0.76		0.77
								Molluscs		0.03		0.01		0.00		0.01		0.01		0.33		0.01		0.04		0.00		0.02		0.04
								Crustaceans		0.00		0.00		0.00		0.00		0.00		0.00		0.36		0.05		0.00		0.00		0.00
								Annelids		0.01		0.02		0.01		0.01		0.02		0.18		0.00		0.03		0.02		0.01		0.05
								Other		0.00		0.00		0.01		0.01		0.00		0.00		0.00		0.00		0.00		0.00		0.00

	Total		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00
Take the top 3 that are above 1%		Insects	0.93	Insects	0.50	Insects	0.58	Insects	0.79	Insects	0.44	Midge	0.32	Insects	0.33	Insects	0.57	Insects	0.53	Insects	0.78	Insects	0.85
		Molluscs	0.03	Midge	0.38	Midge	0.27	Midge	0.07	Midge	0.21	Molluscs	0.44	Crustaceans	0.40	Midge	0.12	Midge	0.34	Midge	0.09	Midge	0.05
		Mayfly	0.04	Mayfly	0.13	Mayfly	0.16	Mayfly	0.13	Mayfly	0.35	Worms and leeches	0.24	Mayfly	0.27	Mayfly	0.31	Mayfly	0.13	Mayfly	0.12	Mayfly	0.10
			1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00

APPENDIX C: SUMMARIES OF CHRONIC STUDIES CONSIDERED FOR CRITERIA DERIVATION

White sturgeon C-2
Sacramento splittail C-12
Fathead minnow C-15
Flannelmouth & razorback suckers C-22
Northern pike C-24
Chinook salmon C-27
Rainbow trout & brook trout C-32
Cutthroat trout C-51
Dolly Varden C-65
Brown trout C-68
Desert pupfish C-86
Eastern and western mosquitofish C-103
Striped bass C-105
Bluegill sunfish C-106
Largemouth bass C-147

See Appendix E for descriptions of other, less conclusive studies with:
Rainbow trout
Fathead minnow
Sacramento splittail
White sucker

See Appendix E for descriptions of invertebrate studies.

Tashjian, D.H., S.J. The, A. Sogomoyan and S.S.O. Hung. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon *(Acipenser transmontanus*). Aquatic Toxicol.79:401-409.

Test Organism: White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Seleno-L-methionine was added to an artificial diet consisting of vitamin-free casein, wheat gluten, egg albumin, dextrin, vitamin mix, BTM-mineral mix, cellulose, corn oil, cod liver oil, choline chloride and santoquin; the measured dietary concentrations were 0.4, 9.6, 20.5, 41.7, 89.8, 191.1 mg Se/kg dw.

Test Duration: 8 weeks

Study Design: 25 juvenile white sturgeon were placed in each of 24 90-L tanks. Treatments were randomly assigned to the 24 tanks resulting in 4 replicates per dietary treatment. Four fish from each tank were sampled after 0, 4 and 8 weeks for weight, length, liver weight, condition factors, hepatosomatic indices, hemocrit, histopathology, and selenium measurement in liver, kidney, muscle and gill tissues. 8 fish after 0 and 8 weeks were sampled for whole body selenium measurement.

Effects Data: Sturgeon survival did not differ significantly among treatment groups after the 8-week exposure with a mean survival rate of 99 across all groups. Fish fed 41.7 to 191.1 mg Se/kg dw exhibited significant declines in body weight (see table). All other endpoints measured were as sensitive or less sensitive to selenium in the diet as body weight.

Mean (SE) white sturgeon moisture, lipid and whole body Se after 8-week exposure

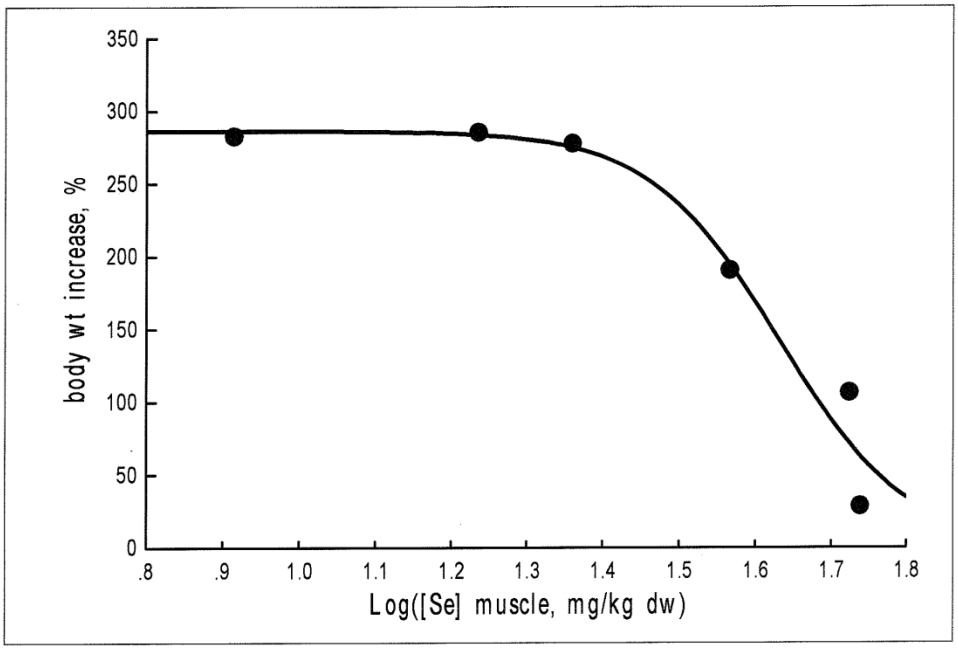
Treatment group	Moisture, % ww	Lipid, % ww	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	76.8 (0.5) b	9.5 (4) abc	8.2 (0.6) e	5.2 (0.4) c
9.6	77.0 (0.7) b	9.5 (0.9) abc	17.2 (0.7) d	11.8 (0.9) b
20.5	76.8 (0.3) b	10.1 (0.4) ab	22.9 (1.5) c	14.7 (0.8) b
41.7	77.3 (0.5) b	9.6 (0.7) abc	36.8 (1.8) b	22.5 (1.4) a
89.8	78.5 (0.3) ab	7.6 (0.4) bcd	52.9 (3.2) a	34.4 (2.3) a
191.1	80.0 (0.4) a	6.1 (0.4) cd	54.8 (2.8) a	27.5 (4.4) a

Mean (SE) white sturgeon body weight increase after 8-week exposure			
Treatment group	Body weight increase (%)	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	282.9 (4.6) a	8.2 (0.6) e	5.2 (0.4) c
9.6	285.5 (9.9) a	17.2 (0.7) d	11.8 (0.9) b
20.5	277.7 (6.1) a	22.9 (1.5) c	14.7 (0.8) b
41.7	191.0 (12.6) b	36.8 (1.8) b	22.5 (1.4) a
89.8	106.5 (5.8) c	52.9 (3.2) a	34.4 (2.3) a
191.1	28.6 (3.6) d	54.8 (2.8) a	27.5 (4.4) a

Letters denote statistical groupings among treatments within each exposure period ($p<0.05$).

Chronic Value: Using the logistic equation with a log transformation of the exposure concentrations (TRAP program), the EC_{10} and EC_{20} values for reduction in body weight are 15.08 and 17.82 mg Se/kg dw whole body and 27.76 and 32.53 mg Se/kg dw muscle tissue.

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

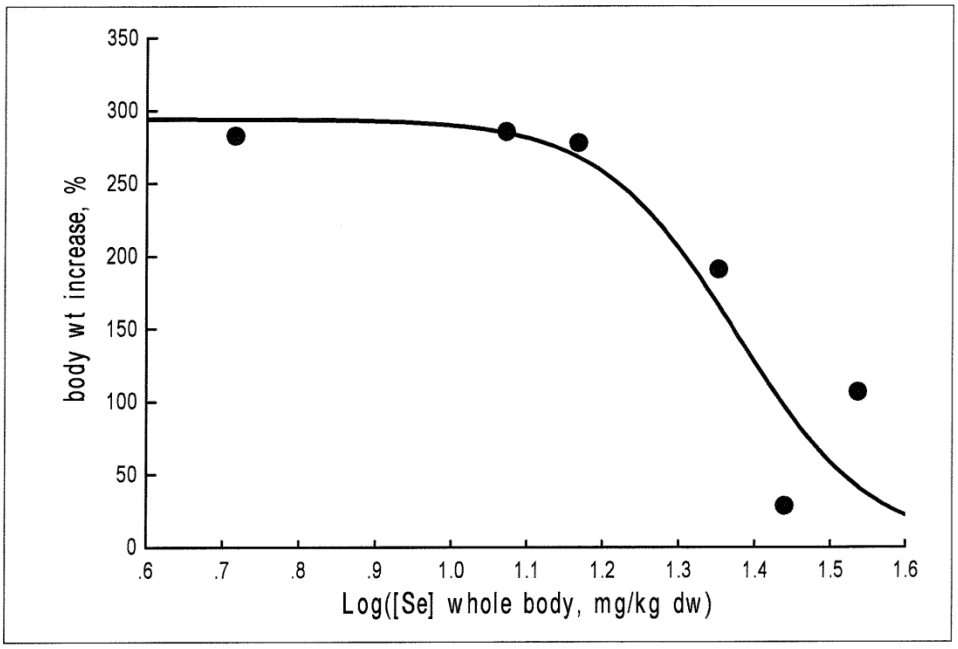
Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.6006	1.6303	0.0314	1.5304	1.7301
S	1.6574	2.938	0.925	-0.005	5.882
Y0	284.2	286.3	18.9	226.1	346.5

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	42.69	33.92	53.72
20.0	32.53	21.17	49.99
10.0	27.76	15.63	49.30
5.0	23.98	11.75	48.93

06/19/2009 10:32 MED Toxic Response Analysis Model, Version 1.03

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3403	1.3750	0.0643	1.1702	1.5797
S	2.283	2.794	1.908	-3.277	8.865
Y0	284.2	294.2	45.0	151.0	437.3

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.71	14.80	37.99
20.0	17.820	6.890	46.090
10.0	15.078	4.160	54.655
5.0	12.926	2.587	64.584

06/19/2009 10:42 MED Toxic Response Analysis Model, Version 1.03

Linville, R.G. 2006. Effects of Excess Selenium on the Health and Reproduction of White Sturgeon (*Acipenser transmontanus*): Implications for San Francisco Bay-Delta. Dissertation. University of California at Davis.

Test Organism: White Sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Selenium was added to the treatment in the form of selenized yeast. Selenized yeast (2.2%; Selenomax®, Ambi Inc.) was added to a commercial salmonid diet and pelleted with fish oil. For the control diet, the selenized yeast mixture contained 1.3% selenized yeast and 98.7 tortula yeast. Only selenized yeast was added to the treatment diet. After pelleting, the diet was allowed to air dry on drying racks.

Test Duration: Females were fed 0.3% body weight/day the experimental diet for 6 months.

Study Design: 16 adult female white sturgeon (approximately 5 years old, mean weight and fork length: 22.71 kg and 134.59 cm) were exposed in a freshwater flow through system to either the control diet (8 females in one tank fed 1.4 mg/kg Se) or treatment (8 females in a separate tank fed 34 mg/kg Se, Se from selenized yeast) for 6 months. After the 6 month dietary exposure, females were induced to spawn and fertilized with non-exposed male milt. Eggs were hatched in jars keeping eggs from each female separate. For each progeny cohort, 3000 larvae were randomly distributed into 3 reps for stage 40 (intestinal portion is void of yolk material, but stomach is not differentiated and is filled with yolk) sampling and 3 reps for stage 45 (yolk sac absorbed, start exogenous feeding) sampling. Se and biological measurements were made in each replicate.

Effects Data: No Se effects were observed for length or weight of larvae. Effects were determined for edema (Table 1), skeletal deformities (Table 2) and larval survival (Table 4). Because the mortalities for each cohort were recorded up to the time the sample was collected for abnormalities, a combined effects variable can be the total proportion of hatched larvae which were both alive and without any abnormalities at stage 45 (Table 4). This was calculated as PS·(1-PA), where PS is the proportion survival in the test chambers prior to sampling and PA is the proportion of the sample of surviving larvae with abnormalities. Binomial confidence limits are included in Table 4 for percent survival and percent abnormalities for each cohort to visualize significant differences among data points and between data points and fitted curves. Such confidence limits cannot be directly calculated for the combined effects variable, for which confidence limits were estimated by combining the lower and upper confidence limits of the individual effects variables using the same equation as above (this slightly overestimates the confidence limit range).

In Table 4, only cohort T2 is significantly different from the controls, based both on larval survival and abnormalities. That this selenium effect is also supported by the microinjection studies of Linville, which showed large abnormality frequencies for egg Se injected with >15 mg/kg, but little or no effect at lower concentrations (this is only supporting information because direct injection of a

specific form of Se is not a complete surrogate for setting effect concentrations for maternally transferred Se). For cohort T3, the data for abnormalities indicate some effects, but cannot be considered a definite effect concentration due to a combination of considerations – overlapping confidence limits with controls, no increase in mortality, limited information on within-cohort variability, and, based on egg concentrations, no effects for cohort T1 at a higher concentration.

EC_{10} Calculations: The combined effects variable is plotted versus Se concentration in the eggs in Figure 1. With only one definite partial effect, TRAP cannot be used to estimate a curve. Instead, the interpolation protocol is applied between the last two points based on specifying the highest no-effect concentration (HNOEC), 11.0 mg/kg, to be the EC_0 in the interpolation equation and specifying the upper control plateau (Y_0 in TRAP) to be average survival of the lower four points. The resultant TRAP slope is 3.0 and the interpolated EC_{10} is 15.6 mg/kg.

The egg EC_{10} of 15.6 mg/kg is slightly lower than the value of 16.3 mg/kg in the previous draft (Figure 3). The lower value was due to the inclusion of larval survival with abnormalities in the endpoint and using interpolation between the last two points rather than a TRAP model of the dataset.

Linville (2006) similarly calculated a 10% effective dose (ED_{10}) of the combined skeletal and edema data of 15.3 mg Se egg/kg dw using a logit regression. Linville (2006) also noted statistically significant differences using a Tukey Honest Significant Difference (HSD) test between Se and control treatments with respect to both the incidence of Stage 45 skeletal and total deformities, respectively, for the maternal transfer study. These author-reported results support the evidence of an effect of selenium in white sturgeon similar to the EC_{10} of 15.6 mg Se/kg egg dw interpolated by TRAP.

The combined effects variable is plotted versus Se concentration in muscle in Figure 2. Unlike for the egg concentration, the muscle concentration for cohort T3, with a small but not significant effect, is greater than that for cohort T1, with no effect, so that TRAP can be used to estimate a curve, although only barely so. This analysis was by tolerance distribution analysis with the log-triangular model. The resultant TRAP estimates are 100% for the control value and 8.8 for the EC_0 (about 11% below the T1 concentration); the standard deviation is 0.14 log units, equivalent to a slope of 3.7. The EC_{10} estimate is 11.9 mg/kg.

Chronic Value: The chronic value for combined deformities and larval survival using egg Se is an EC_{10} of 15.6 mg egg/kg dw. The chronic value for this same endpoint in muscle tissue is an EC_{10} of 11.9 mg muscle/kg dw.

Table 1. Edema deformities.

	Control			Treatment		
	Cohort	Edema (%)	Larval Se (mg/kg dw)	Cohort	Edema (%)	Larval Se (mg/kg dw)
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	6.67 (1)	7.75
Stage 40	C4	0.00 (3)	1.8	T1	0.00 (3)	11.6
	C5	0.00 (3)	2.88	T2	4.44 ± 2.22 (3)	20.4
				T3	1.67 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	15.56 ± 1.11 (3)	19.4
				T3	0.00 (2)	7.61

Table 2. Skeletal deformities.

	Control			Treatment		
	Cohort	Skeletal (%)	Larval Se (mg/kg dw)	Cohort	Skeletal (%)	Larval Se (mg/kg dw)
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	10.00 (1)	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.8	T1	0.00 (3)	11.6
	C5	1.11 ± 1.11 (3)	2.88	T2	14.44 ± 1.11 (3)	20.4
				T3	8.33 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	21.11 ± 1.11 (3)	19.4
				T3	13.33 ± 3.33 (2)	7.61

Table 3. Combined edema and skeletal deformities.

Control					Treatment			
	Cohort	Affected (%)	Egg Se (mg/kg)	Larval Se (mg/kg)	Cohort	Abnormal (%)	Egg Se (mg/kg)	Larval Se (mg/kg)
Stage 36	C3	0.00 (1)	2.46	2.43	T1	0.00 (1)	11	11.6
	C4	0.00 (1)	1.61	1.69	T2	0.00 (1)	20.5	18.4
	C5	0.00 (1)	2.68	2.67	T3	16.67 (1)	7.61	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.61	1.8	T1	0.00 (3)	11	11.6
	C5	1.11 ± 1.11 (3)	2.68	2.88	T2	18.89 ± 1.11 (3)	20.5	20.4
					T3	10.00 ± 0 (2)	7.61	7.22
Stage 45	C4	0.00 (3)	1.61	1.96	T1	0.00 (3)	11	12
	C5	0.00 (3)	2.68	2.59	T2	27.78 ± 2.94 (3)	20.5	19.4
					T3	13.33 ± 3.33 (2)	7.61	7.61

Table 4. Stage 45 data combined abnormalities and percent larval survival.

Cohort	Egg Se (mg/kg)	Muscle Se (mg/kg)	% Survival (95% Binomial CL)	% Abnormal (95% Binomial CL) [# Abnormal][1]	% Alive & w/o Abnormalities (95% Binomial CL)
C4	1.61	1.22	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
C5	2.68	1.48	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T3	7.61	11.1	>99.6 (98.7-99.8)	13.3 (3.7-24.6) [3,5]	86.4 (74.4-96.3)
T1	11	9.93	>99.6 (98.7-99.8)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T2	20.5	15.3	91.6 (90.1-92.8)	27.8 (18.8-38.3) [7,8,10]	66.2 (55.6-75.4)

[1] Bracketed numbers denote abnormal larvae in each of the 2-3 replicates of n=30.

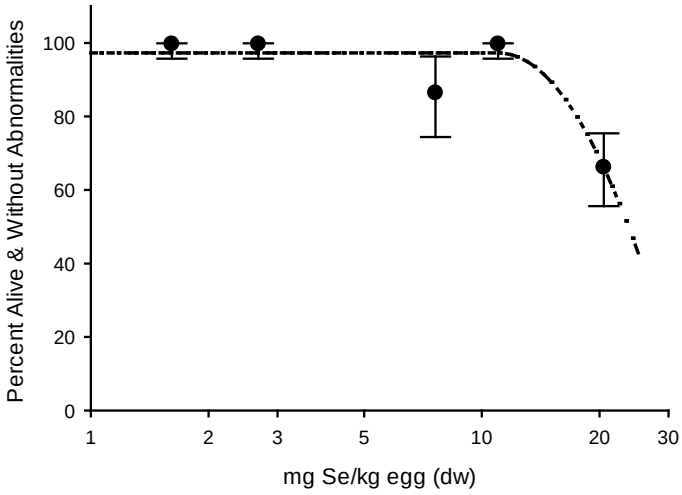


Figure 1. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in eggs. TRAP is used to interpolate between the last two points; EC10 = 15.6 mg Se/kg egg dw.

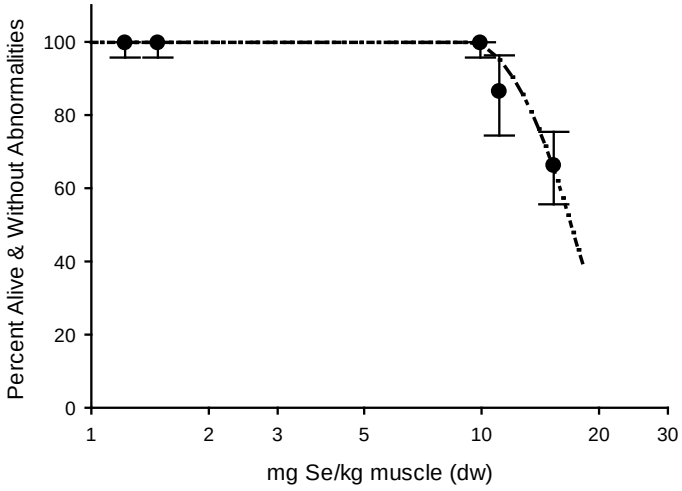
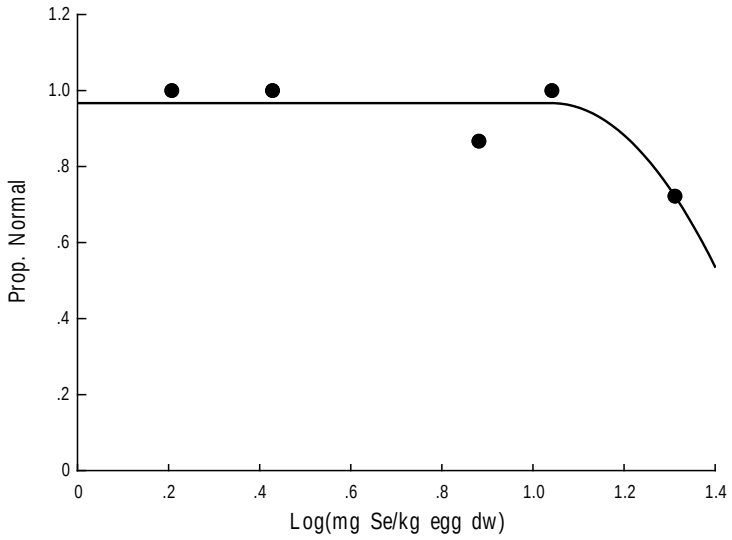


Figure 2. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in female muscle. TRAP tolerance distribution analysis with the log-triangular model; EC10 = 11.9 mg Se/kg muscle dw.



Parameter	Initial	Final
LogX50	1.416	1.4215
S	**2**	**2.6306**
Y0	0.96667	0.96667

Figure 3. TRAP analysis from previous draft. Initial estimate for slope set equal to or less than 2.645 (set to 2 for this figure). EC_{10} = 16.3 mg/kg.

Teh, S.J., X. Deng, D-F Deng, F-C Teh, S.S.O. Hung, T.W. Fan, J. Liu, R.M. Higasi. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (*Pogonichthys macrolepidotus*). Environ. Sci. Technol. 38: 6085-6593.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*); juveniles 7-mos.old

Exposure Route: Dietary only

Dietary Treatments: 8 graded levels of dietary Se; dietary levels obtained by combining selenized yeast with Torula (non-active) yeast. Selenized yeast contained approximately 21% of Se as selenomethionine and proteinaceous Se forms. Diet was formulated as pellets by mixing dry ingredients with water and oil, fan-dried, crumbled and sieved. Analyzed levels: 0.4 (no selenized yeast), 0.7, 1.4, 2.7, 6.6, 12.6, and 57.6 mg/kg.

Fish were fed twice daily with a daily feeding rate of 3% BW in first 5 months and then adjusted to 2% BW thereafter.

Test Duration: 9 months

Study Design: A flow-through system with 40 fish/tank (24 total tanks) was used; each tank held 90 L. Flow rate was 4 L/min. Water temperature was maintained at 23°C for 6 months and then 18°C for last 3 months due to failure of water heating system. 5 fish were sampled from each tank at 5 and 9 months and measured for gross deformities, length, weight, Se in liver and muscle. Sections of the liver were kept for histopathology. Condition factor (100 x BW/length), heptatosomatic index (100 x liver weight/BW), BCF (total organ Se/dietary Se) were determined.

Effects Data: Mortality was observed in the two highest dietary treatments: 10 and 34.3%, respectively. No mortalities were observed in fish fed diets # 12.6 mg/kg. No significant difference in growth of fish fed 12.6 mg/kg Se in diet, but there was in the fish fed 26.6 mg/kg Se. See table below for levels of Se in fish at 9 months and associated effects.

Authors determined prevalence of deformities was higher in fish fed 6.6 and 12.6 mg/kg Se in their diet, however a dose-response relationship did not occur (e.g., no deformities in high concentration). Gross pathology was a more sensitive endpoint than growth.

Summary of effects and assoc. dietary and tissue concentrations in Sacramento splittail after 9 month exp.								
Dietary conc'n mg/kg	0.4	0.7	1.4	2.7	6.6	12.6	26.0	57.6
Se in liver, mg/kg dw	20.1	18.6	20.0	23.0	26.8	31.3	40.4	73.7
Se in muscle, mg/kg dw	6.6	6.9	9.2	10.1	15.1	18.9	29.4	38.7
Liver histopathology (mean lesions scores, N=15)								
Macrophage aggregate	0.13	0.07	0.2	0.27	0.40	0.20	0.20	0.85
Glycogen depletion	0	0	0.2	0	0.4	0.2	0	1.38
Single cell necrosis	0	0	0	0.07	0.13	0	0.07	0.46
Fatty vacuolar degeneration	0	0	0	0.2	0.53	0.07	0.2	0.08
Eosinophilic protein droplets	0	0	0	0	0	0	0.07	0.85
Sum of mean lesion scores	0.13	0.07	0.4	0.54	1.46	0.47	0.54	3.62
Gross Pathology (No. of deformities, N=15)								
Facial deformities (eye, jaw, and mouth)	0	1	0	1	5	3	0	0
Body deformities (kyphosis, lordosis, scoliosis)	0	0	4	2	3	1	1	0
Prevalence of deformity (%)	0	6.7	26.7	20	53.3	26.7	6.7	0

Chronic Value: Using gross pathology as the endpoint (prevalence of deformities, %), the NOAEC is 10.1 mg Se/kg dw and the LOAEC is 15.1 mg/kg Se dw in muscle tissue; MATC or CV = 12.34 mg/kg Se in muscle dw.

The above concentrations in juvenile muscle tissue cannot be exactly translated into an equivalent egg-ovary or whole-body concentration in adult splittail. But using the median egg-ovary to muscle ratio of 1.59 for the family Cyprinidae, the NOEC and MATC would represent 16.1 and 19.6 mg Se/kg egg-ovary. Using the median muscle to whole-body ratio of 1.26 for the family Cyprinidae, the NOEC and MATC would represent 8.04 and 9.83 mg Se/kg whole body. However, appropriateness of these conversion estimates rests upon uncertain assumptions that the muscle concentrations in juvenile splittails equal those of adult splittails under the same exposure conditions, and that splittail tissue ratios are those typical of the family Cyprinidae.

Comments: The authors observed deformities including spinal deformities using fish that were 7-months-old at test initiation. This is the only study in which deformities were observed in fish that were not exposed maternally.

Deng et al. (2008) exposed Sacramento splittail juveniles (21-day post hatch) to dietary selenium and dietary methylmercury in a two factorial design for four weeks. No adverse effects (growth, condition factor, lethargy or abnormalities) were observed in the selenium only exposures. The splittail accumulated approximately 3.5 mg Se/kg ww muscle in the highest dietary exposure (35 mg

Se/kg. Using the average percent moisture in fish muscle of 78.4% (May et al. 2000), the dw Se concentration is 16.2 mg Se/kg muscle indicating the recommended CV does not over-estimate an effect concentration.

Rigby et al. (2010) re-analyzed the juvenile Sacramento splittail data generated in the Teh et al. (2004) study. The authors used logistic regression to estimate EC values for deformities on a culled data set which eliminated the three highest dietary treatments due to their departure from a standard concentration-response relationship. The EC_{10} value for the culled data set was 7.9 mg Se/kg dw muscle which is lower than the recommended CV of 12.3 mg Se/kg dw muscle. Due to the lack of a concentration-response relationship across the entire dietary range and the lack of effects in the Deng et al. (2008) study, an EC_{10} of 7.9 mg Se/kg dw muscle is too uncertain for a recommended CV. Although the recommended CV of 12.3 mg Se/kg dw muscle is based on deformities (an uncertain response), it is considered representative of an effect level for this species because of the significant reductions in growth at the two highest test concentrations.

Bennett, William N., Arthur S. Brooks, and Martin E. Boraas. 1986. Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. Arch. Environ. Contam. Toxicol. 15:513-517.

Test Organism: Fathead minnow (*Pimephales promelas*; 2 to 8 day-old larvae).

Exposure Route: Dietary only
Green alga, *Chlorella pyrenoidosa* were exposed to Se ($H_2{}^{75}SeO_4$) in culture water for 3 days. Rotifers, *Brachionus calyciflorus*, were cultured in chambers with selenium containing green algae at the ratio of 25 µg algae/ml to 50 µg rotifer/ml for 5 hr. The rotifers were filtered to separate them from the algae and immediately heat-killed. The Se concentration in the rotifers was measured for ^{75}Se activity.

Test Duration: 9 to 30 days

Study Design: Selenium uptake by larval fathead minnows was measured in three experiments. Se-contaminated and control rotifers for feeding to larval fish were prepared in advance using the low algae:rotifer ratio. Daily equal volumes of rotifers were divided among five 800 mL polypropylene larval chambers. Three chambers received Se-contaminated rotifers and two received control rotifers. The rotifers were dead at the time of feeding (heat killed).

Larval fish were hatched from eggs spawned in the laboratory. After hatching, active larvae were divided equally among the larval test chambers (daily renewal exposures using dechlorinated Lake Michigan water). Larvae were initially fed rotifers raised on control algae (no selenium). The age of the larvae when first fed Se-contaminated rotifers was 4, 9, and 3 days post-hatch for experiments 1, 2, and 3, respectively. Larval fish were fed Se-contaminated rotifers for 7, 9, and 7 days in the 3 experiments. A post-exposure observation period of 19 and 2 days was used for experiments 1 and 2, respectively. During this time the larvae were fed control rotifers. Daily, larvae from a replicate were removed from the test chamber, washed, placed in a 20 ml vial, and counted for ^{75}Se activity for 20 min. All larvae were then placed in test chambers with fresh food rations. At the end of the study all fish were individually dried and weighed.

	Experiment 1	Experiment 2	Experiment 3
Initial feeding of control diet (days)	3	8	2
Day Se diet first fed	4	9	3
Day Se diet last fed	11	17	9
Observation days on control diet	19	2	0
Age at study termination (days)	30	19	9

Effects Data:

	Experiment 1	Experiment 2	Experiment 3
Mean food Se concentration (mg/kg)	>70	68	55
Food intake (µg rotifers/larva)	50	1330	1190
Initial larvae mean dry wt. at start of Se-laden food (µg)	90	400	100
Final larvae mean dry wt. (µg) at end of test	1470 (Control) 800 (Treatment)[a]	1888 (Control) 1354 (Treatment)[a]	475 (Control) 416 (Treatment)
Final mean larval Se content (µg Se/larva)[b]	0.0062	0.0700	0.0248
Final mean larval Se concentrations (mg Se/kg dw)	43.0	51.7	61.1

[a] Significantly different from the control.
[b] Values when Se-laden feeding was ended.

Selenium was measured in the test water during the feeding exposures, but the concentrations were insignificant (0.84 µg/L). Survival was not affected by the selenium exposures. Preliminary tests showed that fathead minnow larvae would reach plateau concentrations of selenium within the 7- to 9-day exposure periods. The food supply was sufficient to sustain growth of the larvae during the study, according to the authors. The authors state that selenium uptake and higher selenium content in experiment 2 larvae was due to their larger size and ability to consume more rotifers/unit time. Se-exposed larvae were significantly smaller ($p<0.05$) in mass than controls for experiments 1 and 2.

Chronic Value: GM of mean larval Se concentrations measured in the three experiments, i.e., 43.0, 51.7, and 61.1 mg/kg dw WB, respectively, is 51.40 mg Se/kg dw.

Dobbs, M.G., D.S. Cherry, and J. Cairns, Jr. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. Environ. Toxicol. Chem. 15:340-347.

Test Organism: Rotifer (*Brachionus calyciflorus*), and fathead minnow (*Pimephales promelas*) 12 to 24 hr-old at start.

Exposure Route: Dietary and waterborne

Water
Filtered and sterilized natural creek water supplemented with nutrients (Modified Guillard's Woods Hole Marine Biological Laboratory algal culture medium) for algal growth. Sodium selenate (Na_2SeO_4) was added to test water to obtain nominal concentrations of 100, 200, or 400 µg Se/L. Concentrations remained stable and equal in each trophic level.

Control Diet
No selenium was added to the water medium for the alga; green alga was free of selenium for the rotifer; and rotifers were free of selenium for the fathead minnow.

Selenium Diet
Sodium selenate was added to the culture medium for the alga; green alga thereby contained a body burden for the rotifer; and rotifers thereby contained a body burden for the fathead minnow.

Dietary Treatments: Each trophic level had a different treatment. The green alga was exposed directly from the water (1, 108.1, 204.9, 397.6 µg total Se/L); rotifers were exposed from the water (1, 108.1, 204.9, 393.0 µg total Se/L) and the green alga as food (2.5, 33, 40, 50 mg Se/kg dry wt.); and the fathead minnow were exposed from water (1, 108.1, 204.9, 393.0 µg total Se/L) and the rotifer as food (2.5, 47, 53, 60 mg Se/kg dry wt.).

Test Duration: 25 days

Study Design: A flow-through system utilizing a stock solution of filtered and sterilized creek water controlled at 25°C was used to expose three trophic levels of organisms. Approximately one liter of media was pumped from the algal chamber into the rotifer chamber each day. A cell density between 3 and 6×10^6 cells/ml was delivered to the rotifer chambers. Rotifers were started at a density of 151.4 ± 7.7 females/ml and one liter/day of rotifers containing culture water was intermittently pumped into the minnow chamber. (*B. calyciflorus* has a life span of about 7 days at 25°C.) The pump was necessary to overcome the swimming ability of rotifers to avoid an overflow tube. Larval fathead minnows (35/chamber) were prevented from escaping by a screened overflow. Chambers were cleaned daily and aeration was provided. All chambers were duplicated for test replication and water was measured for selenium on days 0, 2, 6, 7, 11, 14, 17, 20, and 24. All algal and rotifer biomass and selenium samples were made on these days. Fathead minnow chambers were measured for biomass, dissolved selenium, and tissue selenium concentrations of days 0, 7, 11, 14, 20, and 24.

Additional measurements were made in the 200 µg Se/L test chambers on the fathead minnow on day 16. Selenium concentrations were maintained near the nominal concentrations and the standard deviation of mean concentrations was less than 4 percent.

Effects Data:

Rotifers. Rotifers did not grow well and demonstrated reduced survival at all selenium exposure concentrations during the 25 day test. By test day 7 only the lowest test concentration (108.1 µg/L) had surviving rotifers which showed a decrease in selenium content from test days 18 through 25. A reduction in rotifer biomass was discernable by test day 4 in the selenium treatments and since all test concentrations had viable rotifer populations present, the effect level was calculated using these data.

Effect of Dietary and Waterborne Selenium on Rotifers after 4 Days Exposure

Se in water, µg/L	Se in diet, mg/kg dw	Se in rotifer tissue, mg/kg dw	rotifer biomass, mg/ml dw
1	2.5	2.5	0.028
108.1	33	40	0.025
202.4	40	54	0.011
393	50	75	0.003

Fathead minnows. Due to the reduction of rotifer biomass in the higher test concentrations, fish mortality and reduction in fish growth observed in the latter days of the test was difficult to discern between effects from starvation and selenium toxicity. The data from test day 8 was selected for determining the effect of selenium on fathead minnows because starvation could be excluded as a variable.

Effect of Dietary and Waterborne Selenium on Larval Fathead Minnows after 8 Days Exposure			
Se in water, µg/L	**Se in diet, mg/kg dw**	**Se in fathead minnow tissue, mg/kg dw**	**Average fish weight, mg dw**
1	2.5	2.5	0.8
108.1	47	45	0.7
202.4	53	75	0.4
393	60	73	0.2

Chronic Value:

Rotifers	42.36 mg Se/kg dw (EC_{20})
Fish	< 73 mg Se/kg dw (LOAEC) - not amenable to statistical treatment; the LOAEC was based on the observation that a >50 percent reduction in mean fish weight occurred at this tissue concentration.

Schultz, R. and R. Hermanutz. 1990. Transfer of toxic concentrations of selenium from parent to progeny in the fathead minnow (*Pimephales promelas*). Bull. Environ. Contam. Toxicol. 45:568-573.

Test Organism: Fathead minnow (*Pimephales promelas*; Adults)

Exposure Route: Dietary and waterborne
Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Study Design: Four Monticello artificial streams were used for the study which lasted from September 1987 to September 1988. For each study, two streams (treated) were dosed continuously to achieve 10 µg/L and two streams served as controls. Mean selenium concentrations at the head of the treated streams were 9.8 ± 1.2 and 10.3 ± 1.7 µg/L, respectively. The concentrations of selenium measured in the water from controls streams were all less than the detection limit, i.e., 2 µg/L. Spawning platforms were submerged into each stream. One subset of six embryo samples (n = 2000 embryos per sample) were collected from the streams for selenium analysis. Another subset of ten embryo samples were reared in incubation cups receiving the same stream water dosed with sodium selenite via a proportional diluter. The treated embryos in egg cups received an average 9.7 ± 2.6 µg Se/L. Samples of hatched larvae were analyzed for selenium content while others were inspected for occurrence of edema and lordosis. Prior to test termination, female parents were seined. The mean selenium content in the ovaries of seven to eight females from the treated and control streams was reported.

Effects Data: Edema and lordosis occurred in approximately 25 percent of the fish spawned and reared in 10 µg Se/L. Corresponding occurrence in control fish incubated in the egg cups was only 1 and 6 percent, respectively. Table 1 provides the abnormality observations and the selenium residues in the embryos and ovaries from the control and treated streams. Although a case can be made that the Se treatment had a higher rate of edema and lordosis, there are some problems that add uncertainty to the estimation of an effect concentration (R. Erickson, pers. comm.). Heavy mortality/loss of embryo/larvae during monitoring and the erratic occurrence of the abnormalities (e.g., there is a significant incidence of edema in only 3 of 10 replicates for the Se treatment) led to the conclusion that results should not be used for criterion derivation. However, the data from this study support the range of reproductive effect levels determined in other studies. The Se concentration in embryos from the 10 µg/L treatment stream of 3.91 mg/kg ww converts to 25.6 mg/kg dw using 15.3% dw (N=3 range 14.7 – 15.6%) for fathead minnow eggs (R. Erickson, pers. comm). The previous draft used the Se concentrations in the ovaries collected at the end of the study for the effect concentration estimate. However, it was determined that the embryos are a more direct representation of Se exposure and toxicity to the larvae.

Chronic Value: The LOEC for embryos is <25.6 mg Se/kg dw.

Table 1. Percent Abnormalities in Fathead Minnow Larvae and the Associated Selenium Concentrations in Embryos and Ovaries.

Treatment	[Se] embryos, mg/kg ww (SD)	[Se] ovaries, mg/kg ww (SD)	Edema, % (SD)	Lordosis, % (SD)
Control	0.31 (0.01)	0.77 (0.14)	0.9 (2.2)	5.6 (8.8)
10 µg/L	3.91 (1.87)	5.89 (2.21)	24.6 (36.1)	23.4 (20.8)

SD = standard deviation

Beyers, D.W. and Sodergren, C. 2001a. Evaluation of interspecific sensitivity to selenium exposure: Larval razorback sucker versus flannelmouth sucker. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval flannelmouth sucker (*Catostomus latipinnis*) and larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage) Continuous flow diluter supplied a range of aqueous test concentrations <1, 25.4, 50.6, 98.9, and 190.6 µg/L selenate. Well water was used as the dilution water. Across the range of aqueous exposure concentrations, each test chamber was fed the same daily ration of living rotifers containing selenium at <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively. Rotifers accumulated selenium from algae (*Chlorella vulgaris*) exposed to 0, 25, 50, 100, and 200 :g/L selenate.

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1st factor - selenium; 2nd factor - species). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data : No survival effects were observed and there were no decreases in fish weight or length. Fish mass was found to increase as a function of selenium concentration.

Chronic Value: The chronic values for the flannelmouth sucker and razorback sucker were >10.2 and >12.9 mg Se/kg dw, respectively, based on the concentrations of selenium measured in whole-body tissue of larval fish at the highest water and dietary selenium concentrations.

Beyers, D.W. and Sodergren, C. 2001b. Assessment of exposure of larval razorback sucker to selenium in natural waters and evaluation of laboratory-based predictions. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage)
Larvae were exposed in a daily static-renewal system to control water (reconstituted very hard) and site waters: De Beque, Orchard Mesa, North Pond diluted 50%, and North Pond. Each water type received either a control diet (rotifers) or a diet previously exposed to the site water (site food: rotifers fed algae exposed to respective site water).

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1^{st} factor - test water type; 2^{nd} factor - rotifers cultured in control water or in site water). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data: No survival effects were observed. There were no significant decreases in growth of fish exposed to both site water and site food compared to fish exposed to control water and control food. There was a significant increase in growth of fish exposed to site water and control food relative to fish exposed to control water and control food ($p<0.0001$). There were reductions in the growth of fish (14%) exposed to site water and site food compared to site water and control food ($p<0.0001$). Due to the lack of a dose-response relationship in both the concentration of selenium in the food (rotifers) and growth, and the concentration of selenium in the fish larvae and growth, the authors did not attribute the effect of site food on the growth of fish to selenium.

Chronic Value: The NOAEC for the razorback sucker larvae in the four site water types based on selenium in whole-body tissue were: De Beque >5.45 mg Se/kg dw; Orchard Mesa >11 mg Se/kg dw; North Pond 50% dilution >41.1 mg Se/kg dw; North Pond >42 mg Se/kg dw. Because no significant effects were observed in larvae exposed to North Pond water at >42 mg Se/kg dw whole-body tissue, this value was selected as the chronic value for the study.

Muscatello, J.R., P.M. Bennett, K.T. Himbeault, A.M. Belknap and D.M. Janz. 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environ. Sci. Technol. 40:6506-6512.

Test Organism: Northern pike (*Esox lucius*)

Exposure Route: Dietary and waterborne - field exposure

Test Duration: Eggs were collected in the field and incubated in the laboratory. The test was terminated when the majority of the fry exhibited swim-up and had absorbed the yolk.

Study Design: The study area was Key Lake uranium milling operation in north-central Saskatoon. Spawning northern pike were collected from four sites, one reference (Davies Creek) and three exposure sites, David Creek near-field (high exposure), Delta Lake (medium exposure), and David Creek far-field (low exposure). The exposure sites were located approximately 2, 10 and 15 km downstream of the effluent discharge. Milt and ova were stripped from ripe fish and eggs were fertilized in the field. Females were saved for metal analysis and age determination. Subsamples of ova (prior to fertilization) were collected for metal analysis.

Although the study sites represent open systems where fish can potentially migrate among sites, radiotelemetry data from tagged adult pike (Muscatello and Janz, unpublished data) indicate high site fidelity at the "high" and "medium" exposure sites (lakes). In contrast, the "low" exposure site likely represents pike that migrated from further downstream sites that were likely of similar Se exposures as the reference site.

Eggs were incubated using a two-way ANOVA experimental design using water collected from reference or exposure sites. So, embryos originating from reference or exposure site females were incubated in either reference or appropriate exposure water. In addition, embryos from reference site females were incubated in water from all four study sites. 50 viable embryos from each individual female were transferred to each of four replicate incubation chambers. Cumulative time to 50% eyed, 50% hatch and 50% swim-up were determined. When the majority of the fry exhibited swim-up and had absorbed the yolk, the remaining fry were preserved and examined for deformities.

Effects Data: Mean egg diameter and fertilization success did not differ among sites. Cumulative embryo mortality throughout incubations was not significantly different among the sites ranging from 45 to 60%. There were no significant differences in the cumulative time to reach 50% eyed embryos, 50% hatch or 50% swim-up among treatments. Differences in the percent total deformities between test waters used during embryo incubation exposures were not significant, so the data were combined for each site (see Table below).

Selenium concentrations in eggs and muscle from female northern pike collected from reference and exposed sites and associated total deformities in embryos					
Site	**Site ID**	**Female**	**[Se] mg/kg dw**		**Total deformities %**
			Egg	**Muscle**	
Davies Creek	Reference	1	3.45	0.86	17
Davies Creek	Reference	2	2.72	1.89	2.5
Davies Creek	Reference	3	3.39	2.56	15.51
Davies Creek	Reference	4	3.72	1.34	7.13
Davies Creek	Reference	5	2.69	1.04	10.41
David Creek (far field)	Low	1	3.39	1.95	20.32
David Creek (far field)	Low	2	4.07	2.04	13.19
David Creek (far field)	Low	3	4.07	1.26	15.33
David Creek (far field)	Low	4	4.07	2.48	18.83
David Creek (far field)	Low	5	3.4	1.26	11.8
Delta Lake	Medium	1	43.19	17	37.8
Delta Lake	Medium	2	24.53	16.52	31.71
Delta Lake	Medium	3	26.14	16.52	26.29
David Creek (near field)	High	1	48.23	47.82	39.5
David Creek (near field)	High	2	N/A*	28.72	N/A*

*female had no eggs

Significant increases in total deformities (edema, skeletal deformities, craniofacial deformities and fin deformities) were observed in fry originating from pike collected at the medium exposure site. Determination of an effect level for the percent total deformities relative to the concentration of selenium in eggs or in female muscle tissue was not amenable to analysis by TRAP. One requirement of TRAP is to have a response greater than 50%, which was not satisfied with the available data.

When data are not amenable to determining an effect level using a software program, such as TRAP, one way to estimate the effect level is to make a direct measurement of effect at an exposure or tissue concentration. For example, if only a control and one exposure concentration, 10 μg/L, were tested in an acute toxicity test and there was 100% survival in the control and 35% in the 10 μg/L, the effect level would be an EC_{35} of 10 **μg**/L. Such an approach was used to estimate effect in the Muscatello et al. data. Because no significant differences were observed in either selenium concentrations in eggs or percent total deformities between the reference and low exposure site, the data from these 10 sites were combined. Similarly, the egg **and muscle** selenium and total deformity data were combined for the 4 medium and high exposure sites. These means, geometric for the selenium concentrations and arithmetic for the percent total deformities, are given in the following table.

	Mean selenium in northern pike egg and muscle and effect values for reference and exposure sites			
Sites	**[Se] in eggs, mg/kg dw (geometric mean)**	**[Se] in muscle, mg/kg dw (geometric mean)**	**Total deformities, % (arithmetic mean)**	**Total deformities, % (accounting for reference deformities and transformed to new scale)[a]**
Reference sites (includes low exposure)	3.462	1.570	13.20	0
exposure sites	34.00	21.70	33.82	23.76

[a] The % total deformities in the reference and exposed sites were normalized to the reference effect (13.2%) and then transformed to a new scale (100%). i.e, Abbott's formula.

The percent affected becomes 24% or an EC_{24} and the effect level is 34.00 mg Se/kg dw in eggs and 21.70 mg Se/kg in muscle**.**

Chronic Value: EC_{24} = 34.00 mg Se/kg dw in eggs. Note: an EC_{10} cannot be estimated with the data.

Hamilton, S.J., K.J. Buhl, N.L. Faerber, R.H. Wiedermeyer and F.A. Bullard. 1990. Toxicity of organic selenium in the diet of chinook salmon. Environ. Toxicol. Chem. 9:347-358.

Test Organism: Chinook salmon (*Oncorhynchus tshawytscha* Walbaum; swim-up larvae)

Exposure Route: Dietary only

Control Diet

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish (1.0 mg Se/kg dw) collected from a reference site.

Selenium Diet #1

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from high-selenium mosquitofish (35.4 mg Se/kg dw) collected from the San Luis Drain, CA, termed SLD diet.

Selenium Diet #2

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish same as in the control diet, but fortified with seleno-DL-methionine (35.5 mg Se/kg dw), termed SeMet diet.

Dietary Treatments: Each selenium diet was formulated to contain about 36 mg Se/kg dw as the high exposure treatment. The remaining treatments were achieved by thoroughly mixing appropriate amounts of high-exposure treatment diet with control diet to yield the following nominal concentrations (3, 5, 10, and 18 mg Se/kg dw).

Test Duration: 90 days

Study Design: Each dietary treatment was fed twice each day to swim-up larvae (n=100) in each of two replicate aquaria that received 1 L of replacement water (a reconstituted experimental water that simulated in quality a 1:37 dilution of water from the San Luis Drain, CA minus the trace elements) every 15 minutes (flow-through design). Mortality was recorded daily. Growth was evaluated at 30-day intervals by measuring the total lengths and wet weights of two subsets of individual fish (n=10x2) held in separate 11.5 L growth chambers within each replicate aquarium. Tissue samples were collected for whole-body selenium determinations (dw basis) at 30-day intervals throughout the study; 10, 5, and 2 fish were sampled from each duplicate treatment after 30, 60, and 90 days of exposure, respectively. Concentrations of selenium measured in water were below the limit of detection (1.5-3.1 μg/L) in all dietary selenium exposure concentrations.

Effects Data: The magnitude of reduced growth was most evident in the weight of the fish, although total length was significantly reduced in fish fed high Se-laden diets as well. The effect of increasing dietary selenium on mean larval weight was similar in both the SLD and seleno-methionine diets.

Effect of San Luis Drain Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	3.3	2.68	97.3
5.3	4.5	2.76	93
9.6	8.4	2.8	95
18.2	13.3	2.62	92.4
35.4	29.4	1.4	89

Effect of Seleno-methionine Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	2	3.08	100
5.3	3.1	3.22	95
9.6	5.3	3.07	94.1
18.2	10.4	2.61	92.4
35.4	23.4	1.25	62.5

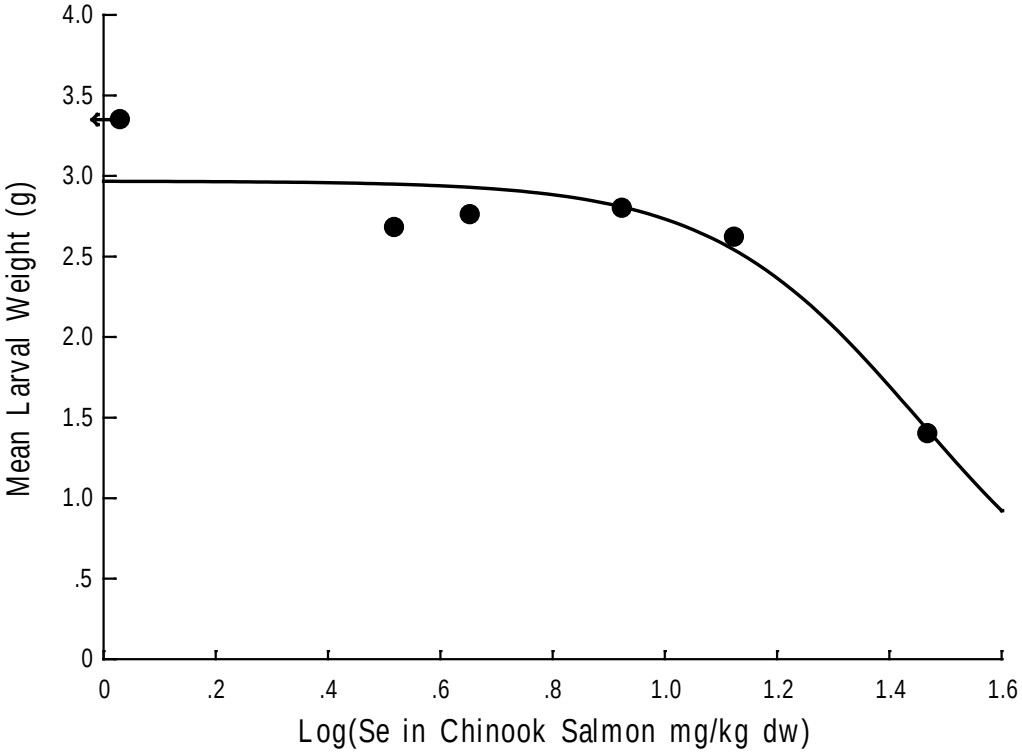
Chronic Value: Due to unacceptable control mortality of swim-up larvae in control treatments after 90 days (33.3 percent - SLD diet; 27.5 percent - SeMet diet), chronic values had to be determined from respective values reported after 60 days (tables above).

Analysis of the elemental composition of the SLD diet indicated that B, Cr, Fe, Mg, Ni and Sr were slightly elevated compared to the control and SeMet diets. No additional analyses were performed to determine the presence of other possible contaminants, i.e., pesticides.

	EC_{20} values		**EC_{10} values**
	Survival (after 60 d of exposure)	**Growth (after 60 d of exposure)**	**Growth (after 60 d of exposure)**
Diet type	**Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**
SLD	NA[a]	15.73	11.14
SeMet	NA[a]	10.47	7.355

[a] The EC_{20} and EC_{10} values for survival of swim-up larvae versus levels of selenium for the SLD and SeMet dietary exposure could not be estimated using non-linear regression.

Hamilton et al (1990) Chinook Salmon fed SLD Diet
Logistic Equation, Three Parameter Model, Se concentrations log_{10} transformed

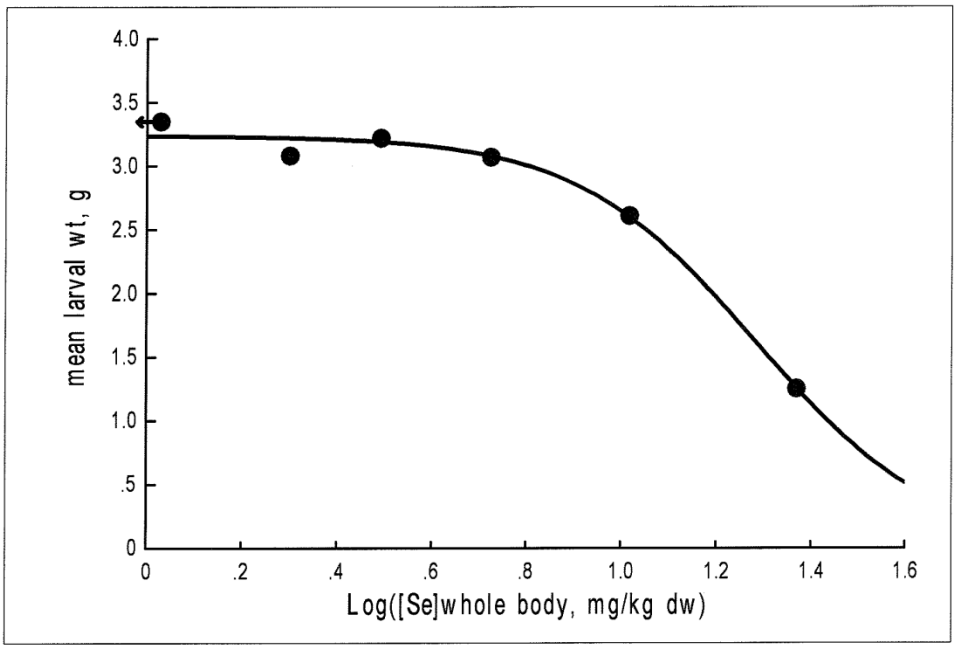


	Guess	FinalEst	SE	95%LCL	95%UCL
LogX50	1.453	1.453	7.30E-02	1.2206	1.6854
StDev	1.353	1.353	6.67E-01	-7.71E-01	3.4769
Y0	2.968	2.968	1.89E-01	2.3651	3.5709

%Effect	Xp Est	95% LCL	95% UCL
50	28.379	16.62	48.458
20	15.734	5.7003	43.431
10	11.143	2.4771	50.127
5	8.1085	1.1213	58.637

	DF	SS	MS	F	P
Total	5	2.0749	0.41498		
Model	2	1.8202	0.91009	10.719	0.95699
Error	3	0.2547	8.49E-02		

Chinook salmon SeMet diet (Hamilton et al. 1990)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3148	1.2823	0.0242	1.2053	1.3593
S	0.6971	1.3214	0.1826	0.7404	1.9025
Y0	3.217	3.239	0.067	3.027	3.452

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	19.156	16.045	22.870
20.0	10.472	7.516	14.591
10.0	7.355	4.595	11.775
5.0	5.312	2.899	9.733

06/19/2009 13:52 MED Toxic Response Analysis Model, Version 1.03

Hilton, J.W. and P.V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). J. Nutr. 113:1241-1248.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only

Low carbohydrate diet (LCD)
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler.

High carbohydrate diet (HCD)
This diet contained cerelose at 25 percent of the diet with cellulose as the filler.

For both diets, the selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The two diets were supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the six different dietary selenium treatments (n = 3 low carbohydrate diet; n= 3 high carbohydrate diet). The six diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw, and the measured concentrations of selenium in the high carbohydrate diet were: 0.7 (control), 6.6, and 11.8 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 weeks

Study Design: Body weights, feed: gain ratios, and total mortalities were determined after each 28-day interval. After 16 weeks, approximately 20 fish were randomly removed from each tank, weighed, and blood was collected for hemoglobin, hematocrit, and plasma glucose, protein, and calcium determination. The livers and kidneys were then dissected. The livers were assayed for glycogen content, and samples of both liver and kidney were assayed for selenium content. Additional subsamples of fish were sacrificed and assayed for selenium content and for ash, crude protein, and moisture content (n=6 per treatment). Finally, 30 fish were killed, their livers and kidneys dissected, and analyzed for Ca, Cu, Fe, Mg, P, and Zn content.

Effects Data: The only overt sign of selenium toxicity was food avoidance observed in trout fed the highest selenium content in both low and high carbohydrate diets, which led to significantly reduced body weight after 16 weeks. There were no significant differences detected between treatment groups in hematological parameters. Kidney, liver, and carcass selenium levels increased with increasing selenium content of the diet, however, only the liver selenium concentrations were significantly affected by dietary selenium level, dietary carbohydrate level, and the interaction between the two treatments. Mineral analysis of the kidney showed significantly higher levels of calcium and phosphorous in trout reared on the two highest levels of dietary selenium. Concentrations of copper in the liver increased significantly with increasing dietary selenium levels and decreasing dietary carbohydrate levels.

Effect of Selenium in Low carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.6	0.8	3.3
6.6	38.3	3.3
11.4	49.3	1.8

Effect of Selenium in High carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.7	0.6	2.7
6.6	21.0	2.3
11.8	71.7	1.4

Chronic Value: The following table lists the NOAEC, LOAEC and MATC for both diets in liver tissue. EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Diet	**NOAEC, mg Se/kg dw liver**	**LOAEC, mg Se/kg dw liver**	**MATC, mg Se/kg dw liver**
Low carb	38.3	49.3	43.5
high carb	21.0	71.7	38.8

Hicks, B.D., J.W. Hilton, and H.W. Ferguson. 1984. Influence of dietary selenium on the occurrence of nephrocalcinosis in the rainbow trout, *Salmo gairdneri* Richardson. J. Fish Diseases. 7:379-389.

(Note: These data are the exact same as reported for the low carbohydrate diet in **Hilton and Hodson 1983**, with the addition of prevalence of nephrocalcinosis occurring in trout after 16 to 20 weeks of consuming the contaminated test diets).

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The test diet was supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the three different dietary selenium treatments. The three diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 to 20 weeks

Study Design: See Hilton and Hodson (1983). After 20 weeks on the test diets, ten fish were randomly removed from each treatment. Tissues for histopathological examination included the stomach, intestine and pyloric ceca (including pancreas), spleen, liver, heart, kidney, skin, muscle, and gills.

Effects Data: Only effects of selenium on kidney tissue are included in the article. The kidneys of the 10 trout fed the highest selenium content in the diet exhibited normal appearance. Five of these trout exhibited precipitation of calcium in the tubules with some epithelial necrosis, but no loss of epithelial continuity. Extensive mineralized deposition of Ca within the tubules, tubular dilation and necrosis of tubular epithelium, ulceration of tubules, and intestinal Ca mineralization was observed in four of the ten fish.

Chronic Value: Same as for growth of rainbow trout reported by Hilton and Hodson (1983). The MATC estimated for growth of rainbow trout relative to final concentration of selenium in liver tissue of trout reared on the low carbohydrate diet is the GM of 38.3 (NOAEC) and 49.3 (LOAEC) mg/kg dw, or 43.45 mg/kg dw.

EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Hilton, J.W., P.V. Hodson, and S.J. Slinger. 1980. The requirements and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J. Nutr. 110:2527-2535.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 1.28 g each)

Exposure Route: Dietary only
A casien-torula yeast diet was formulated to contain geometrically increasing levels of selenium from 0 to 15 mg/kg dw. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Duration: 20 weeks

Study Design: Six test diets were fed to triplicate groups of 75 fish. The trout were fed to satiation 3-4 times per day, 6 days per week, with one feeding on the seventh day. Measured concentrations of selenium in the diet were: 0.07 (control), 0.15, 0.38, 1.25, 3.67, and 13.06 mg/kg dw. The tanks received a continuous flow of dechlorinated tap water from the City of Burlington, Ontario municipal water supply. The waterborne selenium content of this water was 0.4µ g/L. During the experiment, the fish were weighed every 2 weeks with the feeding level adjusted accordingly. Mortalities were noted daily and the feed consumption for each treatment was recorded weekly. After 4 and 16 weeks, three to six fish were randomly removed from each tank, sacrificed, and their livers and kidneys removed and weighed. An additional three to six fish were then obtained from each treatment, killed, and prepared for tissue analysis. Organs and carcasses were freeze-dried for determination of selenium concentration. After 16 weeks, three more fish were removed. Kidney, liver, spleen and dorsal muscle tissue was dissected for examination of histopathology. At the end of 8 and 16 weeks, four to five fish were removed, sacrificed, and a blood sample was taken for hematological measurements (hematocrit, red blood cell count, and blood iron concentration). After 20 weeks, three to four more fish were removed, sacrificed, and a blood sample was taken for measurement of glutathione peroxidase activity.

Effects Data: There were no significant differences detected between treatment groups in histopathology, hematology, or plasma glutathione peroxidase activity. Trout raised on the highest dietary level of selenium (13.06 mg/kg dw) had a significantly lower body weight and a higher number of mortalities (10.7; expressed as number per 10,000 fish days) than trout from the other treatments levels after 20 weeks of exposure.

Effects on Juvenile Rainbow Trout			
Se in diet, mg/kg dw	**Se in Liver, mg/kg dw**	**Weight, g/fish**	**Mortality***
0.07	0.6	3.2	0
0.15	0.95	3.5	0
0.38	2.4	3.7	0.6
1.25	11	4.1	0.6
3.67	40[a]	4.1	0
13.06	100[b]	1.4	10.7

* expressed as number per 10,000 fish-days
[a] NOAEC
[b] LOAEC

Chronic Value: NOAEC = 40 mg Se/kg dw
LOAEC = 100 mg Se/kg dw
MATC = 63.25 mg Se/kg dw

Holm, J. 2002. Sublethal effects of selenium on rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Masters Thesis. Department of Zoology, University of Manitoba, Winnipeg, MB.

Holm, J., V.P. Palace, K. Wautier, R.E. Evans, C.L. Baron, C. Podemski, P. Siwik and G. Sterling. 2003. An assessment of the development and survival of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) exposed to elevated selenium in an area of active coal mining. Proceedings of the 26th Annual Larval Fish Conference 2003, Bergen, Norway. ISBN 82-7461-059-B.

Holm, J., V.P. Palace, P. Siwik, G. Sterling, R. Evans, C. Baron, J. Werner, and K. Wautier. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. Environ. Toxicol. Chem. 24: 2373-2381.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; spawning adults) and brook trout (*Salvelinus fontinalis*; spawning adults)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the high selenium site ranged from 6 to 32 μg/L. Selenium was not measured at the reference streams; selenium concentrations at reference locations in the area ranged from <0.5 to 2.2 μg/L.

Study Design: Spawning fish were collected at low selenium or reference streams (Deerlick Creek, Wampus Creek and Cold Creek), a slightly elevated selenium stream (Gregg Creek), and an elevated selenium stream (Luscar Creek) in the Northeastern slopes region of Alberta, Canada. An active coal mine is the source of selenium in the elevated streams. Eggs and milt from the spawning trout were expressed by light pressure from abdomen. Individual clutches of eggs were fertilized from a composite volume of milt derived from 3-5 males. Fertilized eggs from individual females were reared to swim-up stage and examined for a number of parameters including percent fertilization, mortality, edema, and deformities (craniofacial, finfold, and spinal malformations). Similar studies were conducted in 2000, 2001 and 2002. One notable difference is that the embryos were incubated at 8°C in 2000 and at 5°C in 2001. The authors noted that 5°C is a better representation of the actual stream temperature during embryo development.

Effects Data : Other than selenium, there were no significant differences in the concentrations of other elements (Al, As, Sb, Ba, Be, Ni, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ag, Sr, Tl, Th, Sn, Ti, U, V, Zn) in trout eggs between the low level and elevated selenium streams. There are two ways to approach determination of effects due to selenium in this study and both are presented here. The first approach determines effects based on a comparison of average conditions between streams (*between streams approach*). For example, if there is a significant difference between the average frequency of deformities in a contaminated stream and reference stream, the effect level for the *between streams approach* would be the average concentration of selenium in the tissue from the contaminated stream. The second approach evaluates individual response variables (e.g., edema, deformities) against the individual selenium tissue concentrations for the combined contaminated and reference stream data set with each year (*within streams approach*). This approach, which results in an

EC estimate (e.g., EC_{10}) if the data meet the model assumptions, is explained below.

Between streams approach: For each sampling location (stream), data for the three years (Tables 1 and 2) were combined in the between streams analysis of variance (ANOVA). For rainbow trout embryos, there were no significant differences in fertilization, time to hatch and mortality between the streams with elevated selenium and the reference streams. ANOVA indicated significant differences in the frequency of embryonic effects between streams (Table 3). The analysis did not prove useful; however, due to a higher occurrence of effects in some of the reference streams relative to the exposed streams (Tables 3 and 4). The between streams analysis, therefore, was not used to determine effect concentrations for rainbow trout.

ANOVA of brook trout data indicated the only significant difference in embryonic abnormalities among sites was craniofacial deformities (Tables 5 and 6). Significant differences were also found for fertilization and larval weight. The highest average percent fertilization was observed at the site with the greatest concentration of selenium in eggs, which indicates that the differences in fertilization among sites were not caused by variation in selenium concentrations. Because the percent of embryos with craniofacial deformities in Luscar Creek was 7.9% (2.1% in Cold Creek), it was not considered biologically meaningful. Likewise the significantly lower larval weights at the exposed sites was not large (16% lower than Cold Creek larvae) and again coupled with the low occurrence of abnormalities by the brook trout, a signature of selenium effects, the lower larval weights were not considered biologically meaningful.

Within streams approach: As with the *between streams* analysis, data were combined for the three years of study in the *within streams* analysis (Tables 1 and 2). Craniofacial deformities, skeletal deformities and edema in rainbow trout embryo, as a function of selenium in egg ww, were fitted to a curve using a weighted regression and threshold sigmoidal equation from which EC_{10} values were calculated (see Figures 1, 2 and 3). EC estimates for finfold deformities, length and weight of rainbow trout embryos could not be made because of inadequate dose-response. The brook trout data were not suitable for fitting logistic curves (Figure 5).

Table 1. Rainbow trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference sites (Deerlick Creek and Wampus Creek) in northeastern Alberta over three consecutive years.

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2000	Luscar	11	6.84	7.18	13.26	1.66	4.97
2000	Luscar	12	6.66	1.48	4.43	0.74	1.85
2000	Luscar	14	11.6	14.43	23.71	7.22	85.57
2000	Deerlick	16	1.78	0.63	1.9	0.63	0.63
2000	Deerlick	17	1.39	0	0	0	0
2000	Deerlick	18	1.00	0	0.86	0	0
2000	Deerlick	15	5.01	0	0	0	0
2001	Luscar	1	5.39	7.35	6.76	3.53	2.94
2001	Luscar	3	8.39	6.29	4.97	2.98	6.95
2001	Luscar	4	6.48	22.22	22.22	33.33	26.67
2001	Luscar	8	4.47	12	9.33	2.67	10.67
2001	Luscar	14	10.4	34.55	44.85	4.24	43.64
2001	Luscar	32	5.64	8.24	5.97	3.13	9.09
2001	Luscar	33	3.88	5.26	6.58	9.21	3.95
2001	Luscar	39	5.14	1.91	3.18	0	1.27
2001	Luscar	40	3.36	11.62	7.05	5.39	6.64
2001	Luscar	41	11.7	37.67	83.41	3.59	87
2001	Deerlick	8	3.68	9.55	5.45	1.36	5.45
2001	Deerlick	9	3.08	5.39	4.98	0.41	2.07
2001	Deerlick	10	1.62	7.89	7.89	5.26	10.53
2001	Deerlick	16	2.62	24.24	48.48	3.03	12.12
2001	Deerlick	17	2.79	14.13	15.22	4.35	20.65
2001	Deerlick	21	1.96	13.27	35.71	7.14	25.51
2001	Deerlick	22	3.13	1.09	2.17	0	1.09
2001	Deerlick	23	3.03	9.65	14.04	3.51	7.89
2001	Deerlick	25	3.32	9.25	13.29	7.51	8.09
2001	Deerlick	39	2.43	11.89	9.09	7.69	14.69
2001	Gregg	2	4.57	11.97	7.75	15.49	7.04
2001	Gregg	3	4.49	5.58	9.3	2.33	4.65
2001	Gregg	5	4.05	4.95	5.45	2.48	5.94
2001	Gregg	9	5.09	20	13.85	15.38	16.15
2001	Gregg	18	5.97	16.13	19.35	41.94	35.48
2001	Wampus	9	2.66	16.07	0	1.79	7.14
2001	Wampus	13	2.04	7.84	9.8	1.31	7.84
2002	Luscar	3	5.4	60.47	27.9	93	14
2002	Luscar	8	18.3	94.12	23.5	4.4	97.1
2002	Luscar	10	22	100	64.3	3.6	100
2002	Luscar	12	15.7	82.35	47.1	66.7	52.9

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2002	Luscar	22	20.5	100	42.1	2.1	100
2002	Luscar	23	6.3	5.59	6.6	1.6	2.7
2002	Luscar	24	26.8	100	100	0	100
2002	Luscar	26	6.5	1.72	1.7	4.3	0.9
2002	Deerlick	10	5.9	5.65	7.26	7.26	3.23
2002	Deerlick	18	7.8	10.77	1.54	9.23	3.08
2002	Deerlick	21	5	6.9	6.9	20.69	1.72
2002	Deerlick	24	4.3	2.88	2.88	21.58	0.72
2002	Deerlick	25	4.4	5.3	5.3	6.82	3.03
2002	Deerlick	26	6.6	2.95	1.85	1.11	1.85
2002	Gregg	1	5.8	4.76	3.81	3.81	3.81
2002	Wampus	1	3	18.84	14.49	72.46	11.59
2002	Wampus	2	4	0	0	100	100
2002	Wampus	3	4.6	4.1	3.28	7.58	0.61
2002	Wampus	4	4.7	25	20	70	12.5
2002	Luscar	28	7	19.23	0	76.9	0

Table 2. Brook trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference site (Cold Creek) in northeastern Alberta over three consecutive years.

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2000	Luscar	1	4.78	15.38	0	0	15.38
2000	Luscar	2	4.83	38.06	1.49	3.73	1.49
2000	Luscar	3	5.98	7.39	3.03	0.34	0.5
2000	Luscar	5	3.86	25	5.7	8.77	4.82
2000	Luscar	12	6.06	16.77	1.83	0.7	0
2000	Luscar	13	5.8	4.06	1.42	0.2	0
2000	Luscar	14	5.17	4.13	0.49	0.36	0.12
2000	Luscar	15	9.92	16.22	0.54	0.54	0
2000	Luscar	16	5.03	5.61	0	0.27	0.27
2000	Luscar	17	6.01	9.44	5.83	0.83	1.11
2000	Luscar	18	12.7	14.34	0.72	0	0.36
2000	Cold	21	1.15	3.26	1.48	0.89	0
2000	Cold	22	1.83	4.83	1.38	1.38	0.69
2000	Cold	24	0.97	1.67	0	0.72	0
2000	Cold	25	No data	3.31	1.1	1.66	1.1
2000	Cold	26	0.59	3.45	4.83	6.9	0.69
2000	Cold	33	1.35	6.15	0	1.54	0
2000	Cold	34	2.18	6.45	0	0.81	0
2001	Cold	6	1.79	0	0	0	0
2001	Cold	7	1.36	1.61	0.69	0.46	1.38
2001	Cold	8	0.94	1.36	0	0.27	0.54
2001	Cold	21	1.07	0.43	0	0	0
2001	Cold	51	1.09	0	2.13	0	6.38
2001	Luscar	3	8.4	0	0.93	0	0.46
2001	Luscar	7	7.26	1.35	1.62	0.81	0.27
2001	Luscar	17	14.6	2.22	0.63	0.32	0
2001	Luscar	19	9.79	7.55	2.11	2.42	0.3
2001	Luscar	59	5.8	2.28	0.46	0.91	0.46
2001	Luscar	60	9.03	3.16	0	1.05	1.05
2001	Luscar	61	7.29	0	0	9.09	0
2001	Luscar	64	7.08	1.54	2.19	0	0
2001	Luscar	76	7.1	36.71	13.29	19.65	1.16
2001	Luscar	82	6.06	1.11	0.22	0.88	0.44
2001	Luscar	83	5.82	6	2	5.6	0.8
2001	Gregg	3	7.08	6.32	1.58	20.53	1.58
2001	Gregg	22	7.95	0	0	1.08	0
2001	Gregg	23	9.23	0.5	0.5	2.51	0
2001	Gregg	25	6.46	0.56	0	0.56	0

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2001	Gregg	31	7.35	0.51	1.7	0.17	0
2001	Gregg	32	4.91	7.21	0.48	3.37	0.48
2001	Gregg	33	7.02	1.88	1.88	4.38	0
2001	Gregg	34	5.01	0	0.37	0	0
2002	Luscar	17	6.28	1.7	12.74	0.85	0.21
2002	Luscar	23	5.27	7.34	0.46	0	0.46
2002	Luscar	26	6.36	1.81	0.52	0.26	0.26
2002	Luscar	38	18.9	0.9	0.54	0	0.18
2002	Luscar	42	4.95	2.79	0.44	0.15	0.15
2002	Luscar	44	6.47	0	0.25	0	0
2002	Luscar	54	7.96	0.33	0.33	0	0
2002	Luscar	56	18.8	3.99	0.75	0.5	0.75
2002	Gregg	25	6.27	1.23	1.23	0	0
2002	Gregg	37	4.58	2.99	0	0	0
2002	Gregg	39	6.67	3.57	1.19	1.19	1.19
2002	Cold	32	0.42	0	0.6	0	0
2002	Cold	26	0.89	0	0	0	0.29
2002	Cold	2	0.94	0.96	0.32	0	0
2002	Cold	5	1	0.25	0.5	0.25	0
2002	Cold	29	1.02	0.72	1.09	0.36	0.72
2002	Cold	23	1.2	0.35	0.35	0.35	0.35
2002	Cold	48	1.25	9.52	4.76	2.38	0
2002	Cold	42	1.6	0	0	0	0
2002	Cold	22	1.74	0	0	1.09	1.09
2002	Cold	51	2.11	2.17	2.17	0	2.17

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites

% fertilization

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	77.60	25.8653	0.06336703	0.978935
Residuals	51	20817.33	408.1829		

% mortality

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3751.51	1250.504	1.848008	0.1502207
Residuals	51	34510.50	676.676		

% craniofacial deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8093.97	2697.989	4.430272	0.007732133
Residuals	50	30449.48	608.990		

% skeletal deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3279.30	1093.101	2.773923	0.05094422
Residuals	50	19703.16	394.063		

% finfold deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	6273.17	2091.056	3.888612	0.01417887
Residuals	50	26886.93	537.739		

% edema

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8902.51	2967.502	3.449597	0.0233558
Residuals	50	43012.30	860.246		

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites (continued)

Fry length

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	5.0847	1.694896	0.5694271	0.6377436
Residuals	50	148.8246	2.976493		

Fry weight

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	1721.104	573.7012	3.563888	0.02080915
Residuals	48	7726.859	160.9762		

Table 4. Rainbow trout means (standard deviation) for measurements made in eggs, embryos and larvae spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference sites (Deerlick and Wampus Creeks).

Parameter	Site			
	Luscar Cr.	Gregg Cr.	Deerlick Cr.	Wampus Cr.
egg Se, mg/kg ww	9.93 (6.77)	6.52 (4.11)	3.49 (1.90)	3.5 (1.09)
fertilization, %	77.8 (20.3)	81.2 (12.7)	77.5 (20.9)	77.5 (24.1)
mortality, %	35.0 (29.5)	34.2 (32.5)	18.1 (14.6)	37.3 (34.5)
craniofacial, %	33.3 (37.2)	10.6 (6.5)	7.1 (6.1)	12.0 (9.6)
skeletal, %	25.0 (27.9)	9.9 (5.8)	9.2 (12.3)	7.9 (8.2)
finfold, %	15.0 (27.1)	13.6 (15.2)	5.4 (6.2)	42.2 (43.7)
edema, %	34.5 (40.3)	12.2 (12.3)	6.1 (7.3)	23.3 (37.8)
larval length, mm	18.5 (2.0)	19.4 (1.6)	19.0 (1.5)	19.2 (0.9)
larval weight, mg	53.3 (16.3)	44.6 (10.4)	41.2 (9.3)	40.6 (8.4)

Table 5. Brook trout means (standard deviation) for measurements made in eggs, embryos and larva spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference site (Cold Creek).

Parameter	Site		
	Luscar Cr.	**Gregg Cr.**	**Cold Cr.**
egg Se, mg/kg ww	7.78 (3.80)	6.59 (1.39)	1.26 (0.47)
fertilization, %	92.8 (7.2)	78.4 (18.2)	89.1 (19.6)
mortality, %	6.5 (8.9)	2.9 (2.3)	6.9 (12.1)
craniofacial, %	7.9 (10.1)	2.3 (2.5)	2.1 (2.6)
skeletal, %	2.0 (3.3)	0.8 (0.7)	1.0 (1.4)
finfold, %	1.9 (4.1)	3.1 (6.0)	0.9 (1.5)
edema, %	1.0 (2.9)	0.3 (0.6)	0.7 (1.4)
larval length, mm	17.4 (1.1)	17.9 (0.9)	18.5 (1.2)
larval weight, mg	31.7 (8.6)	31.3 (5.4)	37.8 (7.2)

Table 6. Results of ANOVA comparing brook trout endpoints among sites

% fertilization					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	1683.3	841.67	3.9128	0.0253
Residuals	60	12906.4	215.11		
% mortality					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	131.4	65.72	0.7257	0.4882
Residuals	60	5433.6	90.56		
% craniofacial deformities					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	519.1	259.54	4.9427	0.0103
Residuals	60	3150.6	52.51		

Table 6. Results of ANOVA comparing brook trout endpoints among sites (continued)

% skeletal deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	19.2	9.58	1.5631	0.2179
Residuals	60	367.6	6.13		
% finfold deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	37.5	18.74	1.2562	0.2921
Residuals	60	895.1	14.92		
% edema					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	4.6	2.32	0.4966	0.6110
Residuals	60	280.6	4.68		
Fry length					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	16.1	8.04	6.5265	0.0027
Residuals	60	73.9	1.23		
Fry weight					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	546.2	273.10	4.6644	0.0131
Residuals	60	3512.9	58.55		

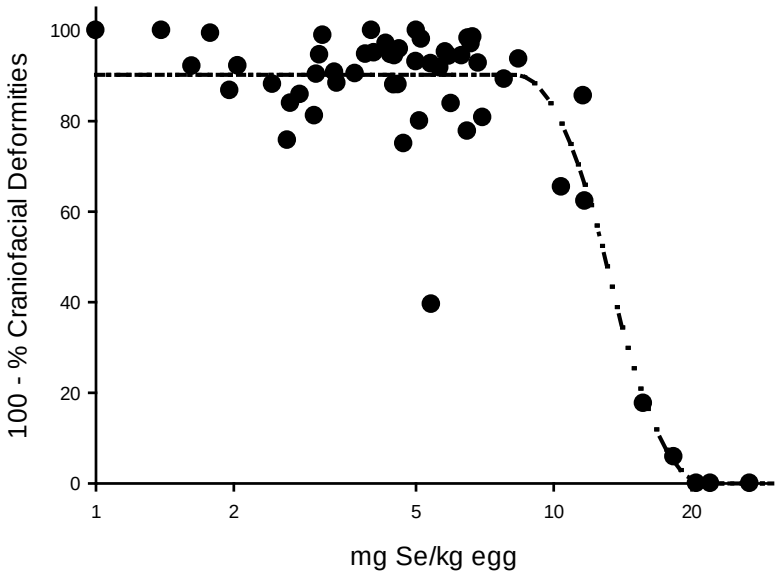


Figure 1. Rainbow trout percent normal (100 - % craniofacial deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 90.2%, the slope 4.8%, and the EC_{10} 10.2 mg Se/kg egg ww.

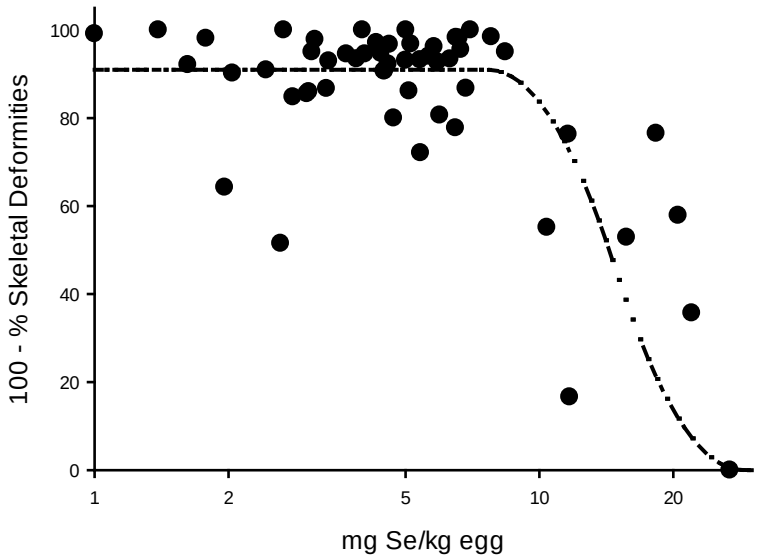


Figure 2. Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 91%, the slope3.5%, and the EC_{10} 10.3 mg Se/kg egg ww.

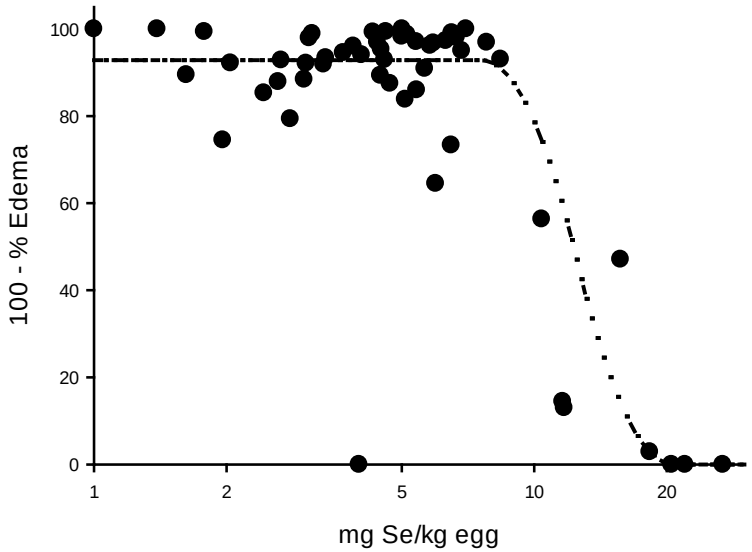


Figure 3. Rainbow trout percent normal (100 - % edema) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoidal equation excluding the one outlier with 100% edema at 4 mg/kg. The background value was estimated to be 92.8%, the slope 4.6%, and the EC_{10} 9.5 mg Se/kg egg ww.

The previous draft used a TRAP logistic regression (Figure 4). A weighted regression using a threshold sigmoidal equation (Figures 1-3) is a better application of these data.

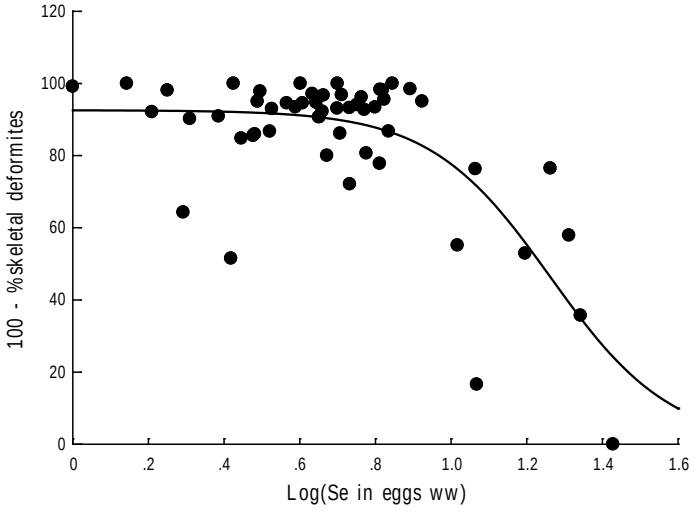
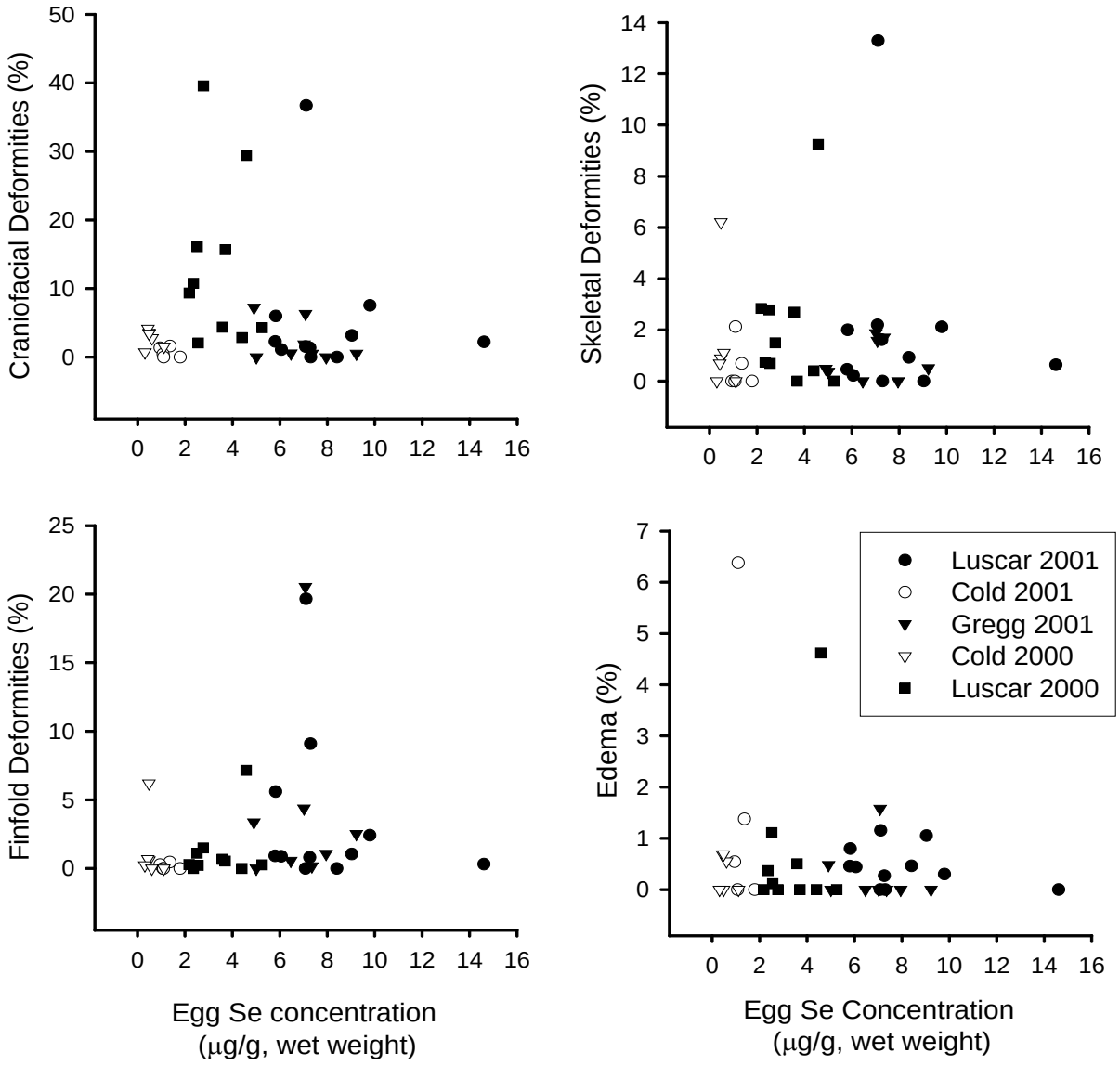


Figure 4. (From Previous Draft) Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). EC_{10} = 8.2 mg/kg ww.

Figure 5. Plot of percent abnormal for craniofacial, skeletal and finfold deformities and edema against selenium concentration in brook trout eggs ww, 2000 and 2001 data.



The effect levels determined using the *within streams* approach resulted in values based on ww in eggs. The primary tissue for which the reproductive effect levels were based, eggs, was converted from ww to dw using the average percent moisture of 61.2% for rainbow trout eggs reported by Seilor and Skorupa (2001).

Chronic Values:

Brook trout: *Between streams approach*
No effects at EC_{10} level at 7.78 mg Se/kg eggs ww or 20.05 mg Se/kg eggs dw; egg. **Chronic value is >20.05 mg Se/kg eggs dw.** Table 3 data, converted to dry weight, suggest no effects at least up to 25-35 mg Se/kg eggs dw.

Rainbow trout: *Within streams approach*
EC_{10} value (edema) at 9.5 mg Se/kg egg ww or 24.5 mg Se/kg egg dw. **Chronic value is 24.5 mg Se/kg eggs dw.**

Kennedy, C.J., L.E. McDonald, R. Loveridge, M.M. Strosher. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarkii lewisi*). Arch. Environ. Contam. Toxicol. 39:46-52.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii lewisi*; spawning adults, 3-6 years)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the time the eggs were taken were <0.1 μg/L from the reference site and 13.3 to 14.5 μg/L at the exposed site.

Study Design: At reference and exposed site (Fording River, BC, Canada which receives drainage from open-pit coal mining), eggs were stripped from females (n=20 from reference site; n=17 from exposed site) and fertilized from milt from one male collected at each site. Fertilized eggs were reared in well water and examined for time to hatch, deformities (craniofacial, finfold, skeletal and yolk sac malformations), and mortalities. Inspection of deformities in eggs was performed using 40X magnification.

Effects Data : No significant correlations between the selenium concentrations in the eggs from either site and: hatching time (reference, 25.5-26.5 days; exposed, 22-25.5 days); percent deformities preponding (reference, 0-2.4%; exposed, 0-0.34%); percent deformities after ponding (reference, 0-0.26%; exposed, 0-0.09%); percent mortalities preponding (reference, 1.5-70.3%; exposed, 1-100%); percent mortalities after ponding (reference, 0.3-4.3%; exposed, 1.5-43.7%); total percent mortalities (reference, 2.8-55.8%; exposed, 3.7-100%). The average selenium residues in tissues were as follows:

Site	Adult fish liver, mg Se/kg dw	Adult fish muscle, mg Se/kg dw	eggs, mg Se/kg dw
Reference	8.2; Range: 3.4-14.6	2.4; 1.4-3.8	4.6
Exposed	36.6; Range:18.3-114	12.5; Range: 6.7-41	21.2

Chronic Value: >21.2 mg Se/kg dw in eggs
>12.5 mg Se/kg dw in muscle

Hardy, R.W. 2005. Effects of dietary selenium on cutthroat trout (*Oncorhynchus clarkii*) growth and reproductive performance. Report for Montgomery Watson Harza. December 14, 2005.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii*, 0.9 g)

Exposure Route: Dietary only
Six experimental dietary treatments were produced by cold extrusion. The formulation of the diet was designed to be similar to commercial trout diets and had a proximate composition of 45% protein and 16% lipid. Seleno-methionine diluted in distilled water (100 µg/L) was added in appropriate volumes to each batch of feed to facilitate pelleting. Measured dietary selenium concentrations were 1.2 (control), 3.8, 6.4, 9.0, 11.5, and 12 mg Se/kg dw. Fry were fed initially at a rate of 10 times per day 6 days each week to apparent satiation. Feeding frequency decreased as fish grew.

Test Duration: 124 weeks (865 days, 2.5 yrs)

Study Design: Groups of 50 fish were placed into triplicate tanks (145 L) receiving 4-15 L/min of hatchery water at 14.5EC and fed one of the six experimental diets. The fish in each tank were bulk-weighed and counted every 14 days for the first 12 weeks of the experiment, and then every 4 weeks until 48 weeks. Samples of fish for whole-body selenium analysis were taken at each sampling date for the first 12 weeks followed by every 3 months thereafter. After six months of feeding, the fish were transferred to 575 L tanks and the number of replicate tanks per dietary treatment was reduced to two. After 80 weeks of feeding, the fish were transferred to 1050 L outdoor tanks each supplied with 70 L/min of constant temperature (14.5°C) spring (hatchery) water. After 2.5 years of the feeding trial, fish were spawned and whole body selenium level , egg selenium level, % eyed eggs, % hatched eggs, and % deformed larvae were examined.

Effects Data: No signs of toxicity (reduced growth or survival relative to controls) were observed in fish fed the highest dietary selenium treatment (12 mg Se/kg dw) after the first 80 weeks of exposure just prior to transfer outdoors. No signs of clinical disease were evident, and no relationship was found between feed conversion ratios and the level of selenium added to the feed. Average whole body selenium levels of female Henry's Lake cutthroat trout at spawning at 2.5 to 3 years of age were 5.87, 9.10, 11.37 and 5.61 mg Se/kg dw in the four highest dietary treatments. Average egg selenium levels in the same four dietary treatments were 6.61, 5.05, 5.18, and 16.04 mg Se/kg dw. Percent survival from the eyed stage to hatching varied among treatment groups, with the control and the highest Se dietary treatment having the second highest survival (85%) and the fifth dietary treatment group the highest (93%). Percent deformed larvae ranged from a low of 5.6% in controls to a high of 20.2% in the 6.4 mg Se/kg dw dietary treatment group; larvae in the two highest dietary treatment groups only exhibited 7 and 6.8 %, respectively.

Chronic Value: The chronic value for embryo/larval deformity is a NOAEC of >11.37 mg Se/kg dw whole-body parent tissue and >16.04 mg Se/kg dw egg.

Rudolph, B-L, I. Andreller, CJ. Kennedy. 2008. Reproductive success, early life stage development, and survival of Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) exposed to elevated selenium in an area of active coal mining. Environ. Sci. Technol. 42: 3109-3114.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)

Exposure Route: Field collected.
In June, 2005, eggs were collected from 12 females from Clode Pond (exposed site) and 16 females from O'Rourke Lake (reference site). Milt was obtained from 3-5 males at each site. Clode Pond is on the property of Fording River Coal Operations in Southeast British Columbia with reported selenium concentrations of 93 µg/L. O'Rourke Lake is an isolated water body into which Westslope cutthroat trout were stocked in 1985, 1989 and 1992 and has selenium levels reported <1 µg/L.

Test duration: Through the end of yolk sac absorption (at swim-up) by the alevins.

Study Design: Individual batches of eggs were fertilized in the field with 2 ml composites of milt. Water-hardened eggs were transported to the rearing laboratory. Eggs and alevins were monitored daily for fertilization, hatching and mortality. After the yolk sacs were absorbed, alevins were sacrificed and preserved in Davidson's solution.

All viable fry (n = 4,922) after yolk absorption were observed for the frequency and severity of skeletal (lordosis, kyphosis, and scoliosis), craniofacial (head, eyes or jaw), and fin malformations as well as edema. The authors used a graduated severity index (GSI) for deformities in which fry were scored 0 (normal) to 3 (severe) based on the level of defect.

Effects Data: Eggs with the four highest Se concentrations (86.3 to 140 mg/kg dw) collected from Clode Pond fish died before reaching the laboratory (Table 1). Excluding the eggs that died from females CP1, CP3, CP4 and CP5, fertilization (total eggs reaching the eyed stage/total eggs x 100) was not related to Se concentrations in the eggs. The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs (Table 1). Note: The data used to estimate the EC_{10} value excluded the variable from OL1 and OL2 (shaded areas in Table 1). These are data from the reference lake in which only 57% of the larvae survived (OL1) or where the % dead eggs plus % hatch did not add up to %100. Alevin survival was meaningfully higher in the other 15 clutches of eggs from the reference site (85.1 to 99.8%). Because there were insufficient partial effects, a TRAP model was not used to estimate the EC_{10} value. The data consist of a cluster background data and a cluster of 100% mortality (Figure 1). With no way to fit a credible curve, the interpolation method is applied here with the EC_0 set to 20.6 mg/kg with background % survival of 95.75% (not including the one low outlier) and the second extrapolation point being 46.8 mg/kg with 0.3% survival. The resultant slope is 5.6 (similar to slopes in other datasets where it was estimated) and the EC_{10} is 24.7 mg/kg. Note: TRAP was used in the previous draft to derive a similar EC_{10} of 24.1 mg/kg, however as stated above, it

was determined that the data are not amenable to a TRAP model because of insufficient partial effects.

An EC_{10} based on Se in maternal muscle was estimated using the same approach as was used for Se in eggs, that is, by interpolation between an EC_0 and a high EC_P. An EC_{10} of 16.6 mg Se/kg muscle dw was interpolated from an EC_0 (HNOEC) of 13.4 mg/kg and the average background survival of 95.75 and the EC_{100} set to 34.7 mg/kg muscle (Figure 2).

Deformity analysis was not performed on the alevins that died prior to the swim-up stage. Therefore, due either to dead eggs or dead alevins, the occurrence and severity of deformities were assessed on four clutches of eggs from Clode Pond (CP2, CP6, CP11 and CP12) with a range of 11.8 to 20.6 :g Se/g dw and 15 of the 16 clutches (all eggs died in OL8) from O'Rourke Lake (Table 1). There was no correlation between egg Se concentration and frequency of deformity or edema. Statistical differences between sites were observed ($p < 0.05$) for skeletal deformities and edema for both the frequency of the occurrence and the severity score (Table 2). Note: the percent and severity score of skeletal deformities were greater in the reference site than in the exposed site.

The effect level for this study was based on the alevin mortality data and not the deformity measurements. Although edema occurred statistically more often at the exposed site (87.7% at Clode Pond, 61.2% at O'Rourke Lake), it was not correlated with selenium levels in the eggs. Also the greater occurrence of skeletal malformations in the reference site confounded the use of statistical differences between sites to determine effect levels for this study.

Effect Concentration: 24.7 mg Se/kg dw in eggs; 16.6 mg Se/kg dw in muscle.

Table 1. Fertilization, egg mortality and alevin mortality for offspring from individual fish collected in Clode Pond and O'Rourke Lake.

Fish ID	Muscle [Se] mg/kg dw	Egg [Se] mg/kg dw	Hatch %	Dead eggs, %	Dead alevins, %	% Survival[1]
Clode Pond (exposed site)						
CP1	38.8	88.3	0	100	NA	
CP2	11.8	16.1	98.2	1.8	0.9	99.1
CP3	40.4	86.3	0	100	NA	
CP4	46.1	121	0	100	NA	
CP5	50.4	140	0	100	NA	
CP6	34.7	51	92.6	7.4	92.6	0.0
CP7	39	65.3	91.1	8.9	91.1	0.0
CP8	7	11.8	63.9	36.1	0.8	98.7
CP9	35.4	46.8	63.4	36.6	63.2	0.3
CP10	35.5	75.4	82.4	17.6	82.4	0.0
CP11	11.3	16.9	77.9	22.1	1.3	98.3
CP12	13.4	20.6	97	3	5.1	94.7
avg	30.3	61.6	55.5	44	42	20.0
SD	15.1	42.4	42.5	42	44	0.0
O'Rourke Lake (reference site)						
OL1	8.28	12.9	71.4	28.6	42.9	39.9
OL2	7.7	13.9	27.7	53.1	6.9	75.1
OL3	8.16	12.5	96.1	3.9	2.4	97.5
OL4	8.03	15	85.5	14.5	12.7	85.1
OL5	8.12	14.9	80.7	19.3	5.3	93.4
OL6	6.61	15.2	68	32	4	94.1
OL7	8.52	12.9	97.9	2.1	0.2	99.8
OL8	7.22	12.3	0	100	NA	
OL9	7.25	16.7	87.2	12.8	4.5	94.8
OL10	7.64	13.1	79.6	2.5	5.5	93.1
OL11	8.74	15.6	89.2	10.8	2.4	97.3
OL12	8.2	13.9	83.6	16.4	3	96.4
OL13	7.86	15.1	74.1	25.9	2.8	96.2
OL14	8.5	13.1	77.8	22.2	0.5	99.4
OL15	7.62	12.3	88.2	11.8	2.6	97.1
OL16	8.13	12.7	54.8	45.2	4.8	91.2
avg	7.9	13.9	72.6	25	7	
SD	0.6	1.4	25.8	25	10	

[1] % Survival based on % hatch

Table 2. Deformity results (frequency and severity) for offspring from O'Rourke Lake and Clode Pond. Values are presented as mean ± SE. * indicates a significant difference ($p < 0.05$) between means from the two sites.

Frequency of deformity, %	O'Rourke Lake	Clode Pond
Skeletal*	37.4 ± 3.6	16.5 ± 2.2
Craniofacial	10.2 ± 2.0	5.7 ± 1.0
Finfold	10.6 ±3.1	7.5 ± 3.84
Edema*	61.2 ± 4.9	87.7 ± 2.0
Severity of deformity, score		
Skeletal*	0.47 ± 0.07	0.18 ± 0.02
Craniofacial	0.12 ± 0.03	0.06 ± 0.01
Finfold	0.15 ± 0.05	0.09 ± 0.05
Edema*	0.61 ± 0.05	0.88 ± 0.02

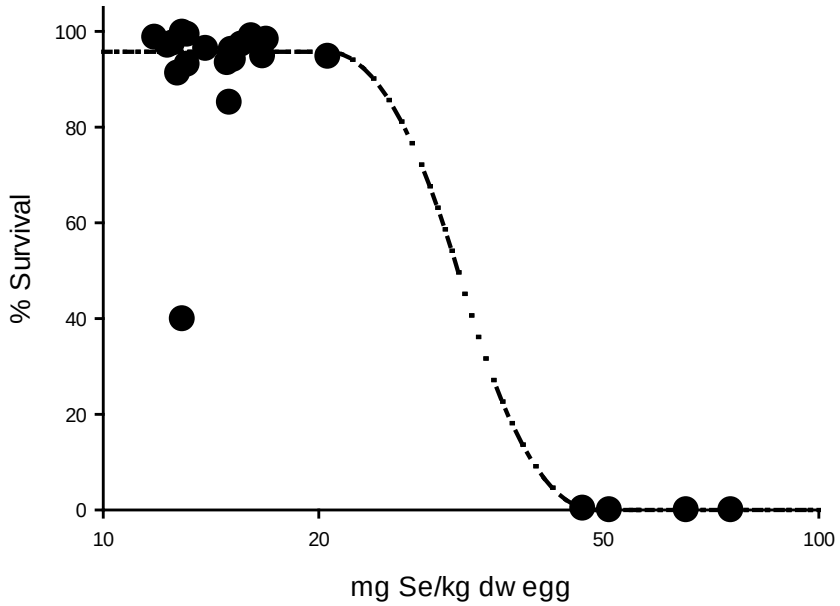


Figure 1. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in eggs.

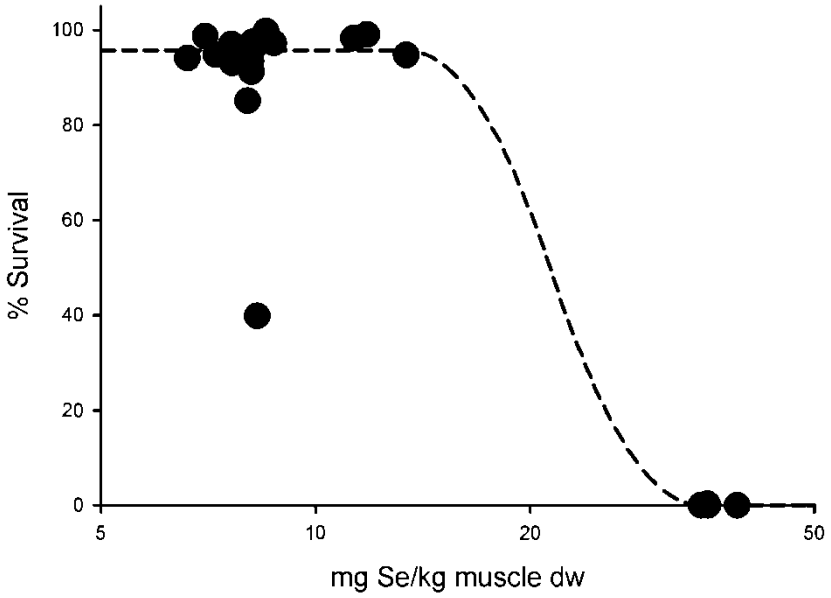


Figure 2. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in maternal muscle.

Nautilus Environmental. 2011. Evaluation of the Effects of Selenium on Early Life Stage Development of Westslope Cutthroat Trout from the Elk Valley, BC. Report to Elk Valley Selenium Task Force, November 24, 2011.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi)*

Exposure Route: Field collected. Adult fish were collected and spawned from lentic and lotic environments in areas proximate to Teck Coal's Fording River Operations. Eggs were also obtained from fish collected from Connor Lake, a lake located within the Elk valley watershed not exposed to mine discharges and considered a reference site and a methodological control.

Test Duration: Fertilized eggs were reared in the laboratory until they reached swim-up fry stage. A subset of fry surviving at swim-up were reared for an additional 28 days.

Study Design: Gametes were stripped from the ripe adults in the field during June and July 2008 and transported immediately to the laboratory in coolers containing wet ice. Eggs were fertilized in the laboratory. After stripping the eggs, female fish were sacrificed and the whole body stored on ice for later Se analysis. For a given female, approximately 240 fertilized eggs were divided into four replicates of 60 eggs. In cases when fewer eggs were available three replicates of 60 eggs were used. If less than 180 eggs were available, either 3 or 4 replicates of 30 were used. Females with less than 90 eggs were not used. The fertilized eggs were maintained in the laboratory until the fry reached swim-up at which point deformities were assessed. Survival was also assessed up to swim-up. In test chambers in which there were at least 40 surviving fish at swim-up, one-half of the surviving fish were maintained for an additional 28 days. Survival, length, weight and deformities were assessed in the 28-day post swim-up test.

The number, type and severity of deformities were measured at swim-up and at the end of the 28-day post swim-up test. Deformity assessments were conducted on recently killed fresh fish to avoid artifacts caused by preservation. A graduated severity index (GSI) was assigned to each of four types of deformity/abnormality: skeletal, craniofacial, finfold and edema. Graduated Severity Index (GSI) methods followed those described in Holm et al. (2003) and Rudolph et al (2006; 2008).

Effects Data: Survival of the larvae from hatch through swim-up spawned from the four fish collected from the reference site, Connor Lake, ranged from 73 to 92% (egg Se 4.32 to 7.31 mg/kg dw) (Table 1). Larval survival at swim-up was also generally high for fish collected in the Se exposed sites up to egg Se concentration 29.6 mg/kg dw (Table 1, Figure 1). Larvae exposed above this egg Se concentration had poor to no survival. Larvae from one fish (P00811) below this threshold did have poor survival (11.7%). The authors noted that the many of the eggs from this fish displayed an unusual distribution of lipid vesicles which resulted in greater than 50% mortality in the first 24 hours due to egg breakage. The remaining eggs may have been compromised due to the organic material released during the egg breakage.

The rate of deformities in larvae at swim-up showed no relationship with Se in egg through 29.6 mg/kg dw (Table 2).

The results of the 28-day post swim-up test showed no relationships between larval survival or deformities and egg Se (Table 3). The authors also measured the length and weight of larvae at the end of the 28 day test; neither of which showed a relationship with egg Se concentration.

Se Tissue Concentrations. Two analytical laboratories (A and B) measured Se in the eggs. The mean difference in egg Se concentrations between the two laboratories was 34.2%. To better understand the difference between the two laboratories, five egg samples (i.e., from five different fish) from this study were sent to both laboratories in 2010. Both laboratories digested the eggs using the methods they used in their own 2008 original analysis. The respective digestates were split and then shared between laboratories. Both labs then measured selenium in their own digestates and the digestate received from the other lab. The results of this follow-up study showed that when each lab used their own digestion procedures Laboratory A had on average 43% higher measurements in the 2008 analysis and 23% higher in the follow-up 2010 analysis. When each lab measured selenium using the same digestate the difference in the Se measurements between labs was on average only 1 to 8%. The authors concluded that although both laboratories employed acceptable and approved practices, Laboratory A used a more efficient digestion process resulting in higher Se measurements. To compensate for the reduced Se measurements in Laboratory B, its values were increased by 34.2%. The measurements made by Laboratory A are marked in Table 1; unmarked values are Laboratory B measurements increased by 34.2%.

Effect Concentration: The most sensitive endpoint determined by TRAP was larval survival at swim-up. Interpolation was used to estimate an effect concentration for larval survival with the entire egg Se dataset that included egg Se measurements from Laboratory A and adjusted measurements from Laboratory B (EC_{10} = 31.1 mg/kg egg dw; Figure 1) and using only the egg Se measurements from Laboratory A (Figure 2). Because the Laboratory A dataset estimated slightly lower EC values, the EC_{10} of 27.7 mg/kg egg dw is the selected effect concentration for this study. Note: In the previous draft, a TRAP model was used to estimate the EC_{10}. However, because of insufficient partial effects, TRAP was determined not appropriate so the EC_{10} was estimated using an interpolation between the HNOEC and the LOEC (see Figure 3 for the TRAP analysis used in the previous draft).

Table 1. Summary of westslope cutthroat trout larvae surviving to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

				Proportion surviving				
Fish ID	Location	Se egg, mg/kg dw	Replicates	Replicate mean	Replicate min	Replicate max	Number survivors	Total number
YO93	Lentic	3.88*	4	0.8125	0.6667	0.9167	195	240
CL1	Reference	4.32	4	0.9167	0.8833	1	220	240
R082	Lotic	5.21	3	0.9056	0.8333	0.95	163	180
CL4	Reference	5.96*	4	0.7333	0.6	0.8	176	240
CL2	Reference	6.82	4	0.8333	0.7	0.9167	200	240
CL3	Reference	7.31	4	0.8542	0.8167	0.8833	205	240
P00815	Lotic	7.6	3	0.8222	0.7167	0.95	148	180
R026	Lotic	12.53	4	0.5792	0.5	0.65	139	240
P00823	Lotic	12.71	4	0.8875	0.85	0.95	213	240
R039	Lotic	12.9	4	0.6042	0.55	0.65	145	240
R086	Lotic	13.4*	4	0.9417	0.85	0.9833	226	240
R077	Lotic	14.29	3	0.6444	0.6167	0.6667	116	180
R042	Lotic	16.44	3	0.8	0.7	0.9	72	90
R055	Lotic	16.5	4	0.8792	0.7833	0.9667	211	240
R043	Lotic	16.85	4	0.8667	0.7667	0.9667	104	120
R074	Lotic	17.8*	4	0.9375	0.8833	0.9833	225	240
P00811	Lotic	19.25	1	0.1167	0.1167	0.1167	7	60
P00809	Lotic	19.72	4	0.7667	0.65	0.8833	184	240
P00803	Lotic	24.8*	4	0.9375	0.9333	0.95	225	240
R078	Lotic	29.61	4	0.8825	0.8333	0.9333	105	119
GO99	Lotic	34.2*	4	0.2083	0.1667	0.2667	50	240
O087	Lentic	54.7*	4	0.07083	0.01667	0.2	17	240
O085	Lentic	56.8*	4	0	0	0	0	240
WO52	Lentic	61.1*	4	0	0	0	0	240
R069	Lotic	65.61	4	0	0	0	0	240
R071	Lotic	72.9	4	0	0	0	0	240
WO94	Lentic	73.1	4	0	0	0	0	240
UT101	Lentic	74.67	4	0	0	0	0	240

*Laboratory A dataset

Table 2. Summary of westslope cutthroat trout larval deformities to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Se egg, mg/kg dw	Skeletal combined	Craniofacial combined	Finfold combined	Edema combined	Deformities combined
YO93	Lentic	3.88*	4.5%	0.9%	4.4%	1.9%	7.7%
CL1	Lentic	4.32	7.6%	1.9%	1.0%	1.0%	9.5%
R082	Lotic	5.21	1.2%	1.3%	2.5%	0.0%	3.7%
CL4	Lentic	5.96*	4.3%	7.3%	1.7%	0.7%	12.6%
CL2	Lentic	6.82	11.1%	3.7%	0.8%	3.0%	15.9%
CL3	Lentic	7.31	5.0%	2.0%	1.0%	0.0%	7.0%
P00815	Lotic	7.6	0.0%	2.7%	0.0%	2.9%	5.6%
R026	Lotic	12.53	2.1%	2.1%	0.7%	1.4%	2.1%
P00823	Lotic	12.71	1.9%	2.9%	1.8%	5.6%	7.4%
R039	Lotic	12.9	2.1%	1.9%	2.9%	4.9%	9.9%
R086	Lotic	13.4*	2.7%	1.0%	0.0%	0.0%	2.7%
R077	Lotic	14.29	1.7%	10.4%	0.9%	12.2%	15.5%
R042	Lotic	16.44	1.2%	0.0%	0.0%	2.6%	2.6%
R055	Lotic	16.5	0.0%	2.8%	1.0%	2.9%	4.7%
R043	Lotic	16.85	0.9%	2.6%	1.8%	1.7%	4.4%
R074	Lotic	17.8*	2.7%	1.8%	0.9%	0.9%	3.6%
P00809	Lotic	19.72	3.9%	2.8%	3.3%	4.7%	9.0%
P00803	Lotic	24.8*	2.7%	0.9%	0.0%	0.9%	4.5%
GO92	Lotic	26.1	0.0%	1.9%	1.9%	4.4%	4.4%
R078	Lotic	29.61	1.8%	0.0%	1.0%	2.9%	5.7%
GO99	Lotic	34.2*	14.5%	53.9%	6.8%	28.2%	64.7%

*Laboratory A dataset

Table 3. Summary of larval survival and rates deformities after the 28-day post swim-up test per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Sample size (n)	Egg Se (mg/kg dw)	Survival (%)	Skeletal (%)	Craniofacial (%)	Finfold (%)	Total (%)
CL1	Reference	112	4.3	99.1	0	0	0	0
CL2	Reference	93	6.8	99	0	0	0	0
CL3	Reference	96	7.3	91.7	0	1	1	2
CL4	Reference	68	6	98.6	0	0	4.3	4.3
Y093	Lentic	93	3.9	95.6	0	0	2	2
R082	Lotic	71	5.2	87.4	0	2.9	0	2.9
P00815	Lotic	69	7.6	91.1	0	1.2	1.4	2
P00823	Lotic	105	12.7	96.3	0	0	0	0
R086	Lotic	112	13.4	97.2	0	0.9	0	0.9
R077	Lotic	36	14.3	92.4	2.8	2.8	2.8	4.2
R055	Lotic	101	16.5	95.9	0	4.6	0	4.6
R074	Lotic	106	17.8	93.1	0	0	0	0
P00809	Lotic	65	19.7	91.7	0	0	0	0
P00803	Lotic	108	24.8	95.7	0	0	1	1

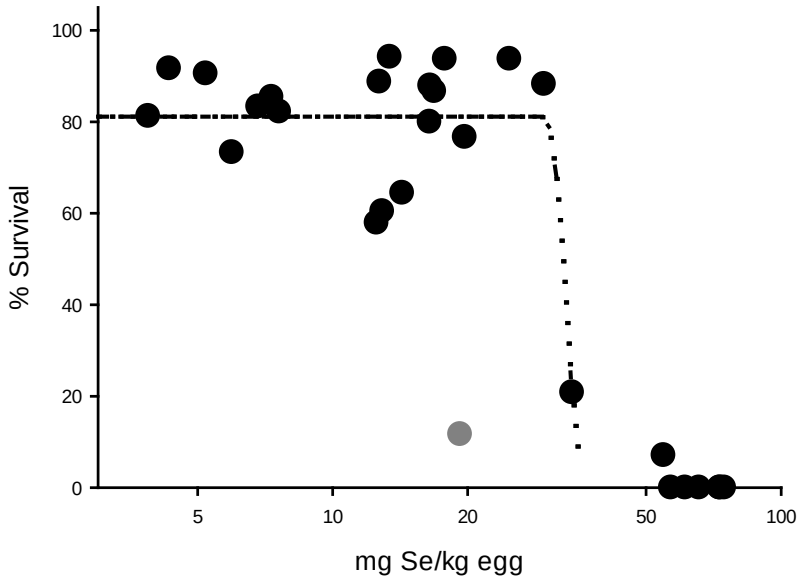


Figure 1. Labs A and B datasets. EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (29.6 mg/kg and 81.1%). The slope is 20.5 and the EC10 is 31.1 mg/kg. Note: the gray point denotes egg batch with quality problems noted by authors and was not used in the analysis.

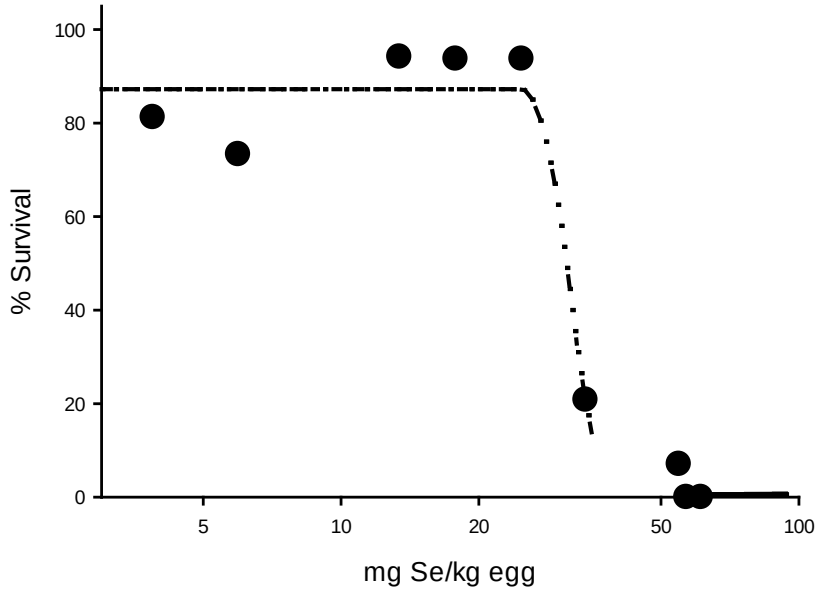


Figure 2. Lab A dataset.* EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (24.8 mg/kg and 87.25%). The slope is 9.4 and the EC10 is 27.7 mg/kg.

*Although some scientists have attempted to explain certain occurrences of improved response with increasing concentration in terms of nutrient selenium sufficiency-deficiency, the concentrations involved in this study are too high to for selenium deficiency to be an explanation. The figure's apparent bi-phasic measured response is thus best explained as being a chance outcome of noise.

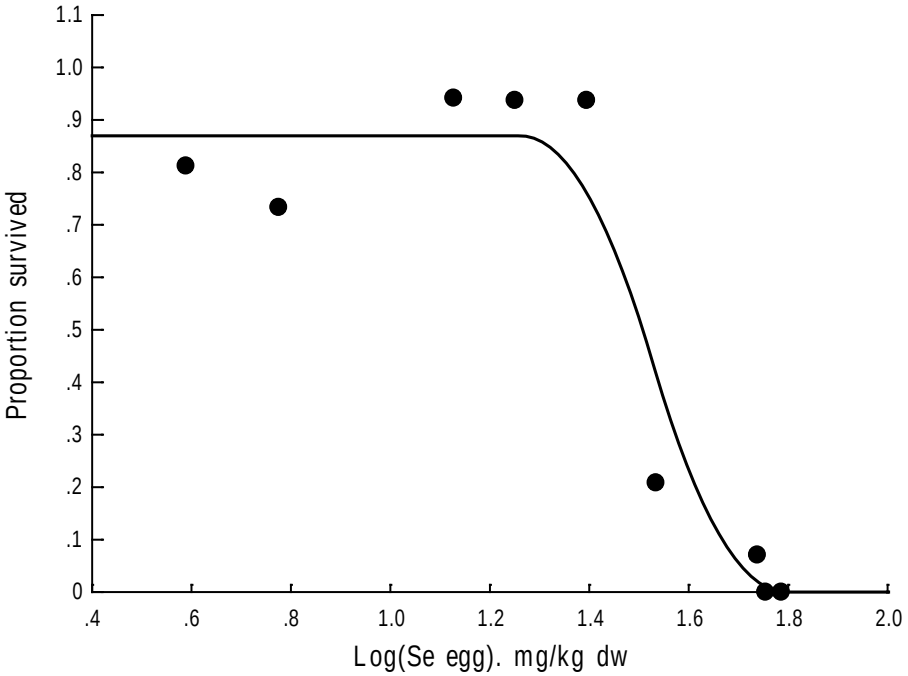


Figure 3. (From previous draft) Tolerance distribution; Model option – Triangular distribution (3 parameter). Includes Laboratory "A" dataset only TRAP EC_{10} estimate = 24.0 mg/kg.

Golder Associates. 2009. Development of a Site-specific Selenium Toxicity Threshold for Dolly Varden Char. Report to Northgate Minerals Corporation, PO Box 3519, Smithers, British Columbia. Report Number 04-1421-101/2000.

Test Organism: Dolly Varden (*Salvelinus malma*)

Exposure Route: Field collected.

Adult Dolly Varden char were collected from reference (North Kemess Creek), high Se exposure (Upper Waste Rock Ponds and Creek) and moderate Se exposure (lower Waste Rock Creek) sites during September 22 to 24, 2008. Eggs were stripped from females and fertilized with milt from males collected from the reference site. Fertilized eggs were taken to the laboratory for testing.

Test duration: The test was terminated when 90% of the larvae reached swim-up, approximately 5 months after fertilization.

Study Design: Approximately 30 fertilized eggs were added to each replicate rearing container. The number of replicates per female parent ranged from one to four depending on the number of eggs available. Embryos were maintained in 4 L containers with 3.5 L dechlorinated tap water in a static-renewal system (3 renewals times/week) at 5°C. The condition of the embryos and alevins were observed daily and any dead individuals were counted and removed. Test termination occurred over a 3-day period during February 11 to 13, 2009. The hatched larvae were sacrificed using an overdose of the anesthetic, clove oil. Individual length and weight were measured on each fry, and deformity analysis was performed on fresh unpreserved larval fish using 40X magnification.

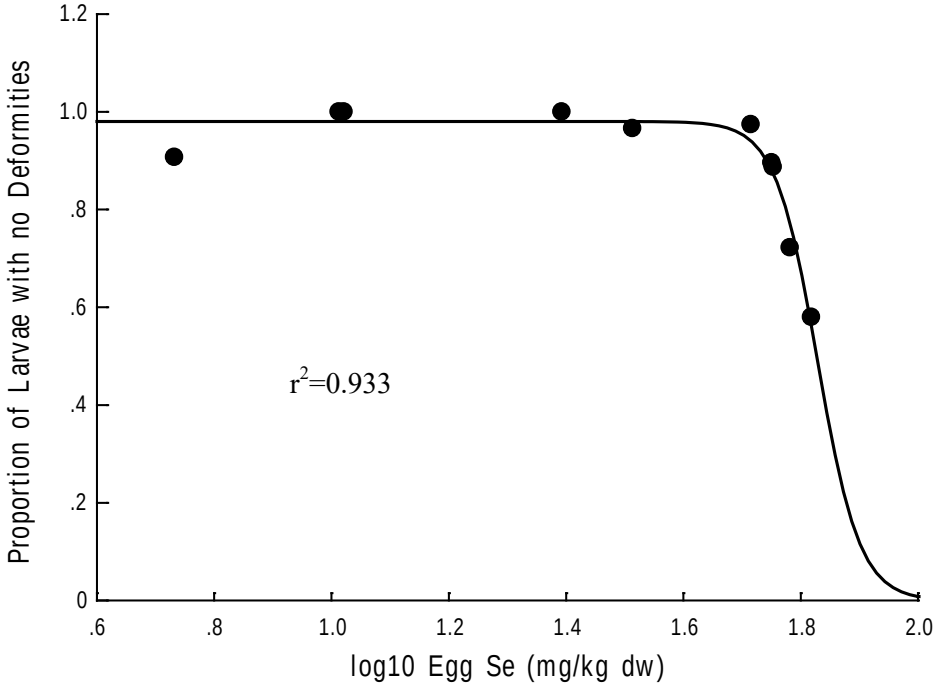
A graduated severity index (GSI) was used for deformity assessment (skeletal, craniofacial, and finfold as well as edema). The narrative criteria were the same as used by Holm et al. (2005) and Rudolph et al. (2008).

Effects Data: Alevin survival was not related to Se concentration in the eggs (Table 1). Almost all of the mortality occurred during the egg stage. Only 4 alevins died during the study, 1 from Fish #19 and 3 from Fish #2, both females collected at an exposed site. The prevalence of deformities increased sharply after the selenium egg concentration exceeded 50 mg/kg dw (Table 1, Figure 1). The proportion of Dolly Varden larvae with any type of deformity (skeletal, craniofacial, and finfold as well as edema) as a function of the log of the selenium concentration in the eggs using TRAP (logistic equation) produced an EC_{10} value of 56.22 mg/kg dw eggs (Figure 1).

Table 1. Selenium concentration in the eggs of Dolly Varden char and the survival of alevins to the swim-up stage and the proportion of larvae without any type of deformity.

Fish #	Sample ID	Location	[Se] eggs mg/kg dw	Survival of eggs to swim-up			Proportion of larvae without any type of deformity
				Initial	End	%	
1	WRC-F105	Waste Rock Creek	56.6	120	71	59	0.89
2	WRC-F61	Waste Rock Creek	65.8	120	81	68	0.58
5	WRC-F103	Waste Rock Creek	32.6	29	29	100	0.97
6	WRC-F83	Waste Rock Creek	51.9	120	115	96	0.97
15	WRC-F104	Waste Rock Creek	56.3	60	48	80	0.90
19	WRC-F86	Waste Rock Creek	60.5	120	115	96	0.72
9	NK-F30	North Kemess Creek	11	30	1	3	a
12	NK-F29	North Kemess Creek	10.5	46	15	33	1.00
17	NK-F21	North Kemess Creek	5.4	90	86	96	0.91
SCD1	Redd #1	Southern Collection Ditch	10.3	30	18	60	1.00
SCD2	Redd #2	Southern Collection Ditch	24.7	40	32	80	1.00

Figure C-1. Proportion of Dolly Varden alevin without any type of deformity as a logistic function of the logarithm of the selenium concentration in eggs (TRAP).



	Guess	Final	SE	95% LCL	95% UCL
LogX50	1.844	1.829	0.007	1.812	1.845
Slope	4.152	6.963	1.252	4.003	9.924
Y0	0.975	0.980	0.017	0.939	1.021

ECx	EC	95% LCL	95% UCL
50	67.42	64.92	70.01
20	60.12	57.96	62.35
10	56.22	53.00	59.64
5	52.85	48.64	57.43
1	46.11	40.13	52.99

	DF	SS	MS	F	P
Total	9	1.74E-01	1.93E-02		
Model	2	1.63E-01	8.13E-02	51.429	0.99993
Error	7	1.11E-02	1.58E-03		

AECOM. 2012. Reproductive success study with brown trout (*Salmo trutta*). Data quality assurance report. Final. December 2012.

Formation Environmental. 2011. Brown Trout Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion. Prepared for J.R. Simplot Company by Formation Environmental. Revised October 2011.

Test Organism: Brown trout (*Salmo trutta*)

Exposure Route: Field collected.

Adult female and male brown trout were collected at three field sites from two streams downstream of the Smokey Canyon mine. In addition, brown trout eggs were obtained from two hatcheries as method controls.

Test duration: Embryo-larval monitoring to 15 days post swim-up.

Study Design: Eggs were collected from 26 ripe female brown trout at three field sites downstream of the Smokey Canyon mine. These included one site on the highly impacted Sage Creek (LSV2C) as well as two sites along Crow Creek (CC-150 and CC-350) downstream of the conflux with Sage Creek. The downstream – most station along Crow Creek (CC-150) was intended to be a field control. Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. Selenium was measured in adult fish (whole body) and in eggs of field collected females. In addition, eggs were collected from 8 ripe females obtained from the Saratoga National Fish Hatchery (SC) to serve as method controls. Similar to field-caught fish, SC hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. As a result of lower than expected hatch rates and fungal contamination in some SC hatchery samples, additional hatchery fish were obtained (as already fertilized eyed embryos) from the Spring Creek Trout Hatchery (SPC), which were divided into four treatments.

Approximately 600 fertilized eggs from each female (or 600 eyed embryos for SPC treatments) were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15-day post swim up feeding trial. Test termination ranged from 83 to 88 days after hatch for all but the Spring Creek Hatchery egg treatments, which occurred 50 days after the arrival of fertilized, eyed embryos from that hatchery.

Endpoints measured in the laboratory study were fecundity, hatch, growth, survival/mortality, and feeding success (growth) post swim up. Larval brown trout were also evaluated for deformities (craniofacial, vertebral, fin) and edema. For this study, deformities were combined and assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Se concentrations in eggs ranged from 6.2-12.8 mg Se/kg dw at CC150, 6.9-14.0 mg Se/kg dw at CC350, and 11.2-40.3 mg Se/kg dw at LSV2C. Se concentrations in hatchery eggs ranged from 0.76-1.2 mg Se/kg dw at the SC hatchery, and were 0.73 mg Se/kg dw at the SPC hatchery. The Se whole body concentration in field collected fish ranged from 7.2-22.6 mg/kg dw at LSV2C, 4.7-8.4 mg/kg dw at CC150, and 5.5-9.2 mg/kg dw at CC350. Se whole body concentrations in SC hatchery fish ranged from 2.5-4.3 mg/kg dw. Hatchery data were combined with field data and included in all analyses.

Three endpoints were considered for purposes of calculating an EC_{10}. These were percent survival, percent fully free from deformities, and percent surviving and normal. Initially, data for these endpoints were combined and analyzed for both portions of the test: hatch through swim up and the 15-day post swim feeding trial. Data for these endpoints over both portions of the test are shown in Tables 1-3.

A U.S. Fish and Wildlife (2012) review of the Formation Environmental (2011) report suggested that fish lost due to an overflow even resulting from a drain the became clogged with food during the 15-day post swim up portion of the test were more likely to have been dead or deformed, and proposed that all treatments that lost fish to the overflow event should be excluded from the EC_{10} calculation. In the 2014 and 2015 draft Se documents, endpoints assessed for the hatch through 15-day post swim up test were analyzed using two scenarios. In the "worst-case" scenarios, the hypothesis from the USFWS review was examined, by treating all fish lost to overflow as either dead or deformed, rather than excluding those treatments altogether. In the "optimistic" scenario, the overflow event was treated as a random technician error unrelated to selenium toxicity, and any lost fish were removed from the calculation. In other words, fish lost to overflow were assumed to be equally likely to have been dead or deformed compared to fish that were not lost.

Because of the importance of these data for the numeric criterion calculation, and because of several experimental factors that resulted in the calculation of several reasonable EC_{10}s, such as the loss of fish due to an overflow event described above, EPA conducted a careful and thorough reanalysis of the study data and subjected the reanalysis to independent, external peer review (ERG 2012) to confirm the validity and scientific robustness of the approach taken by EPA in the reanalysis and use of the reanalyzed data. Those assessments were then superseded by a reanalysis of a more complete enumeration of the deformity counts provided by AECOM (2012). All analyses reported in the 2014 and 2015 draft Se documents and the current Se document used values from the updated dataset provided by AECOM (2012).

Hatch Through 15-Day Post Swim Up Combined Data

In the 2014 and 2015 draft Se documents, data for three endpoints, survival, deformities, and combined survival+deformities were considered for both portions of the test. The first portion of the test was from hatch through swim up, lasting 88 days (on average). The second portion was the 15-day post-swim up

feeding trial. None of the fry from the five treatments with Se concentrations of 26.8 mg/kg and higher reached swim-up. However, surviving fry from those treatments were included in the post-swim up feeding trial.

Combined Survival and Deformity Endpoint

Selenium concentrations and counts of total larvae, and counts of proportions of fully normal larvae (alive and normal) are included in Table 1. Background percentages of live and normal individuals were extremely variable and often low (Figure 1). In the 2014 draft document, EC_{10}s for the optimistic (21.16 mg/kg) and worst case (20.65 mg/kg) scenarios were calculated, and these were also reported in the 2015 draft document. Although there is a clear demarcation between treatments equal to or less than 20.5 µg/L and treatments equal to or greater than 26.8 µg/L, suggesting an effect level between these concentrations, a careful reanalysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} cannot be calculated because of the high background variability.

Table 1. Brown trout selenium concentrations and survival + deformity data (combined endpoint) from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
SC-001	3.6	0.76	63		63	115	8		123	0.512
SC-002	4.1	0.94	72		72	113	4		117	0.615
SC-003	3.7	0.83	131		131	302	7	9	309	0.424
SC-004	4.3	0.92	46		46	140	28		168	0.274
SC-005	3	1.2	23		23	42	6		48	0.479
SC-006	3.1	1.2	457		457	535	8		543	0.842
SC-007	2.7	1	93		93	137	30		167	0.557
SC-008	2.5	0.96	283		283	359	6	10	365	0.775
SPC-001[c]		0.73	427		427	570	8		578	0.739
SPC-002[c]		0.73	371		371	545	20		565	0.657
SPC-005[c]		0.73	400		400	561	8		569	0.703
SPC-006[c]		0.73	427		427	556	17		573	0.745
CC-150-009	8.4	12.8	106		106	142	11		153	0.693
CC-150-011	5.6	8.4	87		87	266	2		268	0.325
CC-150-012	6.7	8.5	156		156	282	12		294	0.531
CC-150-013	5.9	8.4	137		137	310	46	26	356	0.385
CC-150-015	6	9.1	210		210	445	14		459	0.458
CC-150-016	7	7.5	13		13	23	3	43	26	0.500
CC-150-017	5.6	6.6	99		99	163	7	33	170	0.582
CC-150-018	4.7	6.9	195		195	486	16		502	0.388
CC-150-020	7.2	6.2	453		453	558	6		564	0.803
CC-350-006	9.2	14	120		120	386	26		412	0.291
CC-350-007	5.5	6.9	68		68	131	10	20	141	0.482
CC-350-008	8.5	9.5	269		269	338	21	28	359	0.749
LSV2C-002	8.9	12.8	483		483	544	4	16	548	0.881
LSV2C-003	13.8	40.3	2	2	0	0	395		395	0.000
LSV2C-004	17.9	36	16	16	0	0	289		289	0.000
LSV2C-005	13.6	26.8	8	8	0	0	267		267	0.000
LSV2C-008	9.6	17.7	147		147	194	4	45	198	0.742

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
LSV2C-010	22.6	38.8	5	5	0	0	97		97	0.000
LSV2C-012	7.2	13.2	217		217	554	17		571	0.380
LSV2C-016	9.2	13.4	440		440	530	20		550	0.800
LSV2C-017	13.2	20.5	110		110	150	28	19	178	0.618
LSV2C-019	8.6	12.5	267		267	390	22	39	412	0.648
LSV2C-020	11.3	11.2	240		240	296	5	36	301	0.797
LSV2C-021	20	28.1	8	8	0	0	404		404	0.000

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

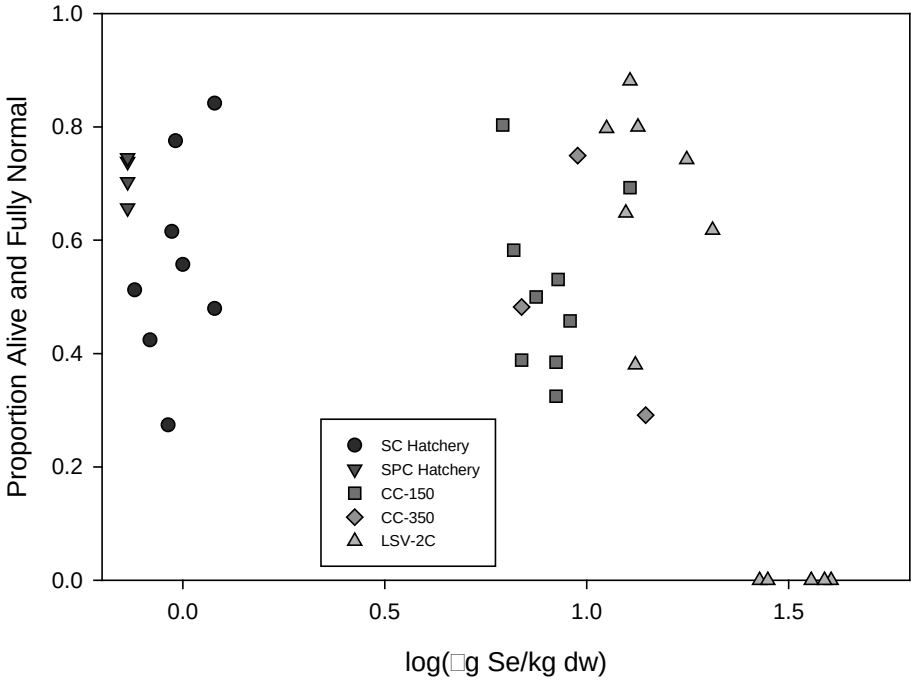


Figure 1. Proportion of alive and normal larvae plotted against Se concentrations in eggs. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

Deformity Endpoint

Selenium concentrations, counts of larvae assessed for deformities, and counts and proportions of normal larvae are included in Table 2. As with the combined endpoint, background (at or below 20.5 mg/kg) proportions of deformities were highly variable (Figure 2). In the 2014 draft document, EC_{10}s were calculated for both the optimistic and worst case scenarios, and the EC_{10} of 15.91 mg/kg for the worst case scenario was used as the EC_{10} for Salmo. During the review phase following the release of the 2014 draft Se document, several public commenters noted that because of the high variability, more than one EC_{10} could be calculated by TRAP for both the optimistic and the worst case scenarios depending on the initial model conditions, in particular the slope of the falling limb of the concentration-response curve. For the optimistic scenario, EC_{10}s based on initial conditions ranged from 16.36-21.95 mg/kg, and for the worst case scenario, EC_{10}s based on initial conditions ranged from 15.91-21.58 mg/kg. In order to evaluate the most appropriate EC_{10} for the deformity endpoints, models were evaluated based on residual sum of squares, and the EC_{10} for the model with the lowest residual sum of squares was selected as the most appropriate. For the worst case scenario deformity endpoint, the model with the lowest residual sum

of squares was the EC_{10}=21.58 mg/kg model, and for the optimistic deformity endpoint, the model with the lowest residual sum of squares was the EC_{10}=21.94 mg/kg model.

These variable EC_{10}s were the result of large variability in background concentration, with several treatments at low Se concentrations experiencing greater than 60% deformities (Figure 2). Although there is clear evidence of an effect between the 20.5 and 26.8 mg/kg concentrations, because of this high background variability, a careful re-analysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} could be calculated for the deformity endpoint.

Some of the background variability in deformities appears to be the result of differences among field sites. For example, deformity rates among field samples appear to be greater for fish hatched from eggs collected in the two Crow Creek sites (CC-150, CC-350) compared to Sage Creek (LSV-2C) (Figure 2). If the result of higher background deformities among Crow Creek sites is not a random artifact, it suggests a confounding factor, unrelated to selenium exposure. Whether the higher deformity rates represent random variation, population differences, other environmental quality differences (unrelated to Se), or methodological issues is unclear**.**

Table 2. Brown trout selenium concentrations and deformity data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
SC-001	3.6	0.76	63	115		0.548
SC-002	4.1	0.94	72	113		0.637
SC-003	3.7	0.83	131	302	9	0.434
SC-004	4.3	0.92	46	140		0.329
SC-005	3	1.2	23	42		0.548
SC-006	3.1	1.2	457	535		0.854
SC-007	2.7	1	93	137		0.679
SC-008	2.5	0.96	283	359	10	0.788
SPC-001[c]		0.73	427	570		0.749
SPC-002[c]		0.73	371	545		0.681
SPC-005[c]		0.73	400	561		0.713
SPC-006[c]		0.73	427	556		0.768
CC-150-009	8.4	12.8	106	142		0.746
CC-150-011	5.6	8.4	87	266		0.327
CC-150-012	6.7	8.5	156	282		0.553
CC-150-013	5.9	8.4	137	310	26	0.442
CC-150-	6	9.1	210	445		0.472

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
015						
CC-150-016	7	7.5	13	23	43	0.565
CC-150-017	5.6	6.6	99	163	33	0.607
CC-150-018	4.7	6.9	195	486		0.401
CC-150-020	7.2	6.2	453	558		0.812
CC-350-006	9.2	14	120	386		0.311
CC-350-007	5.5	6.9	68	131	20	0.519
CC-350-008	8.5	9.5	269	338	28	0.796
LSV2C-002	8.9	12.8	483	544	16	0.888
LSV2C-003	13.8	40.3	2	100		0.020
LSV2C-004	17.9	36	16	142		0.113
LSV2C-005	13.6	26.8	8	149		0.054
LSV2C-008	9.6	17.7	147	194	45	0.758
LSV2C-010	22.6	38.8	5	80		0.063
LSV2C-012	7.2	13.2	217	554		0.392
LSV2C-016	9.2	13.4	440	530		0.830
LSV2C-017	13.2	20.5	110	150	19	0.733
LSV2C-019	8.6	12.5	267	390	39	0.685
LSV2C-020	11.3	11.2	240	296	36	0.811
LSV2C-021	20	28.1	8	172		0.047

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

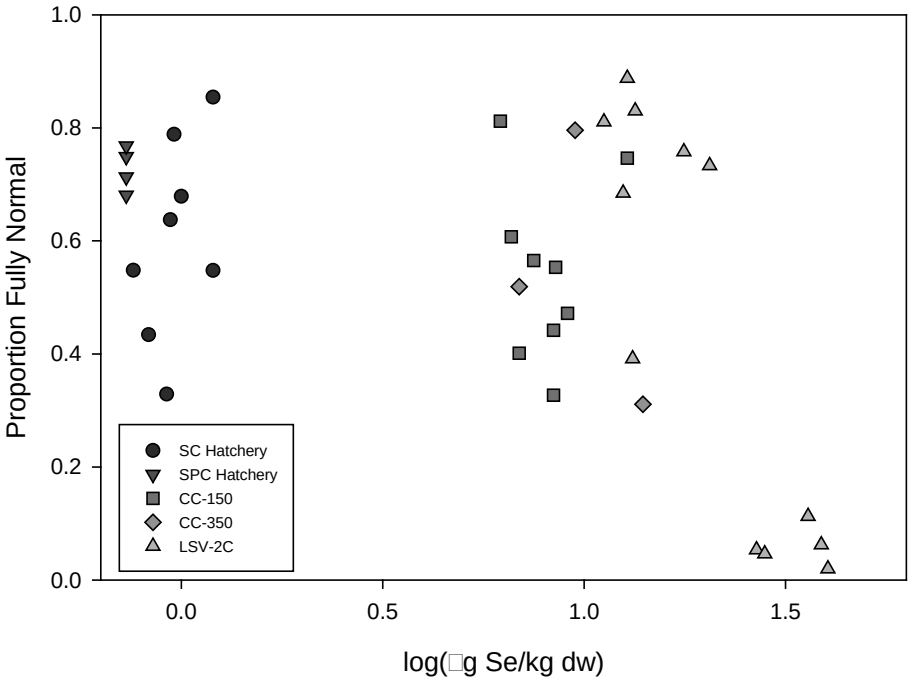


Figure 2. Proportion of normal (free from deformities) larvae plotted against Se concentrations in eggs, hatch through 15-days post swim up. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

Survival Endpoint

Selenium concentrations and estimated counts and proportions of larvae surviving from hatch through 15 days post swim up are included in Table 3. Estimated counts and proportions were reported for survival through the 15-day post swim up test because larvae were thinned to a target of 100 individuals/treatment prior to the onset of the post swim up test, and final full test survival is calculated as the product of survival from hatch to swim up and survival during the 15-day post swim up test. In the 2014 draft document, EC_{10}s were calculated for the worst case (16.78 mg/kg) and optimistic (20.40 mg/kg) survival scenarios, and these were also reported in the 2015 draft document. For both scenarios, the assumption was made that fry that failed to swim up would not have survived, and so the survival for the post swim up portion of the test in the 5 treatments with the highest selenium concentrations (26.8 mg/kg and above) was set to zero. The EC_{10} of 16.78 mg/kg for the optimistic is nearly identical to the EC_{10} for the worst case survival scenario of 16.76 mg/kg presented in the response to the FWS review of the Formation Environmental study (Taulbee et al. 2012), peer reviewed by ERG (2012).

In contrast to the deformity and combined deformity+survival endpoints, background survival (concentrations up to and including 20.5 mg/kg) was much less variable. Despite the lower variability among background effect levels, a careful re-examination of these data following the release of the 2015 draft Se

document determined that a meaningful EC_{10} cannot be calculated by TRAP so long as the assumption is made that fry failing to reach swim up are assumed to be dead. This is because TRAP requires at least 2 partial effects to calculate an EC_{10}, and this dataset has no partial effects, but rather, a background range with high and relatively stable survival through 20.5 mg/kg, and then no survival at concentrations of 26.8 mg/kg and above (Figure 3). In order to calculate an EC_{10} for survival, the assumption regarding fry that failed to swim up was removed. In addition, in order to remove the uncertainty introduced by the clogged drain leading to the overflow and loss of fish from some of the treatments in the post swim up test, the EC_{10} for larval survival was calculated for the much longer hatch through swim up portion of the test, as described below.

Table 3. Brown trout selenium concentrations and survival data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
SC-001	3.6	0.76	144	0.951	0.990	0.942
SC-002	4.1	0.94	138	0.978	0.990	0.968
SC-003	3.7	0.83	340	0.982	0.989	0.971
SC-004	4.3	0.92	189	0.868	0.971	0.842
SC-005	3	1.2	70	0.914	1.000	0.914
SC-006	3.1	1.2	564	0.988	0.990	0.978
SC-007	2.7	1	188	0.856	0.970	0.830
SC-008	2.5	0.96	396	0.985	1.000	0.985
SPC-001[c]		0.73	598	0.987	1.000	0.987
SPC-002[c]		0.73	20	1.000	1.000	1.000
SPC-003[c]		0.73	585	0.966	1.000	0.966
SPC-004[c]		0.73	21	1.000	1.000	1.000
SPC-005[c]		0.73	589	0.986	1.000	0.986
SPC-006[c]		0.73	593	0.971	1.000	0.971
CC-150-009	8.4	12.8	173	0.942	0.990	0.933
CC-150-011	5.6	8.4	288	0.993	1.000	0.993
CC-150-012	6.7	8.5	314	0.965	0.990	0.955
CC-150-013	5.9	8.4	402	0.891	0.973	0.866
CC-150-015	6	9.1	479	0.971	1.000	0.971
CC-150-016	7	7.5	89	0.966	1.000	0.966
CC-150-017	5.6	6.6	223	0.969	1.000	0.969
CC-150-018	4.7	6.9	522	0.969	1.000	0.969
CC-150-020	7.2	6.2	584	0.990	1.000	0.990
CC-350-006	9.2	14	432	0.944	0.980	0.926
CC-350-007	5.5	6.9	181	0.950	0.988	0.938
CC-350-008	8.5	9.5	407	0.951	0.986	0.938
LSV2C-002	8.9	12.8	584	0.993	1.000	0.993
LSV2C-003[d]	13.8	40.3	404	0.079	0.281	0.022
LSV2C-004[d]	17.9	36	309	0.414	0.477	0.197
LSV2C-005[d]	13.6	26.8	287	0.387	0.622	0.240
LSV2C-008	9.6	17.7	263	0.989	0.982	0.971
LSV2C-010[d]	22.6	38.8	108	0.231	0.440	0.102
LSV2C-012	7.2	13.2	591	0.971	1.000	0.971

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
LSV2C-016	9.2	13.4	570	0.965	1.000	0.965
LSV2C-017	13.2	20.5	217	0.885	0.963	0.852
LSV2C-019	8.6	12.5	471	0.953	1.000	0.953
LSV2C-020	11.3	11.2	357	0.986	1.000	0.986
LSV2C-021[d]	20	28.1	424	0.288	0.730	0.210

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.[d] Survived but failed to reach swim up. Assumed dead in all hatch to 15-day post swim up analysis.

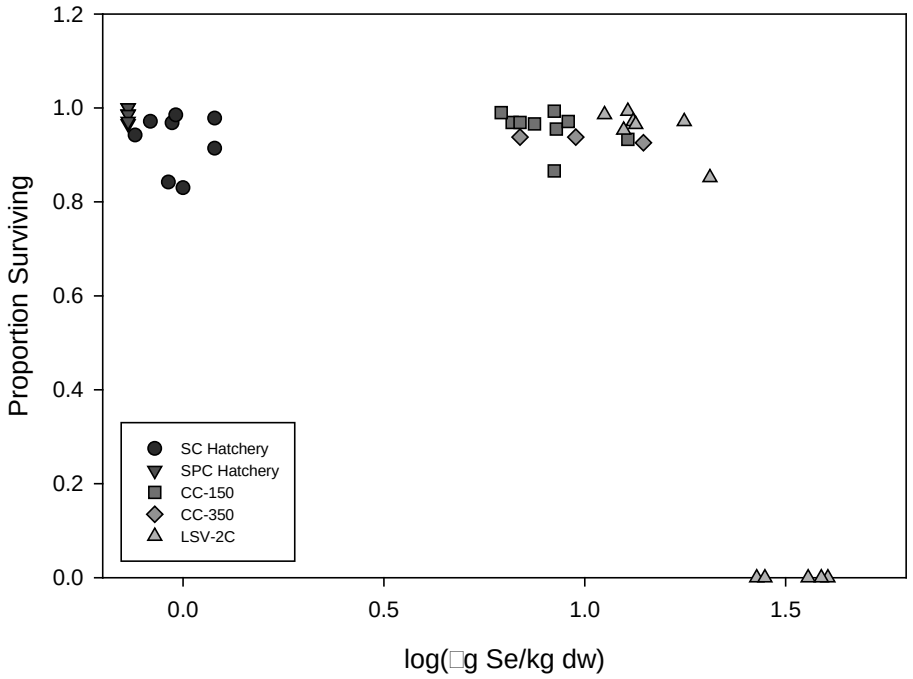


Figure 3. Proportion of larval survival plotted against log transformed Se concentrations in eggs, hatch through 15-day post swim up. Larvae from the five highest Se concentration treatments failed to reach swim up and were assumed to not have survived in the wild.

Assessment of Overflow Loss During 15-day Post Swim Up Feeding Trial

In the 2015 draft Se document, an assessment was made to determine whether the loss of fish from the overflow event during the 15-day post swim up portion of the test was related to survival or to Se treatment concentration measured during the first portion of the test. In this assessment, data were examined from the perspective of whether the overflow loss of brown trout during the second stage of the test could reflect dead, dying, or weak organisms. This was done to examine the hypothesis proposed in the U.S. FWS review that fish lost to overflow were either dead or dying.

First, the relationship between larval survival in the first and second stages of the test (hatch to swim up, 15 days post swim up) were compared for all treatments where larvae successfully reached the swim up stage (Figure 4). Overall, survival in the second stage tracks survival in the first stage (r^2=0.6), but survival in the second stage was noticeably higher in than in the first stage. This result is consistent with the following statement made by the principle scientist of the brown trout study in the public comments to the 2014 selenium draft document submitted for external peer review: "escaped fry were observed swimming in the water bath where the treatment containers were being held. These fry congregated near the treatment cells. Dead or dying fish were not observed."

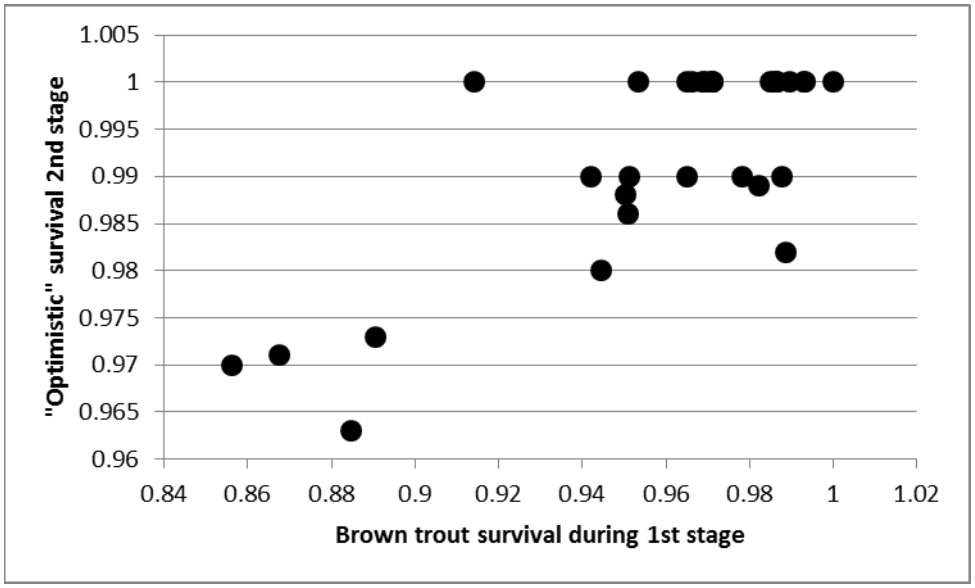


Figure 4. Relationship between survival during the first and second portions of the test. All treatments where larvae successfully reached swim up (Se concentrations of 20.5 mg/kg and lower).

Second, the relationship between larval mortality in the first stage and overflow loss in the second stages of the test (hatch to swim up, 15 days post swim up) were compared separately for all treatments (field and hatchery) and for all field collected treatments (Figure 5). As with figure 4, these correlations were made for treatments where larvae successfully reached the swim up stage. In these instances, there is no apparent relationship between health, as reflected by mortality in the first stage, and overflow loss in the second stage, whether considering all individuals or wild-only: r^2 for both graphs is 0.0. The lack of a relationship in these correlations suggests that overflow loss has a likelihood of being a random noise variable.





Figure 5. Relationship between mortality during the first stage of the test and overflow loss during the second stage of the test. Upper figure – all hatchery and field treatments. Lower figure – field treatments only. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

Finally, the relationship between overflow loss and selenium concentrations in eggs was examined (Figure 6). As with previous correlations, only larvae from treatments where individuals reached swim up were considered.

Figure 6 shows a clear difference between hatchery (far left) and field treatments, but across the concentration range for the offspring of field collected fish there is no apparent relationship between overflow loss and Se concentration. Within the field treatments, the r^2 of the correlation between Se concentration and overflow loss is 0.01. Although there are no known genetic differences between hatchery

and wild fish, if leaving the aquarium required swimming over the rim, one might speculate that previous generations of hatchery fish might have developed a tolerance to remaining in conditions that might seem crowded to wild organisms. (That is, however, purely speculative.) Otherwise, the difference between hatchery and wild fish would seem only to reflect a random artifact, since the Se concentrations at which the wild fish displayed high overflow losses are low.

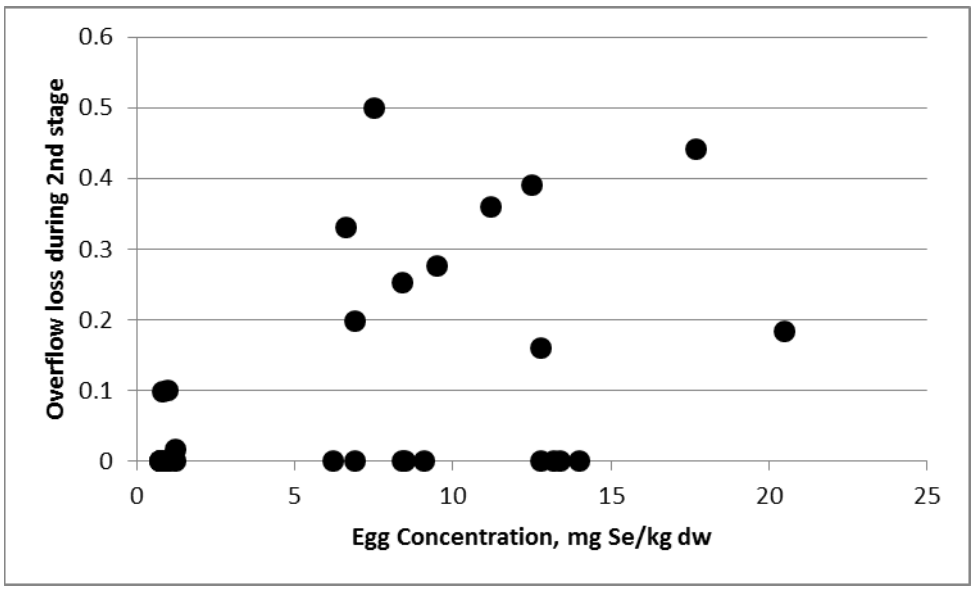


Figure 6. Relationship between egg Se concentration and overflow loss during the second stage of the test. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

In summary, the positive correlation between survival during the hatch to swim up portion of the test and survival during the 15-day post swim up portion of the test, combined with the lack of a correlation between mortality during the hatch to swim up portion of the test and overflow loss during the second stage of the test, suggests that the overflow loss likely represents a random technician error not related to the health of the individuals lost. The relationship between selenium egg concentrations and overflow loss was lower for the larvae hatched from hatchery fish compared to the larvae hatched from field collected fish; however, among field treatments ranging from 6.0-20.5 mg/kg there was no correlation, further supporting the hypothesis that the overflow event was a random occurrence unrelated to the health of larval fish.

The results of the above assessment of the overflow event strongly suggest that the overflow event was a random technician error unrelated to selenium toxicity, and that the "optimistic" scenario is also likely more realistic.

Survival Endpoint – EC_{10} for the first portion of the test

Because larval survival was measured at the end of the first portion of the test (hatch to swim up), an alternative approach to measuring survival would be to

calculate the brown trout EC_{10} for survival for only the first portion of the test. Selenium concentrations and counts of total larvae and larvae that survived the first portion of the test are included in Table 4. The hatch to swim up portion of the test was much longer than the second portion (88 days on average compared to 15 days), and more importantly, it avoids the experimental confound introduced by the loss of fish during the overflow event. With this approach, the second portion of the test would be rejected as inconclusive due to the laboratory accident.

Unlike survival, deformities could not be analyzed for the first portion of the test because of a bias introduced during the thinning process prior to the initiation of the 15-day post swim up portion of the test. During the thinning process, visibly deformed larvae were selectively removed, so that the fish used in the 15-day post swim up test were less likely to have been deformed. Because of this selection bias, only survival could be evaluated from hatch to swim up. Nevertheless, survival appears to be as sensitive an endpoint as deformities or survival+deformities, as all endpoints exhibit background effects (with differing levels of variability) through 20.5 mg/kg, and severe effects at concentrations between 26.8-40.3 mg/kg.

In contrast to survival endpoints measured from hatch through 15 days post swim up, survival for all treatments were included, including larvae from the five treatments of 26.8 mg/kg and higher, where larvae failed to reach swim up. This avoids any potential inconsistency stemming from not knowing whether small percentages of individuals did not swim up in other treatments. In contrast to the previous EC_{10} calculations, this approach is free from all assumptions about individuals lost in the lab accident. In the 2015 draft document, an EC_{10} of 18.09 mg/kg was calculated for this endpoint in TRAP, and this EC_{10} was used as the GMCV for Salmo. During a subsequent review, this EC_{10} was determined to be inappropriate, because it is lower than the 20.5 mg/kg concentration, which with 88.5% survival falls within the variability of the 32 data points at lower concentrations. Compared to the average survival for all 33 background concentration treatments, the survival at 20.5 mg/kg represents an approximately 8% effect.

In order to calculate an EC_{10} that would not fall below the background concentration of 20.5 mg/kg, a weighted least squares linear regression was calculated in TRAP, using a threshold sigmoid model (Figure 7). The model was weighted using the standard deviation of the 33 background concentrations (all concentrations between 0.73-20.5 mg/kg), and the residual standard deviation of the five concentrations between 26.8-40.3 mg/kg. This was done to provide less weight to the more variable, and more uncertain, high Se treatments relative to the less variable background treatments. The EC_{10} for survival using the weighted regression model is 21.0 mg/kg.

One issue with the above TRAP analysis is that to fit the 5 higher effects data well, the EC_0 estimate is pushed down to 16.4 mg/kg, below two of the points in the background range. Also, the fitted curve goes through the data point at 20.5 mg/kg, so that this point is considered to be an EC_8. This is not unreasonable because the response is so steep at concentrations above this point that some effect at this point is plausible. Nevertheless, this point is within the range of the

background and there are insufficient data to say that this concentration is an effect level. Thus, to accept this analysis and use the EC_{10} from this curve requires making a slightly conservative risk management decision that the point at 20.5 mg/kg should be treated as having some effect.

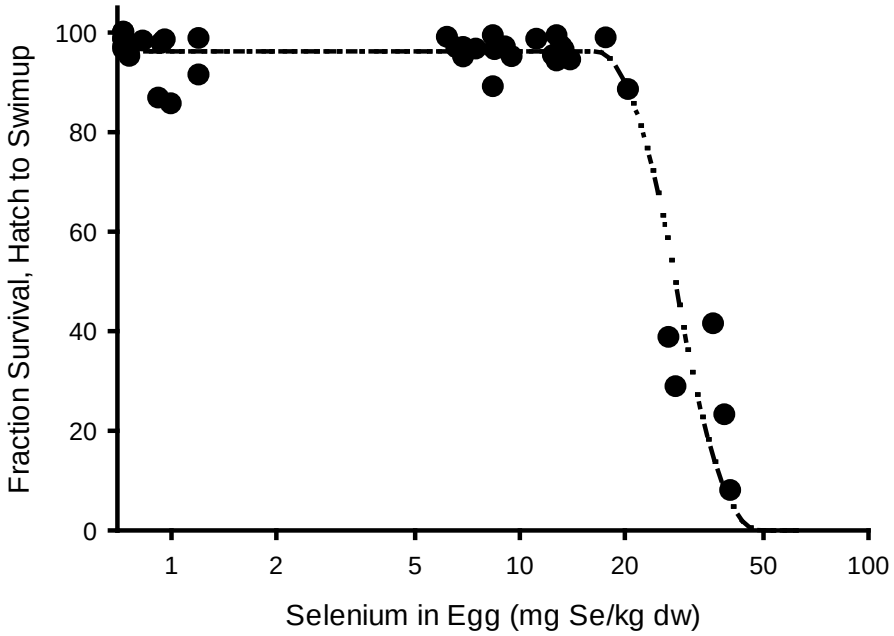


Figure 7. Brown trout survival, hatch to swim up. EC_{10} of 21.0 mg/kg calculated using a weighted nonlinear regression model.

Table 4. Brown trout selenium concentrations and survival data from hatch to swim up (first portion of the test).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SC-001	3.6	0.76	144	137	95.1
SC-002	4.1	0.94	138	135	97.8
SC-003	3.7	0.83	340	334	98.2
SC-004	4.3	0.92	189	164	86.8
SC-005	3	1.2	70	64	91.4
SC-006	3.1	1.2	564	557	98.8
SC-007	2.7	1	188	161	85.6
SC-008	2.5	0.96	396	390	98.5
SPC-001[b]		0.73	598	590	98.7
SPC-002[b]		0.73	20	20	100
SPC-003[b]		0.73	585	565	96.6
SPC-004[b]		0.73	21	21	100
SPC-005[b]		0.73	589	581	98.6

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SPC-006[b]		0.73	593	576	97.1
CC-150-009	8.4	12.8	173	163	94.2
CC-150-011	5.6	8.4	288	286	99.3
CC-150-012	6.7	8.5	314	303	96.5
CC-150-013	5.9	8.4	402	358	89.1
CC-150-015	6	9.1	479	465	97.1
CC-150-016	7	7.5	89	86	96.6
CC-150-017	5.6	6.6	223	216	96.9
CC-150-018	4.7	6.9	522	506	96.9
CC-150-020	7.2	6.2	584	578	99
CC-350-006	9.2	14	432	408	94.4
CC-350-007	5.5	6.9	181	172	95
CC-350-008	8.5	9.5	407	387	95.1
LSV2C-002	8.9	12.8	584	580	99.3
LSV2C-003	13.8	40.3	404	32[c]	7.9
LSV2C-004	17.9	36	309	128[c]	41.4
LSV2C-005	13.6	26.8	287	111[c]	38.7
LSV2C-008	9.6	17.7	263	260	98.9
LSV2C-010	22.6	38.8	108	25[c]	23.1
LSV2C-012	7.2	13.2	591	574	97.1
LSV2C-016	9.2	13.4	570	550	96.5
LSV2C-017	13.2	20.5	217	192	88.5
LSV2C-019	8.6	12.5	471	449	95.3
LSV2C-020	11.3	11.2	357	352	98.6
LSV2C-021	20	28.1	424	122[c]	28.8

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

[c] Survived, but failed to reach swim up.

Whole Body Concentration

The whole-body concentration response curve for survival, hatch to swim up is shown in Figure 8. These data are not amenable to TRAP modeling, and Figure 8 shows the interpolation procedure, the first interpolation point being an EC_0 at 13.2 mg/kg and 96% survival and the second point an LOEC at 13.6 mg/kg and 39% survival. Because the HNOEC (13.2 mg/kg) and LOEC (13.6 mg/kg) are so close, the chronic value for whole body selenium is the HNOEC of 13.2 mg/kg dw.

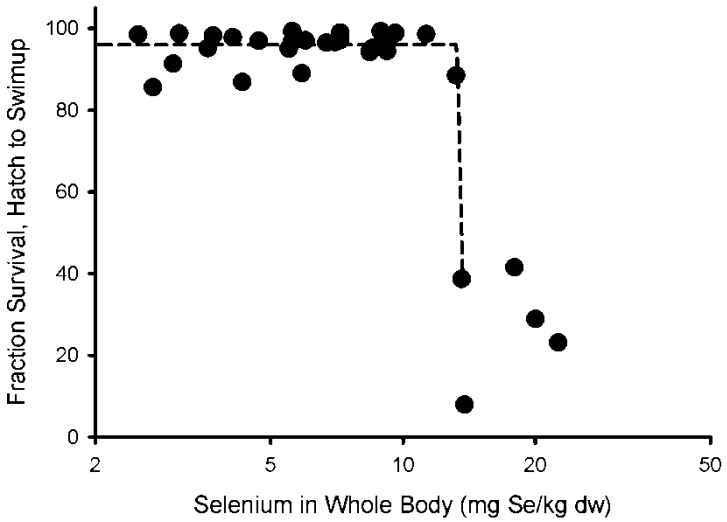


Figure 8. Fraction survival of brown trout larvae as a function of selenium in eggs.

Effect Concentration: For this study the most appropriate, least confounded endpoint is survival, hatch to swim up. For egg selenium, EC_{10} is 21.0 mg Se/kg egg dw, calculated for survival from hatch to swim up using a weighted nonlinear regression model. Expressed as whole body, the chronic value is 13.2 mg Se/kg WB dw.

Besser, J.M., W.G. Brumbaugh, D.M. Papoulias, C.D. Ivey, J.L. Kunz, M. Annis, and C.G. Ingersoll. 2012. Bioaccumulation and toxicity of selenium during a life-cycle exposure with desert pupfish (*Cyprinodon macularius*): U.S. Geological Survey Scientific Investigations Report 2012–5033, 30 p. with appendixes.

Test Organism: Desert pupfish (*Cyprinodon macularius*)

Exposure Route: Dietary and waterborne. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, which had been grown on a diet of selenized yeast.

Test Duration: 180 days life cycle, 21 days F1 larvae, 58 days F1 juveniles and adults.

Study Design: Desert pupfish (*Cyprinodon macularius*), a federally-listed endangered species, were exposed simultaneously to waterborne and dietary selenium at six exposure levels (controls and five selenium treatments) in a three-phase life cycle exposure study. Aqueous exposures were prepared using sodium selenate and sodium selenite salts at an 85%-15% proportion, respectively. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, daily to satiation (25 to 30% rations based on wet weights). Prior to being fed to the pupfish, the oligochaetes were exposed to aqueous selenium and fed selenized yeast at appropriate concentrations to attain the target dietary tissue concentrations. The measured concentrations in water, oligochaetes (pupfish diet), and pupfish tissues for the control and five treatments during the life cycle exposures.

Treatment	water µg/L	oligochaetes mg/kg dw	pupfish, mg/kg dw		
			F_0 WB	eggs	F_1 WB
Control	nd	1.6	0.75	1	1.2
Se–1	3.4	5.1	2.5	3	3.4
Se–2	6.2	7.3	3.4	4.4	3.7
Se–3	14	14	6.7	8	6.7
Se–4	26	24	12	13	12
Se–5	53	52	24	27	31

The 85-day Phase 1 exposure was initiated with approximately five week old juvenile pupfish (F_0). Phase 1 consisted of two separate groups with one group (started two weeks prior to the second group) used for determining survival, growth and whole body selenium concentrations, and the other group used for survival assessment and to provide adults for the main reproduction exposure. Both groups in Phase 1 were similarly exposed to all six treatments, with each treatment having 8 replicates and 10 fish in each replicate.

At the end of the 85-day Phase 1 exposure, the pupfish were reproductively mature and were used for the Phase 2 exposure, the main reproduction study. A preliminary reproduction study was conducted with adults from the first exposure group of F_0 pupfish. These fish were divided into two spawning groups and eggs were collected on four dates during a 9-day period. The main purpose of the preliminary study was to confirm the reproductive maturity of the pupfish, but samples of larvae from this study were used for assessment of deformities. The main reproduction study in Phase 2 was started with adults from the second F_0 exposure. These fish were sorted into spawning groups (1 male and 3 females) in

7-L exposure chambers, with eight replicate spawning groups per selenium treatment. Spawning activity was monitored by removing (and replacing) spawning substrates from each chamber three times a week (Monday-Wednesday-Friday). There were 23 egg collection dates during a 60-day period. All eggs were counted and eggs collected from eight Wednesdays were used for hatching success, deformities and F_1 larval and juvenile growth and survival in the 58-day Phase 3 exposure. Larvae were examined for developmental endpoints including edema, delayed development, and skeletal, eye, craniofacial, and fin deformities.

Effects Data: A summary of the endpoints by each treatment level is shown below.

Table 1. Summary of pupfish toxicity endpoints by exposure treatment (average across all replicates). There were no statistically significant differences across controls and selenium amendment treatments for any of the endpoints shown here (1-way ANOVA, α=0.05).

Endpoint[a]	Control	Se-1	Se-2	Se-3	Se-4	Se-5
F0 survival, day 28	100	100	100	100	100	98
F0 survival, day 56	100	100	100	100	100	100
F0 survival, day 85	100	100	100	100	100	100
F0 survival, day 150	91	94	94	94	91	97
F0 growth, day 28	213	206	204	198	213	203
F0 growth, day 56	535	526	486	469	509	447
F0 growth, day 85	935	998	941	934	914	1053
F0 growth, day 150	1718	1763	1776	1755	1673	1606
F1 survival, day 30	100	100	100	100	98	98
F1 survival, day 58	100	100	93	90	95	88
F1 growth, day 30	73	73	76	78	77	58
F1 growth, day 58	260	264	286	286	288	255
total number eggs	6845	6331	4143	4386	3337	5225
% reduction eggs	NA	8	39	36	51	24
avg % deformities, main	5.3	2.7	4.9	2.4	11.4	8.1
avg % deformities, preliminary	4.4	8.8	11.6	14.3	10.7	21

[a] Endpoint units: survival, %; growth, mg wet weight; % reduction eggs is relative to the control.

The authors observed no significant differences in pupfish survival or growth among treatments. The authors hypothesized the lack of statistically significant acute effects was because the pupfish in this study were near their chronic toxicity threshold, as suggested by the (non-significant) mean reductions in growth (7% in F_0 day 150) and survival (12% in F_1 day 58) in the highest selenium treatment (Se-5), relative to controls (Table 1).

Egg hatching and larval survival in all selenium treatments (not listed in Table 2) were within 10 percent of control means, and differences among treatments were

not related to selenium exposure. The authors noted that the highest selenium treatment, Se-5, did have the lowest larval survival (84%) and lowest combined egg hatching and larval survival (76 percent). The means frequencies of deformities were higher in the two highest Se treatments (Se-4 and Se-5, Table 1); however % deformities across treatment levels were not statistically significant (1-way ANOVA, p=0.13; Beckon et al. (2012). However, overall deformity rates were statistically significantly higher in a preliminary reproduction than in the main reproduction test. Beckon et al. (2012) hypothesized that the reason for the difference in deformity rates between the two tests was related to the time the eggs were collected relative to the time the respective spawning groups were isolated. Eggs were collected in the preliminary reproductive study 1 - 9 days after the spawning groups were isolated, whereas spawns used to characterize deformities in the main reproduction test were collected at least 14 days after the onset of spawning. The larvae produced from the earlier collected eggs may have been exposed to higher selenium concentrations in the egg. The pattern of a gradual decrease in egg selenium concentration over time was observed in the life cycle study.

Egg production varied considerably over the 23 collection dates (Table 2 and Figure 1). Although each of the selenium treatments had a lower total number of eggs relative to the control, one-way ANOVAs of cumulative egg production did not indicate significant differences among treatments on either a per-replicate basis (p=0.34) or on a per-female basis (p=0.20). Similarly, repeated measures ANOVA indicated no differences between treatments, but the authors indicated significant differences among sampling dates and significant interactions of treatment and date. Because of the lower number of eggs in the selenium treatments and the significance of the interaction of treatment and time, the authors concluded that pupfish egg production was adversely affected by elevated selenium exposure and reported significant reductions in egg production at treatment levels Se-2 through Se-5 (4.4 to 27 mg/kg dw Se in eggs). The authors recognized that typically larval survival and deformities are the most sensitive reproductive endpoint for selenium toxicity and not egg production and suggested more study is needed to confirm the unusual sensitivity of pupfish egg production to selenium.

Table 2. Number of pupfish collected on each sampling date throughout the study, by treatment level. Values represent the sum of all eggs collected on a given date for a given Se treatment.

Day	Control	Se-1	Se-2	Se-3	Se-4	Se-5
2	136	112	90	67	122	94
4	275	173	123	142	188	162
7	307	273	301	283	160	432
9	265	252	226	169	271	283
11	401	136	424	319	265	380
14	417	359	333	246	198	401
17	448	456	206	163	145	232
21	303	664	404	204	163	400
23	287	205	141	143	177	175
25	340	308	94	143	150	228
28	366	273	103	101	95	181
30	130	164	104	52	82	132
32	323	304	271	78	75	151
35	320	427	81	150	74	223
37	236	176	41	113	38	38
39	326	151	159	184	113	140
42	507	140	55	193	101	140
44	251	133	66	152	69	137
51	380	359	227	338	305	370
53	278	63	38	197	56	188
56	199	478	138	195	238	222
58	202	329	331	410	143	320
60	148	396	187	344	109	196

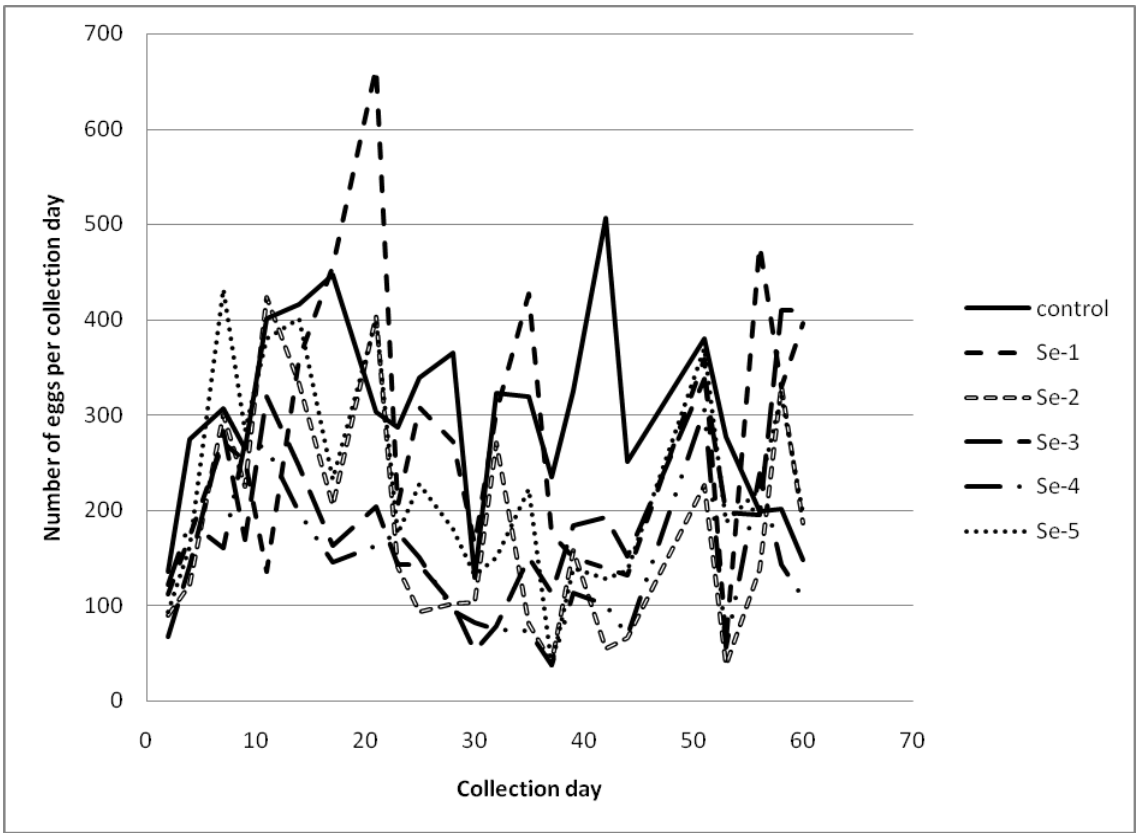


Figure 1. Pupfish egg production by sampling date

Several findings from the pupfish study put a clear demonstration of effect due to selenium in question. The fact that the typical sensitive endpoints for selenium, larval survival and deformities, were not demonstratively responsive to selenium through the highest treatment level, the fact that the egg production data did not show significance among treatments alone, and the fact that egg production increased at the highest selenium treatment level provide sufficient doubt of a clear effect due to selenium. These issues are discussed below.

Examination of the Repeated Measures Analysis:

Analysis Using the Full Dataset: The effects of selenium treatment and sampling date on pupfish egg production (eggs per female per day) were reanalyzed. First, the data were reanalyzed using repeated measures ANOVA. Results of the repeated measures ANOVA analysis were qualitatively similar to those reported in Besser et al. (2012) and are shown in the following table.

Between Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Se treatment	2,202.6	5	440,5	1.755	0.143
Error	10,543.5	42	251.0		

Within Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Sampling Date	1,867.5	22	84.89	4.973	<0.001
Se Treatment x Sampling Date	2,566.3	110	23.33	1.367	0.010
Error	15,771.8	924	17.07		

As with the results reported in Table 7 of Besser et al. (2012), there was no main effect of Se treatment (note – for purposes of these analyses and associated text, "Se treatment" is defined as the control plus the 5 treatments that received Se amendments), but there was a statistically significant ($p \leq 0.05$) effect of sampling date and a significant date by Se treatment interaction. Results were qualitatively similar because the p-values for Se treatment and sampling day were identical in both analyses, yet the p-values for the day by Se treatment interaction term were nearly identical.

A statistically significant sampling date effect means that there were significant differences in overall egg production on different sampling dates. Daily egg production per female ranged from 2.176 on day 2 to a high of 7.294 on day 11, and was variable throughout the study. Of greater interest is the statistically significant day x Se treatment interaction. What this means is, although there was not an overall significant effect of Se treatment on egg production per female, there was a significant Se treatment effect ($p<0.05$) on egg production per female on at least one of the 23 sampling dates.

Analysis after Removal of Control Replicate Outlier: Repeated measures ANOVA analysis confirmed the results reported in Besser et al. (2012). However, as shown on Figure 8b of Besser et al. (2012), one replicate chamber (replicate g) within the control treatment had only one surviving female pupfish from day 7 through the end of the test (day 60), and that replicate also had the highest overall egg production per female of any test chamber. All replicate chambers in all treatments began with three female pupfish, and the replicate described above was the only one with only one surviving female. All three females survived the 60 day test in the majority of the replicate chambers. In order to determine whether the significant date by Se treatment interaction was an artifact of this one test chamber, data were reanalyzed after removing this replicate.

One requirement of repeated measures ANOVA is that the model cannot contain any missing values. An alternative to repeated measures ANOVA when data are missing, and the most commonly followed procedure under these circumstances, is to analyze the data using a mixed model. This was the procedure followed here.

The results of a fully balanced mixed model (no missing data) should be identical to repeated measures ANOVA. As an initial check, the full dataset was reanalyzed as a mixed model. Sample chamber was the random effect parameter, and Se treatment, sampling date, and Se treatment by sampling date were the fixed effect parameters. As expected, the F-ratios for the effects of selenium treatment, sampling date, and the sampling date by Se treatment interaction were identical. Next, the data were reanalyzed after removing data from control replicate g from all sampling dates. Results of this analysis are reported in the table below.

Mixed Model – Fixed Effect

Effect	Numerator df	Denominator df	F-ratio	p-Value
Se Treatment	5	902	1.087	0.366
Sampling Date	22	902	6.042	<0.001
Se Treatment x Sampling Date	110	902	1.310	0.023

T

The statistically significant interaction between Se Treatment and Sampling Date persisted after removal of the potentially anomalous control treatment chamber with one female pupfish. In other words, even after removing the one potentially anomalous control replicate, there were still some individual sampling dates where the effects of Se treatment were statistically significant ($p<0.05$).

Se Treatment x Sampling Date Interaction: When a significant interaction is observed in a repeated measures ANOVA, the next recommended step in the process is to examine each of the repeated measures (sampling dates) separately to identify those dates where the significant difference in Se treatment level occurred. When individual dates for the full dataset (including the replicate with one surviving female) were analyzed separately, there were significant ($p<0.05$) effects of Se treatment level on egg production on days 28, 35, 37, 42, and 53 (1-way ANOVA, $df_{5,42}$). There were no significant Se treatment effects on the remaining 18 sampling dates. ANOVA results are summarized in the table below.

Sampling Date	F-ratio	p-value
28	2.501	0.045
35	2.704	0.033
37	3.351	0.012
42	4.294	0.003
53	3.352	0.012

Because of the large number of comparisons (23 individual ANOVA models for each sampling date), an alpha of 0.05 is inappropriate for this particular analysis. This is because an alpha of $p<0.05$ means that a statistically significant result will be observed 5% of the time due to chance alone (Type I error). In order to control for the increased likelihood of a Type I error when making multiple comparisons, the alpha level of 0.05 was adjusted using Sidak's correction (Abdi 2007). For 23 comparisons and an alpha of 0.05 for one comparison, the adjusted alpha using Sidak's correction is as follows:

$$1 - (1 - 0.05)^{\frac{1}{23}} = 0.0027$$

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As a result, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Each of the 23 sampling dates for the dataset where the replicate chamber from the control treatment with one surviving female pupfish was excluded were also analyzed using one-way ANOVA to determine which sampling dates had significant Se treatment effects. Significant differences among Se treatment levels at alpha 0.05 are shown in the table below.

Sampling Date	F-ratio	p-value
35	2.839	0.027
42	3.164	0.017
53	2.549	0.042

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As with the full dataset, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Summary of Repeated Measures Analysis: This analysis demonstrated that although there was a significant Se treatment by sampling date interaction, regardless of whether or not the control treatment chamber with one female pupfish was excluded, differences among Se treatment levels were only observed for a small subset of the 23 sampling dates. Furthermore, after adjusting alpha to account for multiple comparisons, one-way ANOVA analyses conducted separately for each sampling date to locate the source of the Se Treatment x Sampling Date interaction determined that there were no statistically significant differences among Se treatment levels on any sampling date, precluding the need to perform *post hoc* comparison of means tests to identify significant differences among individual Se treatments.

Combining Effect Metrics Using a Population Model: To improve the certainty of any conclusions to be made about the sensitivity of pupfish to selenium, it is also worthwhile to consider the biological (as opposed to statistical) significance of the observations. But for total egg production, survival, and deformities, the concentration-response curves did not show a sufficient concentration-related effect to calculate an EC10. Nevertheless, because Besser et al. (2012) raised the issue of an interaction of egg production with time, there is a particular concern that there could be a delay in egg production that would reduce population growth rate, even while total numbers of eggs were not significantly affected. This question was evaluated by constructing a population model corresponding to data available from the test.

This modeling approach allows for combining and properly weighting effects on egg production, timing of egg production, and survival. Percent hatch and percent deformities were also considered in alternate calculations. Because the model is only intended for combining the lab data into a unified concentration-response curve, it cannot be interpreted as making real-world population predictions. The relevant data were taken from spreadsheets Besser et al. (2012b and 2012c), which were provided by Besser.

The reproduction and larval endpoints spreadsheet, Besser et al. (2012b), presents egg production at 23 time points. This information thus allows for 23 adult life stages, each assigned its own fecundity. Another page of this spreadsheet provides larval survival data, thus defining survival of the early life stage. The juvenile and adult survival spreadsheet, Besser et al. (2012b), defines a survival rate shared by these life stages.

For each treatment, the data from the test thus provide *all* the needed input for 25 life stages: (1) an embryo-larval stage with its own daily survival probability (along with hatching and deformity percentages, when considered in alternative calculations), (2) a non-reproducing juvenile stage sharing its treatment's daily survival probability with the adult stages, and (3 – 25) 23 short-duration adult stages each with its own egg production, but sharing its treatment's daily survival probability with the treatment's other adult stages. Use of the data is detailed below.

Egg Production: Egg production at the test's 23 observation time points is from the spreadsheet Besser et al. (2012b), expressed as eggs per female per day. The intent of Besser et al. (2012) was for each treatment to have eight replicates, and each replicate was to have one male and three females. Only replicates matching that design were used. Early in the test Control Replicate "g" ended up with only one female, and was therefore not used here. Se-1 Replicate "h" and Se-3 Replicates "d" and "h" had been inadvertently stocked with two males and two females, and were likewise not used here. Table 3 shows the time course of egg production incorporated into the population model. For each treatment, model fecundity, m_i, for life stages i = 3 – 25, is the observed egg production *divided by 2*, in order to provide *female eggs* per female per day.

Percent Hatch: The spreadsheet Besser et al. (2012b) presents percent hatch for eggs collected at selected time points. Within each treatment these were averaged. In selenium reproductive studies percent hatch is often treated as a noise variable unrelated to selenium exposure. Consequently, the population growth calculations were run with and without including percent hatch. When hatch was incorporated into the calculation, daily fecundity was reduced by multiplying by percent hatch.

Deformities: The Besser et al. (2012b) spreadsheet also provides deformity counts for the study's preliminary test and for its main test. Only the main test results were used here. Counts were totaled for each treatment, and a percentage calculated. Population growth calculations were performed both with and without consideration of deformity percentage. For simplicity when considered, a worst case assumption was made that deformed individuals do not contribute to the

population. Percent deformity was thereby handled in manner parallel to percent hatch, by multiplying daily fecundity by percent free of deformity.

Table 3. Life stage durations, and observed eggs per female per day at observation time points for control and selenium treatments, *only with replicates having the design three females and one male*. Model fecundity, m, is set at one-half the observed, to yield female eggs per female.

Repro Study Observation Day	Assigned Life Stage Number	Life Stage Duration	Observed Eggs/Female/Day					
			Control	Se-1	Se-2	Se-3	Se-4	Se-5
-	1	35	-	-	-	-	-	-
-	2	85	-	-	-	-	-	-
2	3	2	2.690	2.571	1.875	1.319	2.542	1.958
4	4	2	5.548	4.048	2.563	2.153	3.917	3.375
7	5	3	4.333	4.302	4.181	3.185	2.222	6.000
9	6	2	5.762	5.524	4.708	3.639	5.646	5.896
11	7	2	8.024	3.238	8.833	4.528	5.521	7.917
14	8	3	6.540	4.905	4.625	2.296	2.750	5.569
17	9	3	6.429	7.143	2.861	1.481	2.014	3.222
21	10	4	3.345	7.881	4.208	1.764	1.698	4.167
23	11	2	5.786	4.643	2.938	3.806	3.688	3.646
25	12	2	6.905	7.286	1.958	2.792	3.125	4.750
28	13	3	4.794	4.317	1.431	1.306	1.319	2.514
30	14	2	1.881	3.881	2.167	1.403	1.708	2.750
32	15	2	5.464	7.286	5.646	1.444	1.563	3.146
35	16	3	4.373	7.310	1.132	2.880	1.028	3.097
37	17	2	5.631	4.417	0.927	1.556	0.792	0.792
39	18	2	6.119	3.917	4.240	3.556	2.354	2.917
42	19	3	7.349	2.222	1.056	2.500	1.403	1.944
44	20	2	4.798	3.274	1.719	3.194	1.438	2.854
51	21	7	1.847	2.139	1.571	2.532	2.060	2.202
53	22	2	6.310	1.512	0.823	5.403	1.333	3.917
56	23	3	3.183	7.317	2.076	2.491	3.528	3.083
58	24	2	3.405	7.810	8.469	9.597	3.104	7.656
60	25	2	3.810	8.226	4.115	6.347	2.271	4.271
Total as $\sum$ (duration · eggs/f/d) =			281.6	294.3	181.9	174.7	142.0	220.1

Larval Survival: The Besser et al. (2012b) spreadsheet also has data for larval survival after 14 and 21 days for eggs collected at three time points. The fraction surviving 21 days was used here. For each treatment, the probability of the early life stage (i=1) surviving each day equals the fraction surviving for 21 days, raised to the 1/21 power: $\sigma_1 = \sigma_L = (\text{21-d Surv})^{1/21}$, shown in Table 4.

Juvenile and Adult Survival: A second spreadsheet, Besser et al. (2012c), has data on juvenile and adult survival after 30 and 58 days. The fraction surviving 58 days was used (Table 4). Parallel to the handling of larval survival, for each treatment the juvenile-adult daily survival probability, σ_{JA} = (58-d Surv)$^{1/58}$, as shown in the table. This value applies to life stages i=2-25 (σ_2 through σ_{25}).

Table 4. Pupfish observed survival and modeled daily survival; fraction hatching and fraction free of deformity.

Treat-ment	Conc	21-d Larval Surv	Larval Daily Surv (σ_L)	58-d Juv+Adlt Surv	Juv+Adlt Daily Surv (σ_{JA})	Fraction Hatch	Fraction Free of Deformity
Control	1	0.9038	0.9952	1.0000	1.0000	0.9023	0.9489
Se-1	3	0.9770	0.9989	1.0000	1.0000	0.9026	0.9727
Se-2	4.4	0.9109	0.9956	0.9250	0.9987	0.8197	0.9563
Se-3	8	0.9600	0.9981	0.9000	0.9982	0.8922	0.9750
Se-4	13	0.9586	0.9980	0.9500	0.9991	0.8988	0.9048
Se-5	27	0.8396	0.9917	0.8750	0.9977	0.9104	0.9174

Formulation of the Population Model: The population growth equation is shown below, in abbreviated form.

$$\begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_t = \begin{bmatrix} \sigma_1(1-\gamma_1) & 0 & \sigma_3 m_3 & \cdots & \sigma_{25} m_{25} \\ \sigma_1\gamma_1 & \sigma_2(1-\gamma_2) & 0 & \cdots & 0 \\ 0 & \sigma_2\gamma_2 & \sigma_3(1-\gamma_3) & \cdots & 0 \\ \vdots & \vdots & \vdots & \cdots & 0 \\ 0 & 0 & 0 & \cdots & \sigma_{25}(1-\gamma_{25}) \end{bmatrix} \begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_{t-1}$$

The diagonal of the 25x25 projection matrix has $\sigma_i(1-\gamma_i)$, the sub-diagonal has $\sigma_i\gamma_i$, and the top row has $\sigma_i m_i$. All other elements are 0. For life stage i, σ_i is the daily survival probability, γ_i is the daily probability of graduating to the next life stage, and m_i is the fecundity expressed as number of female eggs produced per female per day, set at one-half the observed eggs/female/day.

The graduation probability, γ_i, for individuals in each life stage was calculated as follows:

$$\gamma_i = \frac{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - \left(\frac{\sigma_i}{\lambda}\right)^{(Dur_i - 1)}}{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - 1}$$

where λ is the population growth rate and Dur_i is the duration of the life stage. In a 2-day duration life stage, were survival 100% (σ=1) and were the population not growing (λ=1), exactly one half (1/*Dur*) would graduate each day from the 2-day life stage. However, with σ<1 and λ>1, there would be a slight youthful bias

within the life stage, such that slightly more than half would be only 1 day into the life stage and not ready to graduate, and slightly less than half would be in their second day and ready to graduate. The above function adjusts for that.[1] The projected population growth rate for each treatment was calculated as follows. The 25x25 projection matrix was placed on an Excel spreadsheet. Each cell in the diagonal was then modified to subtract the eigenvalue, λ, which represents the population growth rate. That is, each cell in the diagonal was rewritten as $\sigma_i(1-\gamma_i) - \lambda$. The determinant of the 25x25 matrix was then calculated by function MDETERM. To obtain the population growth rate, Excel's Solver was then tasked with finding a value for λ that yielded a value of zero for the matrix determinant. In this case,$-10^{-18} <$ MDETERM $< +10^{-18}$ was deemed sufficiently close to zero. Introducing the constraint to look for λ values between 1.01 and 1.04 was found helpful for Solver to find the dominant eigenvalue. When Solver occasionally could not get the determinant within 10^{-18} of zero, probably due to a solution oscillation that can occur because the input values γ_i are expressed as a function of the solution output λ, digits were removed from Solver's best estimate for λ, to provide a new starting value with which Solver could complete the solution.

Effects on Projected Population Growth Rates: Table 5 and Figure 2 show the model results. Figures 2-B, -C, and -D are almost indistinguishable from Figure 2-A, because hatch and deformity rates varied so little across treatments. Although population growth rates at 4.4 – 27 mg Se/kg are less than at 1 – 3 mg Se/kg, the 6-fold increase in concentration from 4.4 – 27 mg Se/kg yields no change in response. Consequently, the results do not suggest a selenium-related effect, and no EC_{10} can be calculated. Based on the combined influences of egg production and timing, and survival (with or without percentage hatch and deformities), pupfish does not appear to be among the most sensitive species.

[1] The formula for γ is undefined (0/0) under the condition σ=1 and λ=1, so it is not obvious from inspection how it behaves. This function addresses a model artifact that is called numerical dispersion when it occurs in pollutant transport models. It prevents overoptimistic rates of moving through the life stages, particularly in the 35-day and 85-day larval and juvenile stages, and allows a 25-stage model of life duration 180 days to yield precisely the same growth rate as a 180-stage (one day per stage) model, which was also constructed and checked for comparison. However, in this application where absolute growth rates have no particular meaning and only relative differences between treatments are of interest, the function does not change the overall perspective.

Table 5. Model output: daily population growth rates as λ (factor increase) and r (=ln λ), for models that account for survival, fecundity and its timing, and optionally also hatch and/or deformities. Because λ is responding to all the treatment parameters included in the model, its treatment-to-treatment variations do not exactly track the variations in any single input.

Treat-ment	Conc	Factors included in model: All account for survival (σ_L , σ_{JA}) and fecundity (*m*) and its timing -		Hatch		deformity		hatch & deform.	
		λ	r	λ	R	λ	r	λ	r
Control	1	1.0337	0.0332	1.0330	0.0324	1.0334	0.0328	1.0326	0.0321
Se-1	3	1.0346	0.0340	1.0338	0.0333	1.0344	0.0338	1.0336	0.0331
Se-2	4.4	1.0299	0.0294	1.0284	0.0280	1.0295	0.0291	1.0281	0.0277
Se-3	8	1.0285	0.0281	1.0277	0.0273	1.0283	0.0279	1.0275	0.0271
Se-4	13	1.0291	0.0287	1.0283	0.0279	1.0283	0.0279	1.0276	0.0272
Se-5	27	1.0294	0.0290	1.0288	0.0283	1.0288	0.0284	1.0281	0.0277

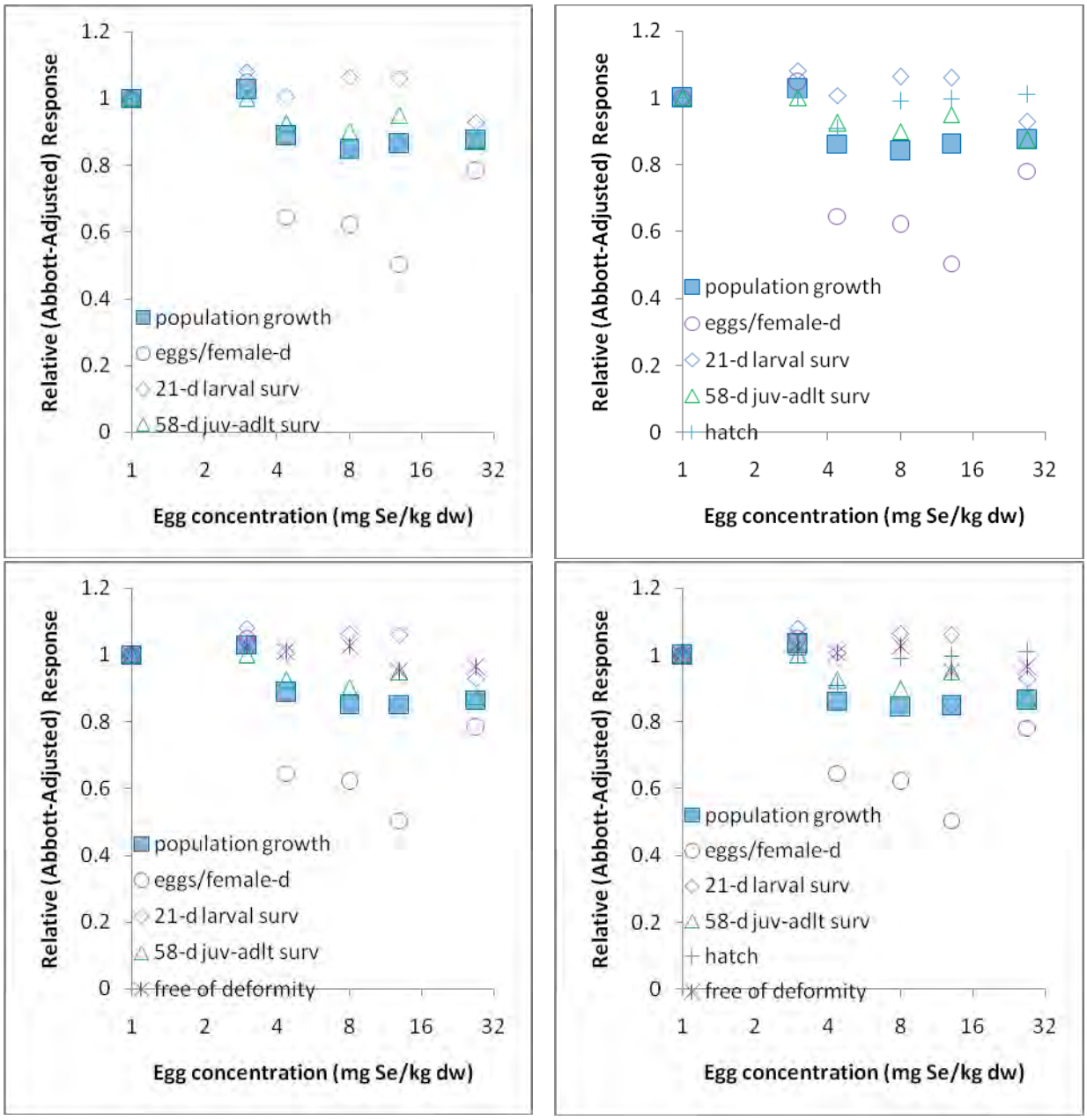


Figure 2. Abbott-adjusted pupfish response as modeled population growth rate (solid-filled symbols) and observed eggs per female per day, larval survival, and juvenile and adult survival (open symbols). Where used in the population model (to modify fecundity), hatch and deformity are shown as open symbols. Some open-symbol points are obscured beneath solid-symbol points. (A) Upper left, egg production and survival only, (B) upper right, adds in influence of percent hatch, (C) lower left, adds in influence of deformities, and (D) lower right, adds in influence of percent hatch and deformities.

Isolating the Influence of Timing of Egg Production: By combining survival with egg production and its timing in the above analysis, the assessment obscures the influence of timing: the issue that was the main reason for undertaking population modeling in the first place. The concern is whether selenium exposure could delay reproduction, thereby yielding reduced population growth. To help isolate the influence on the timing of egg production, two population model runs were performed where all treatments were assigned one of two daily survival rates (0.99 or 0.999) spanning the full range of daily survival rates observed in the 21 and 58 day survival calculations. That is, with survival held constant, the only factors varying across treatments were egg production and timing.

The results are shown in the table below. The Abbott-adjusted results are plotted in Figure 3. Although the relative differences in Figure 3 population growth rates are subdued compared to the wider variation in egg production, this is merely a consequence of the predicted population growth rate being more responsive to survival than to reproduction. It is still apparent that the variations in total egg production are affecting growth rate. The question to be addressed here is whether increasing selenium concentration yields a decline in growth rate beyond the pattern reflecting total egg production.

Population growth rates, as influenced only by differences in egg production and timing					
Treat-ment	**Conc**	**With only egg production (*m*) and its timing variable across treatments**			
		σ=0.999		**σ=0.99**	
		λ	**r**	**λ**	**r**
Control	1	1.0339	0.0334	1.0246	0.0243
Se-1	3	1.0338	0.0333	1.0245	0.0242
Se-2	4.4	1.0310	0.0306	1.0217	0.0215
Se-3	8	1.0293	0.0289	1.0201	0.0199
Se-4	13	1.0293	0.0288	1.0200	0.0198
Se-5	27	1.0324	0.0318	1.0231	0.0228

Inspection of Figure 3 indicates that when survival is assigned a constant value across treatments, the pattern of population growth differences across treatments does not suggest an additional selenium-accentuated factor depressing population growth rate. Population growth at 13 and 27 mg Se/kg is slightly higher than might be expected from total egg production, when compared to lower concentrations. The lack of influence of selenium exposure on timing of egg production is also illustrated by comparing each treatment's cumulative proportion of egg production over the course of the test, as shown in Figure 4. Although the treatments differ somewhat in the temporal pattern of their egg production, there is no consistent relationship with selenium exposure.

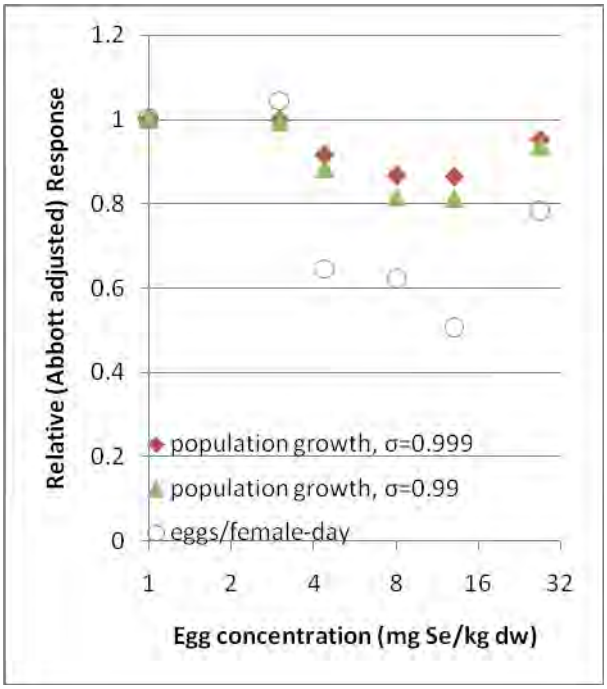


Figure 3. Predicted population growth rate calculated considering differences only in egg production and timing (having assigned uniform survival rates across treatments).

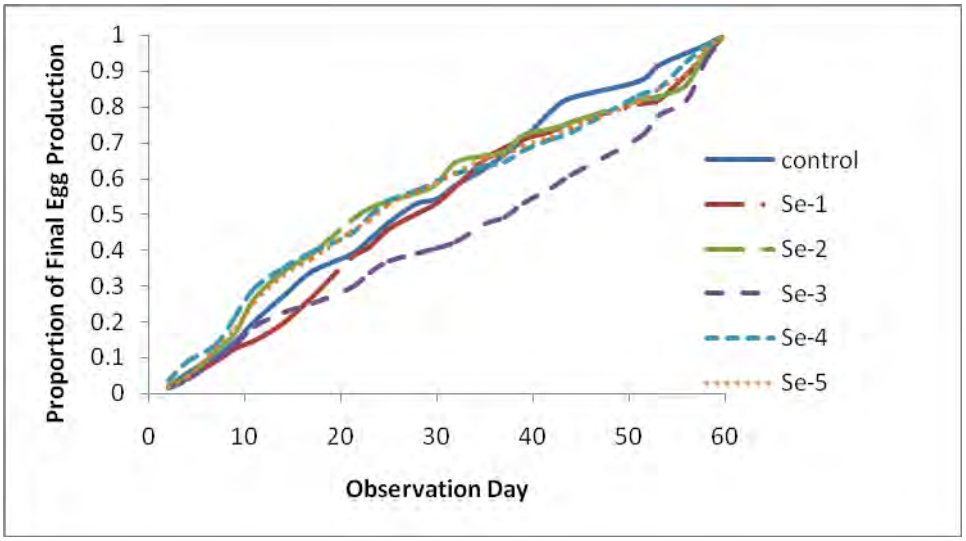


Figure 4. Cumulative pattern of egg production over time. (Control: continuous line. Se-1: dot, dot, long dash. Se-2: long dashes. Se-3: medium dashes. Se-4: short dashes. Se-5: dots.)

Chronic Value: In other selenium studies, egg production and percent hatch have not generally been thought to be related to selenium exposure. Although Besser et al. (2012) noted that repeated measures ANOVA indicated a potential interaction between selenium treatment and egg production on particular sampling dates, a thorough examination of the study data from multiple perspectives indicates no statistically significant or biologically apparent effect of selenium on egg production, timing of egg production, or percent hatch at or below the highest tested concentration of 27 mg Se/kg (dw). Likewise there was no discernible effect on deformity rates.

In the separate tests of F1 larval survival at 21 days and of F1 juvenile-adult survival at 58 days, the highest treatment, 27 mg Se/kg (dw), displayed lower survival than any other treatment. Although the reduction was not sufficient to be statistically significant, Besser et al. (2012) suggest that this is indicative of a threshold. Note that among toxicity tests in general, the 10% effect level of the EC_{10} might or might not be statistically significant from the perspective of hypothesis testing.

Shown below are the survival rates for the 27 mg Se/kg treatment adjusted to the control (Abbott-adjusted), or similarly adjusted to the average survival at all lower treatments (some of which had better survival than the controls). Either way the adjustment is done, results are similar. (These survival data, Abbott-adjusted, are included in Figure 2.)

27 mg Se/kg treatment:	**Larval Surv at 21 days**	**Juv-Adlt Surv at 58 days**
adjusted to control	92.9%	87.5%
adjusted to all lower treatments	89.1%	91.6%

The effect level at 27 mg Se/kg was thus 7% – 13% in the above comparisons. While the concentration response curve is not sufficiently defined to allow confident assignment of an EC_{10}, the data suggest a chronic value in the general neighborhood of 27 mg Se/kg.

An effect level of 27 mg Se/kg egg for the pupfish in this study is consistent with the findings of Saiki et al. (2012a) who evaluated selenium in two related species in the Salton Sea, California. These authors measured 3.09 to 30.4 mg/kg whole body Se levels in mosquitofish and sailfin mollies and based on a lack of a negative relationship with the catch-per-unit-effort deduced these species were not adversely affected by selenium. They extrapolated the finding of selenium tolerance to the pupfish based on the results of another study (Saiki et al 2012b) in which mosquitofish and sailfin mollies accumulated similar levels of selenium to the pupfish. Note: the ratio of selenium in whole body to egg tissues in the pupfish was approximately 1:1 in the Besser study (see first table in the pupfish study summary above).

Staub, B.P. W.A. Hopkins, J. Novak, J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Arch. Environ. Contamin. Toxicol. 46:96-101.

Test Organism: Eastern mosquitofish (*Gambusia holbrooki*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from a contaminated ash basin (ASH) and a reference pond (REF)

Study Design: In July 1999, male eastern mosquitofish were collected from ASH and REF (n=26, n=20, respectively) for measurement of standard metabolic rate (SMR). In July 1999, gravid female eastern mosquitofish were collected from ASH and REF and transported to a laboratory for testing. To ensure all females were fertilized in the field, all offspring used in testing were limited to three weeks after collection. (Eastern mosquitofish are live-bearers with a four week gestation period.) Response variables compared between ASH and REF were (1) SMR of males, (2) brood size of females, (3) percent of live offspring at parturition, and (4) trace element concentration in females and offspring.

Effects Data: SMRs of males, brood size of females, and offspring viability were not significantly different between sites. Average (n=5) concentrations of selenium in females were 11.85 and 0.61 mg/kg dw in ASH and REF sites respectively. The average concentrations of selenium in offspring were 15.87 mg/kg dw and below detection in ASH and REF sites, respectively. The authors point out that the selenium concentrations are an under-estimate of the field levels since the females were allowed to depurate during their time in the laboratory prior to parturition.

Chronic Value: >11.85 mg Se/kg dw whole body

Saiki, M.K., B.A. Martin, and T.M. May. 2004. Reproductive status of western mosquitofish inhabiting selenium-contaminated waters in the grassland water district, Merced County, California. Arch. Environ. Contamin. Toxicol. 47:363-369.

Test Organism: Western mosquitofish (*Gambusia affinis*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from selenium-contaminated sites and reference sites in the San Joaquin River watershed.

Study Design: Western mosquitofish were collected in June and July 2001 from San Luis Drain (SLD) at Gun Club Road (Se-contaminated site), North Mud Slough at Gun Club Road (MSN1; reference site); North Mud Slough at State Highway 140 (MSNs; Se-contaminated site); San Joaquin River at Lander Avenue (SJR; reference site). 20 gravid females from each site were held in the laboratory for two weeks to quantify live and dead births and to make other measurements. Only 17 females from SLD were collected. Live and dead fry were visually examined under low magnification with a binocular microscope for evidence of external abnormalities (teratogenic symptoms such as spinal curvature, missing or deformed fins, eyes and mouths and edema).

Effects Data: The percentage of live births was high at both Se-contaminated sites (96.6 to 99.9%) and reference sites (98.8 to 99.2%). There were no obvious anomalies (e.g., deformities, edema) observed during the study. The concentration of selenium in 4 postpartum females from the site with the highest selenium concentration, SLD, ranged from 13.0 to 17.5 mg Se/kg dw (geometric mean of the high and low is 15.1 mg Se/kg dw. The concentration of selenium of western mosquitofish collected at each site is in Table D-8.

Chronic Value: >15.1 mg Se/kg dw whole body

Table D-8. Selenium in whole body samples of western mosquitofish from study sites

Site	N	[Se], mg/kg dw
SLD	8	18.1
MSN2	24	9.31
MSN1	20	2.72
SJR	22	0.907

Coughlan, D.J. and J.S. Velte. 1989. Dietary toxicity of selenium-contaminated red shiners to striped bass. Trans. Am. Fish Soc. 118:400-408.

Test Organism: Striped bass (*Morone saxitilis*; adults from Lake Norman, NC, approximately 250 g each)

Exposure Route: dietary only
Treated fish were fed selenium contaminated red shiners (1 g) from Belews Lake, NC (9.6 mg Se/kg ww or 38.6 mg Se/kg dw based on a mean reported moisture content of 75.1 percent). Control fish were fed golden shiners from a local bait dealer (0.3 mg Se/kg ww or 1.3 mg Se/kg dw based on a mean reported moisture content of 76.3 percent).

Test Treatments: Test treatments were as described above. Two tanks contained treated fish (n = 20 fish total), and one tank of fish served as the control (n = 10 fish). Each tank received a continuous flow of soft well water (hardness and alkalinity approx. 30 mg/L as $CaCO_3$) throughout the exposure.

Test Duration: 80 days

Study Design: During the experiment, all striped bass (n = 10 per tank) were fed to satiation three times per day. Pre-weighed rations of live red shiners (treated fish) and golden shiners (controls) were added to the tanks and allowed 5 hours to feed. Uneaten prey was removed and weighed. Composite whole-body samples of each prey fish were collected at regular intervals throughout the study for whole-body tissue selenium analysis. The final selenium concentration in epaxial white muscle was determined for surviving striped bass at the end of the test. Moribund striped bass were sacrificed so as to obtain muscle tissue samples for selenium analysis. Samples of liver and trunk kidney of these and the surviving striped bass were dissected for observations of histopathology.

Effects Data: Striped bass fed selenium-laden red shiners exhibited changes in behavior (lethargy, reduced appetite), negligible weight gain, elevated selenium concentrations in muscle, histological damage, and death. Control fish ate and grew well, and behaved normally. Average selenium ingestion was between 60 and 140 Φg Se/fish per day until day 30. Appetite of the treated fish appeared to be significantly reduced beyond this point compared to the appetite of the control group. By day 78, all striped bass fed the Se-laden red shiners either had died or were moribund and sacrificed for analysis. The final selenium concentration in muscle of treated striped bass averaged from 3.5 (tank 1) and 4.0 (tank 2) mg/kg ww, or 16.2 and 18.5 mg/kg dw, respectively, assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species. The final selenium concentration in muscle of control striped bass fed uncontaminated golden shiners averaged 1.1 mg/kg ww, or 5.09 mg/kg dw (assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species).

Chronic Value: The chronic value for percent survival of striped bass relative to final selenium in muscle tissue after being fed Se-laden red shiners is <16.2 mg/kg dw.
An EC_{20} value could not be calculated for this data set because the data did not meet the assumptions required for analysis.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1984. Roxboro Steam Electric Plant 1982 Environmental Monitoring Studies, Volume II, Hyco Reservoir Bioassay Studies. Environmental Technology Section. Carolina Power & Light Company.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* embryos and larvae)

Exposure Route: dietary and waterborne - field exposure
Native adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. No selenium values were given for Hyco Reservoir, total selenium was not detected in the control lake (<1 µg/L). A mean selenium for the ash pond effluent from a previous study was 53 µg/L (N=59; range 35-80 µg/L).

Study Design: All combinations of crosses between the Hyco and control fish were made using gametes from the collected fish. Fertilized eggs were exposed in egg cups to 0, 20 and 50 percent ash pond effluent under flow-through conditions. Percent hatch and swim-up successes were measured. Swim-up larvae were released to exposure tanks where there were fed zooplankton collected from Hyco and the control lake. Larvae were observed for 28 days at which time survival and weight were measured.

Effects Data: Survival to the swim-up stage was different between larvae from Hyco females fertilized with either male type and those larvae from control females fertilized with either male type. All crosses involving a Hyco female resulted in larvae exhibiting 100 percent mortality prior to reaching swim-up. Percent survival from hatch to 28 days for larvae from control females exposed to control water and fed control lake zooplankton was only 5 and 12 percent for the two replicates so no meaningful comparisons can be made to the different dilution exposures or diet exposure. The mean concentrations of selenium in the ovaries, female liver and female muscle were 49, 130, and 84 mg/kg dw, respectively.

Effect level: <49, <130 and <84 mg Se/kg dw in adult ovaries, liver and muscle, respectively

Chronic Value: <49.65 mg Se/kg dw in whole body using the muscle to whole body equation
<84 mg Se/kg dw maternal muscle
<49 mg Se/kg dw ovary

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; 30-day old larvae)

Exposure Route: Dietary and waterborne - field exposed adults
Juvenile bluegill from crosses with females in 0, 20 and 50 percent ash pond effluent were transferred to control water and fed zooplankton from either Hyco or the control lake. Selenium in Hyco and control zooplankton was 45 and 1.9 mg/kg dw, respectively. Duration was not given.

Study Design: Survival and observations on pathology and morphology were made in the two diet treatments.

Effects Data: Mortality in larvae fed control zooplankton was 23.7 percent, whereas mortality in larvae fed Hyco zooplankton was 97.3 percent. There were no differences in survival (for two diet treatments) in larvae that were raised for the 30 days prior to the test in different effluent concentrations (0, 20 50 percent). The average selenium concentrations in the larvae fed control and Hyco zooplankton were 1.9 and 24.7 mg/kg dw, respectively.

Effect level for larval survival: <24.7 mg Se/kg dw in larvae

Chronic Value: None recommended for larval tissue.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1985a. Roxboro Steam Electric Plant Hyco Reservoir 1983 Bioassay Report. Environmental Services Section. Carolina Power & Light Company. September 1985.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; embryos and larvae)

Exposure Route: dietary and waterborne - field exposed
Resident adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. For embryo/larval study up to swim-up stage, control fish were collected from the unaffected portion of Hyco.

Study Design: Repeat of 1982 28-day Embryo/Larval Study. Three crosses between: Hyco female and Hyco male; control female with Hyco male; and control female with control male. Gametes were fertilized and maintained for the 28-day test in ash pond effluent dilutions of 0, 20 and 50 percent. Percent hatch, percent swim-up success and survival were measured to 28 days post hatch. Two treatments were replicated and fed zooplankton collected from Hyco-affected and Hyco-unaffected (control). Larvae were observed for 28 days at which time survival and weight were measured.

Embryo/Larval Study up to Swim-up Stage. Five crosses were made between fish collected from the affected and unaffected areas. Percent hatch, percent swim-up and survival were measured until swim-up (approximately 3-4 days after hatch).

Effects Data: 28-day Embryo/Larval Study. All larvae that hatched from eggs obtained from Hyco females died prior to completing swim-up (see table below).

Effect level (larval survival): <30, <33 and <59 mg Se/kg dw for adult female bluegill in ovaries, liver and muscle, respectively

Summary of 28-day embryo larval study										
% effluent	Parent source in cross M X F	% hatch	% swim-up	% survival, 28-days	Adult tissue, mg Se/kg dw					
					Gonad		Liver		Muscle	
					M	F	M	F	M	F
0	H X H	92	0	0	33	30	43	33	62	59
20	H X H	98	0	0	33	30	43	33	62	59
20	H X H	92	0	0	33	30	43	33	62	59
50	H X H	97	0	0	33	30	43	33	62	59
0	H X C	89	87	18	33	2.2	43	4.4	62	2.7
20	H X C	96	96	34	33	2.2	43	4.4	62	2.7
50	H X C	60	84	58	33	2.2	43	4.4	62	2.7
0	C X C	79	95	40	nd	2.2	37	4.4	27	2.7
20	C X C	90	96	36	nd	2.2	37	4.4	27	2.7
20	C X C	88	97	25	nd	2.2	37	4.4	27	2.7
50	C X C	72	92	42	nd	2.2	37	4.4	27	2.7

Chronic Value: <36.49 mg Se/kg dw in whole-body using the muscle to whole body equation.
<59 mg Se/kg dw muscle
<30 mg Se/kg dw ovary

Embryo/larval study to swim-up. Percent swim-up of larvae from parents collected in non-affected Hyco averaged 93 percent, whereas percent swim-up from larvae collected from affected Hyco was 12 percent. Effect levels were determined for adult female and larval tissues. Larval tissues were averaged across effluent concentrations (geometric mean).

Effect level (percent swim-up):
Adult female ovaries: >9.1 mg/kg dw; <30 mg/kg dw
Adult female liver: >26 mg/kg dw, <33 mg/kg dw
Adult female muscle: >25 mg/kg dw, <59 mg/kg dw
Larvae: >12.8 mg/kg dw; < 165 mg/kg dw

Summary of Embryo/Larval Study up to Swim-up - Affected vs Unaffected Hyco											
date of fert.	Parents' capture location in Hyco	Percent hatch			Percent swim-up			Selenium in tissue, mg/kg dw			
		at % effluent			at % effluent			Adult female			
		0	20	50	0	20	50	Ovary	Liver	Musc	Larvae
6-24	affected	93	98	94	0	0	0	30	33	59	0: 130 20: 120
6-27	affected	99	88	77	0	0	0	30	33	59	0: 130 20: 120
6-28	affected	29	34	35	25	14	3	30	33	59	0: 130 20: 120
6-28	affected	98	86	91	5	0	0	30	33	59	0: 130 20: 120
6-29	affected	88	93	85	59	42	25	30	33	59	0: 130 20: 120
7-14	unaffected	92	80	84	79	92	89	9.1	26	25	0: 19 20: 11 50: 10
7-26	unaffected	99	94	93	100	98	98	9.1	26	25	0: 19 20: 11 50: 10
7-27	unaffected	76	84	86	100	89	91	9.1	26	25	0: 19 20: 11 50: 10

Chronic Value: The chronic value estimated for the percentage larvae reaching the swim-up stage is presented as a range:
>25 mg Se/kg dw (unaffected area) and <59 mg Se/kg dw muscle (affected area)
>30 mg Se/kg dw (unaffected area) and <9.1 mg Se/kg dw ovary (affected area)

Bryson, W.T., K.A. MacPherson, M.A. Mallin, W.E. Partin, and S.E. Woock. 1985b. Roxboro Steam Electric Plant Hyco Reservoir 1984 Bioassay Report. Environmental Services Section. Carolina Power & Light Company

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile- hatchery raised)

Exposure Route: Dietary only

Test Treatments: 5 diets: Se form (nominal selenium concentration in base diet)
seleno-DL-cystine (5 mg/kg)
seleno-DL-cystine (10 mg/kg)
seleno-DL-methionine (5 mg/kg)
sodium selenite (5 mg/kg)
Hyco zooplankton (5 mg/kg)

Test Duration: 60 days

Study Design: Each treatment contained 40 fish which were maintained in a flow-through system. Fish were fed at 3 percent of their body weight. Length and weight were measured on days 30 and 60. Total selenium was measured in liver and whole-body.

Effects Data: No decreased length or weight in any of the Se-diets relative to the control.

Chronic Value: all values are whole-body
seleno-DL-cysteine: >2.16 mg Se/kg dw
seleno-DL-cysteine-2X: >3.74 mg Se/kg dw
seleno-DL-methionine: >2.46 mg Se/kg dw
sodium selenite : >1.21 mg Se/kg dw
Hyco zooplankton: >2.35 mg Se/kg dw

Because none of the selenium-spiked diet formulations affected growth of juvenile fish at the concentrations tested, the chronic value selected for this study is >3.74 mg Se/kg dw for the seleno-DL-cysteine-2X formulation.

Source and Exposure Embryo-Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* Adults from Hyco and a control lake)

Exposure Route: Dietary and waterborne - field exposure

Test Treatments: Four treatments:
Hyco–collected fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.
Control fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.

Test Duration: Adult fish were in spawning tanks 4-7 months

Study Design: Eggs from each treatment were observed for percent hatch and percent swim-up.

Effects Data: Fish collected from the control lake did not spawn. Percent hatch and percent swim-up from Hyco fish in Hyco and control water are given in the table below. The percent hatch and percent swim-up were >83 and >83 for all the Hyco fish suggesting no effect for these endpoints.

Source of parents	Se in parental liver tissue, mg/kg dw	Water type for eggs and larvae	N	Percent hatch	Percent swim-up
Hyco	18.6	Hyco	16	86.6	91.1
Hyco	18.6	well water	10	83.8	95.5
Control	13.8	Hyco	a	a	83.3
Control	13.8	well water	12	86.0	97.4

[a] percent hatch unknown.

Chronic Value: The chronic value for this study is >18.6 mg Se/kg dw liver tissue.

Gillespie, R.B. and P.C. Baumann. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans. Am. Fish. Soc. 115:208-213.

Test Organism: Bluegill sunfish, wild-caught (*Lepomis macrochirus*; adults; embryos and larvae)

Exposure Route: dietary and waterborne - field exposure

Test Treatments: High selenium adult fish were collected (electrofishing and with Fyke nets) from Hyco Reservoir. Low selenium adult fish were collected from Roxboro City Lake, Roxboro, NC.

Study Design: All possible combinations of bluegill parents from Hyco Reservoir and Roxboro City Lake were artificially crossed in June and July, 1982 and 1983, respectively. Fertilization success was assessed by stripping subsamples of 100 to 500 eggs per female and combining them with 2 ml of sperm. All zygotes were reared in Roxboro City Lake water and percent fertilization was estimated 2-3 hours later as the proportion of mitotically active zygotes. To estimate hatching success, gametes were combined as before and subsamples of 100 to 300 embryos per cross were transferred to egg cups and maintained in closed aquaria receiving re-circulated Roxboro City Lake water. Percent hatch (approx. 2d at 22 to 25^{o}C) was based on the number of yolk-sac larvae. In 1982, about 200 embryos from 8 crosses were observed and preserved at intervals up to 40 h after fertilization, and about 450 larvae were preserved at intervals of 40 to 180 h after fertilization. In 1983, about 1,800 larvae were observed and preserved from 40 to 150 hr from crosses involving females from Hyco Reservoir, and about 40-300 hr for crosses involving females from Roxboro City Lake (10 crosses total).

Effects Data: No significant differences were found in percent fertilization or in percent hatch among parent combinations from the 18 crosses made in June 1982 and July 1983. In contrast, larvae from all crosses involving a Hyco female were edematous; 100 percent of the larvae were abnormal in 7 of 8 crosses. Note: This outcome was observed when the same female from Hyco Reservoir was crossed with males from either Hyco Reservoir or Roxboro City Lake. The range of selenium concentrations in the ovaries of Hyco Reservoir females used for the cross experiments was from 5.79 to 8.00 (GM = 6.945 mg/kg ww; n=7). The reported concentrations of selenium in ovaries and carcasses of females collected from Hyco Reservoir in 1982 and 1983 were 6.96 and 5.91 mg/kg ww (n=22 and 28, respectively). The reported concentrations of selenium in ovaries and carcasses of females collected from Roxboro City Lake in 1982 and 1983 were 0.66 and 0.37 mg/kg ww (n=14 and 19, respectively). The mean selenium concentration in bluegill larvae (n=222) from artificial crosses of parents from Hyco Reservoir was 28.20 mg Se/kg dw.

Chronic Value: <46.30 mg Se/kg dw ovary using 85 percent moisture for ovaries measured in study.

Doroshov, S., J. Van Eenennaam, C. Alexander, E. Hallen, H. Bailey, K. Kroll, and C. Restrepo. 1992. Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River; Part II, Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (*Lepomis macrochirus*). Final Report to State Water Resources Control Board, State of California. Contract Number 7-197-250-0.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); Population A: selenium bioaccumulation observations used 113 g (range 30-220 g) obtained from Rainbow Ranch Fish Farm, California. Population B: spawning performance observations used 106 g (range 65-220 g) females and 164 g (range 80-289 g) males obtained from Chico Game Fish Farm.

Exposure Route: Dietary only
Dietary
Seleno-L-methionine added to trout chow; the three nominal dietary concentrations of 8, 18 and 28 mg/kg seleno-L-methionine were measured at 5.5, 13.9, and 21.4 mg/kg Se (moisture content 13 to 16%).

Test Duration: 140 days

Study Design: Population A fish and Population B females were fed nominal dietary treatments 8, 18 and 28 mg/kg seleno-L-methionine; Population B males were fed untreated diets until the start of spawning. Population A fish were sampled on days 0, 30, 58, 86 and 114 for Se measurement. At least 3 females were sampled each event. Fish remaining after day 114 were transferred to an outdoor pond fed untreated diet and sampled on day 144 for depuration analysis.

On day 120 Population B males and females were paired for natural spawning which had limited success. Fish were maintained in treatment tanks and females were monitored for egg ripeness. When ripe, females were induced to ovulate and ova were fertilized *in vitro* with semen stripped from males. Fertilized eggs were sampled for fertilization success, Se content, and two live sub-samples for bioassay, one a 30-day embryo-larval test and another for larval development during first 5 days after hatching.

Larval development: after hatching, 100 larvae were transferred to beakers and samples were examined daily for normal, abnormal and dead were recorded.

Larval bioassay: 90 fertilized eggs from each female were placed in groups of approximately 30 eggs. Larvae and fry were fed rotifers and brine shrimp nauplii through the 30 day observation.

Effects Data: Selenium concentrations in parental tissues for Populations A and B are given in Tables 1 and 2, respectively. Treatment effects were only observed on early development bioassays. In the 5-day larval bioassay, systemic edema and underdeveloped lower jaw were apparent in all larvae in the 28 mg/kg dietary treatment by day 3 and complete mortality by day 5, except for two progenies where 10% of the larvae appeared normal. No abnormalities were observed in control and 8 mg/kg treatment. 3 of the 6 progenies in the 18 mg/kg treatment exhibited 10 to 20% larvae with similar abnormalities (Table 3). The average proportion of larvae with edema were 5% in 18 and 95% in 28 mg/kg, both of

these were statistically different from the control (0% edema).

For analysis of the effect level determination, 4-day edema observations were used (Table 4) rather the 5-day data because the latter were difficult to interpret relative to edema because of almost complete mortality at the highest concentration (although the 4-day and 5-day edema observations were almost identical). Of the 33 edema measurements, only 15 could be used because not all the individual-replicate egg concentrations were reported. Table 4 also shows the treatment averages, which are only slightly different than the 5-day edema data. These averages do not match the average of the individual replicates in this table because they are for all the replicates, not just those with which concentrations could be paired.

The Se egg and edema data from Table 4 are plotted on Figure 1. The individual replicates are analyzed using TRAP. TRAP warns about inadequate partial responses because the partial responses are less than 10% or greater than 90%, and there are no data between 10 and 90%. However, for this dataset, these partial responses at both ends, albeit small, are sufficiently informative based on multiple lines of evidence (e.g., same response on both days 4 and 5, other endpoints that show effects at treatment 18, and several instances of edema at treatment 18 in contrast to absolutely none for many observations at any lower concentration). And because treatment 18 does have an effect of several percent or so, estimating the EC_{10} near these points is defensible; the EC_{10} is 22.6 mg/kg egg. The EC_{10} of 22.6 mg Se/kg egg dw was selected for the chronic value because it was determined using the individual replicates rather than treatment averages as was done in the previous draft document. The EC_{10} of 22.6 mg Se/kg egg is slightly higher than that in the previous draft which used means rather than replicate data (Figure 3).

In the 30-day larval survival bioassay, statistical difference was only in the highest test treatment for survival and growth measurements, length and weight (Table 5). The proportion of abnormal larvae was higher in the selenium-treated diets but was not significantly different from the control. The percent of abnormal larvae in the 18 mg/kg treatment (7.2%) was only slightly higher than the control (6.3%).

Authors present the effect level for bluegill at the 18 mg/kg dietary treatment (NOEC 8 mg/kg) based on proportions of edema and delayed resorption of the yolk sac. The latter endpoint is based on significantly greater yolk area and oil globule area in the 18 and 28 mg/kg treatments.

The most sensitive endpoint, percent edema, as a function of selenium in maternal muscle dw, was fitted to a TRAP tolerance distribution analysis using the individual replicates (Figure 2). The response is steep and the EC_{10} estimate is 15.7 mg/kg. This basically is setting the EC_{10} to the average of the two replicates with nominally 10% edema (15.4 and 16.6 mg/kg), with 90% edema occurring at only a slightly higher concentration (17.3 mg/kg).

Chronic Value: EC_{10} value (edema) at 22.6 mg Se/kg egg dw or 15.7 mg Se/kg muscle dw Chronic Value is 22.6 mg Se/kg eggs dw.

Table 1. Selenium Concentrations (mg/kg dw) in Bluegills from Population A Day 113 of				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Ovary	2.17 (0.05)	10.89 (1.83)	26.17 (0.07)	40.32 (2.44)
Female liver	2.51 (0.32)	NA	22.75 (2.96)	40.68 (2.14)
Testis	2.65 (0.21)	9.87	16.38 (0.71)	29.70 (5.02)
Male liver	4.10 (0.37)	14.32	24.28 (4.54)	52.47 (5.23)

Table 2. Selenium Concentrations (mg/kg dw) in Bluegill Parents (Population B) Used in Larval Toxicity Tests				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Male liver	4.07 (0.23)	6.94 (1.58)	20.46 (3.46)	31.63 (1.75)
Testis	1.87 (0.11)	3.64 (0.47)	9.96 (0.45)	15.25 (0.45)
Female liver	4.00 (0.26)	12.33 (1.09)	25.98 (4.28)	47.60 (4.11)
Female muscle	**1.47 (0.14)**	**5.80 (0.79)**	**10.41 (2.02)**	**23.64 (2.04)**
Ovary	2.23 (0.11)	6.34 (0.47)	14.10 (2.62)	30.63 (3.23)
Eggs	**2.81 (0.14)**	**8.33 (0.63)**	**19.46 (3.83)**	**38.39 (3.14)**
Larvae	NA	NA	NA	35.30 (4.16)
Fry	1.48 (0.11)	1.25 (0.02)	1.37 (0.06)	1.46 (0.03)

Table 3. 5-day Larval Development Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Free of Edema, %	100	100	95 (2)*	4.3(2.7)*

Table 4. 4-day Edema Observations by Replicate from 5-day Larval Toxicity Test			
Treatment/Replicate ID	**Se egg, mg/kg dw**	**Se muscle, mg/kg dw**	**Percent edema (n=10)**
08-2C	3.54	2.25	0
18-4C	3.25	0.95	0
8-1S	11.49	7.07	0
8-2S	8.31	5.80	0
8-6S	6.18	1.41	0
18-1S	8.55	2.75	0
18-3S	22.06	15.44	10
18-6S	30.20	16.58	10
28-1S	44.02	NA	100
28-2S	36.31	31.10	100
28-3S	25.21	17.28	90
28-4S	52.18	27.40	100
28-5S	42.40	24.00	100
28-6S	38.47	24.66	100
28-7S	30.12	17.42	90
Treatment	**Se egg, mg/kg dw treatment avg**		**Percent edema treatment avg**
C	2.81	--	0 (n=140)
8	8.33	--	0 (n=50)
18	19.5	--	6.67 (n=60)
28	38.4	--	97.1 (n=70)

Table 5. Results from 30-day Embryo-larval Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Larval survival, %	71 (8.5)	51.9 (26.5)	64.4 (3.4)	2.5 (3.5)*
Larval length, mm	19.1 (1.2)	19.9 (1.2)	19.3 (0.8)	16.6 (2.5)*
Larval weight, mg	114 (24)	133 (27)	119 (16)	81 (37)*
Abnormalities in larvae, %	6.3 (7.9)	15.0 (5.8)	7.2 (3.1)	25.0 (43.3)

* Statistically significantly different from control

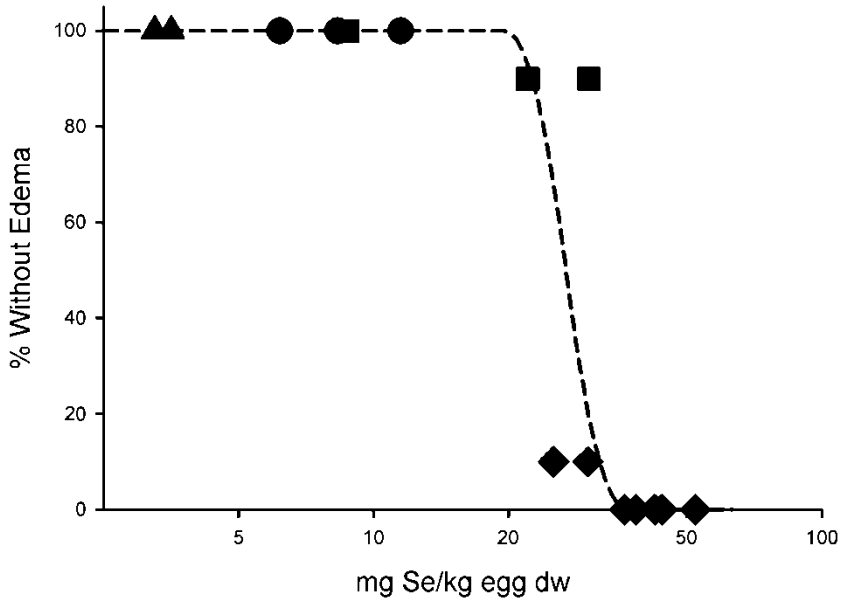


Figure 1. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

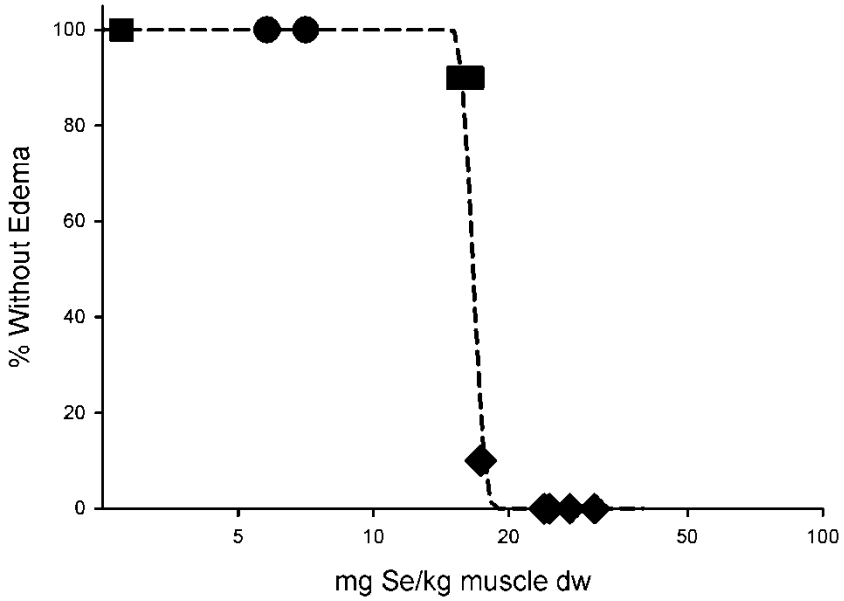
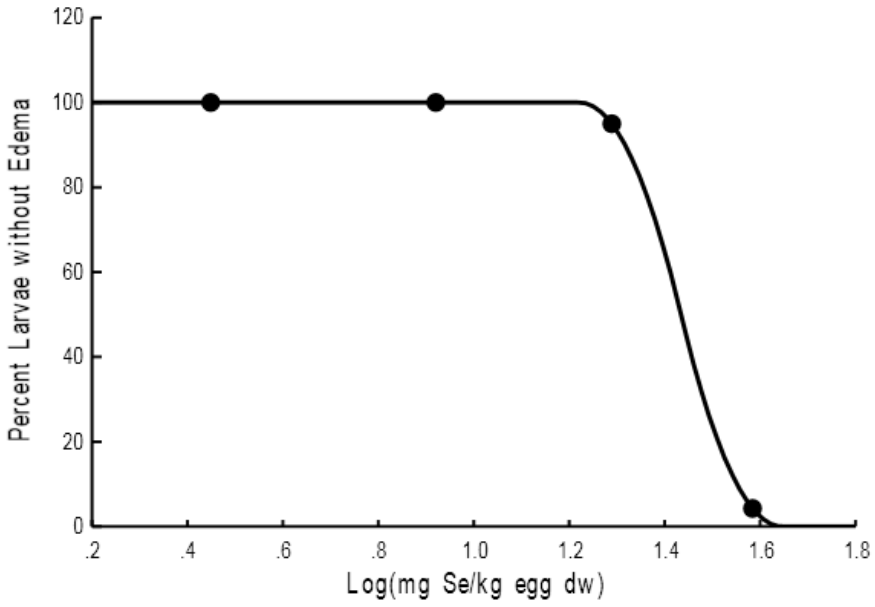


Figure 2. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in maternal muscle. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

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Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4428	1.4342	0.0000	1.4342	1.4342
S	5.452	4.712	0.000	4.712	4.712
Y0	98.33	100.00	0.00	100.00	100.00

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	27.18		
20.0	22.71		
10.0	20.75		
5.0	19.460		

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Figure 3. (From previous draft document) TRAP analysis of bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs.

Hermanutz et al. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ. Tox. & Chem. 11: 217-224

Hermanutz et al. 1996. Exposure of bluegill (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. EPA Report. Mid-Continent Ecology Division. Duluth, MN.

Tao, J., P. Kellar and W. Warren-Hicks. 1999. Statistical Analysis of Selenium Toxicity Data. Report submitted for U.S. EPA, Health and Ecological Criteria Div. The Cadmus Group.

Test Organism: Bluegill (*Lepomis macrochirus*; 3 to 4-year old adults)

Exposure Route: Dietary and waterborne followed by dietary only

Dietary and waterborne

Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Dietary only

Recovering streams exposed bluegill to selenium in prey organisms. Selenite addition to water was ceased (selenium in water was below detection level).

Study Design: Eight Monticello artificial streams were used for three separate studies between 1987 and 1990.

Table 1. Study Design.

Stream	**Study I**	**Study II**	**Study III**
Dates **BG[a] put in station 0-2** **BG transferred to sta. 6 End of study**	 **9-1-87** **5-16-88** **8-22-88**	 **10-88** **5-89** **8-89**	 **11-89** **5-90** **7-90**
1	Unused	Control	Control
2	Unused	2.5 µg/L	Recovering
3	10 µg/L	10 µg/L	Recovering
4	30 µg/L	Recovering	Recovering
5	Control	Control	Control
6	30 µg/L	Recovering	Recovering
7	Control	2.5 µg/L	Recovering
8	10 µg/L	10 µg/L	Recovering

[a] BG = Bluegill

The design of the three Hermanutz et al. studies is included in Table 1 and a schematic diagram of an artificial stream is provided below (Figure 1). For each study, a random sample of 22-50 adult bluegill were transferred from stations 0-2 (provided temperatures above 4°C during winter) to station 6 (most suitable for nests) during mid-May for spawning. Spawning activity was monitored in the streams. Embryo and larval observations were made *in situ* and in the laboratory from fertilized eggs taken from the streams and incubated in the lab.

Figure 1. Schematic Design of One of the Artificial Streams in the Monticello Study

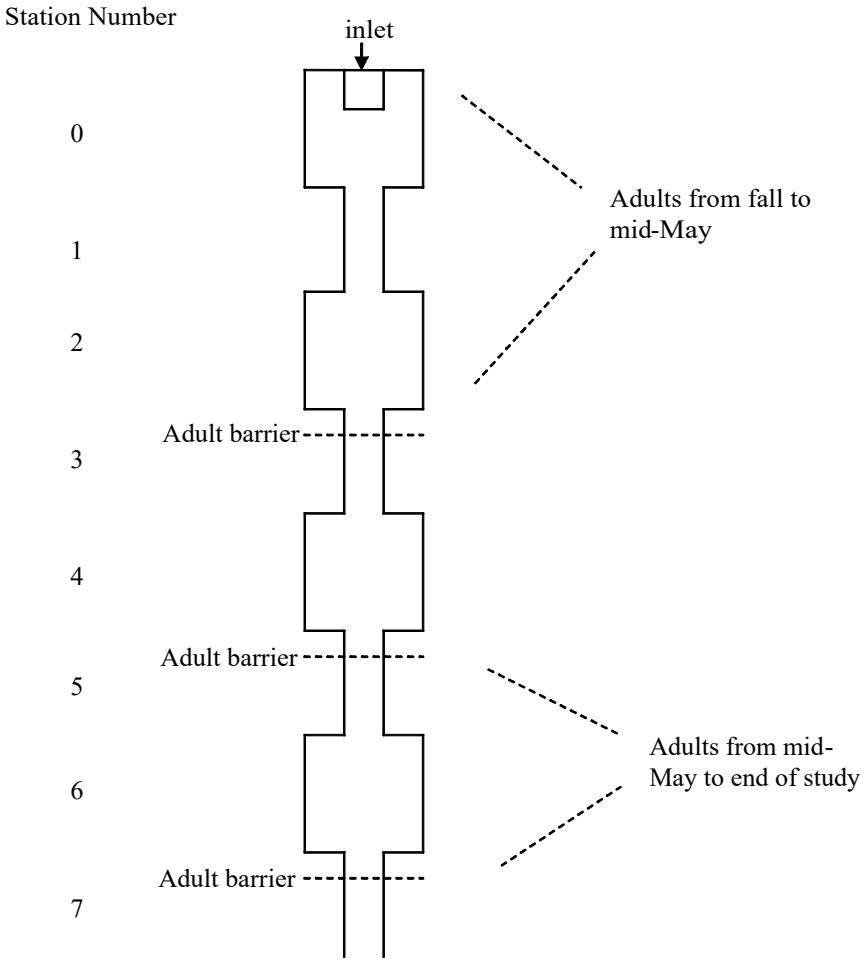


Table 2. Effects on Progeny - Study I[a]

Egg cup observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	% hatch mean ± SD	% survival to 4th day mean ± SD	% edema mean ± SD	% lordosis mean ± SD	% hemorr mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	93.3 ± 9.1	69.7 ± 13.9	0.1 ± 0.2	1.8 ± 2.6	0.1 ± 0.3
control	7	0.47	0.01	0.07	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	71.5 ± 22.5	28.8 ± 23.1	80 ± 1.0	11.6 ± 15.9	28.5 ± 40.6
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L	4	3.71	NA	3.71	15.46	15.46	60.3 ± 25.8	9.1 ± 12.9	50.3 ± 64.1	6.3 ± 1.8	26.8 ± 20.2

Nest observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	# active nests mean ± SD	# embryos Collected mean ± SD	% dead Embryos mean ± SD	# larvae Collected mean ± SD	% dead Larvae mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	6.5 ± 2.1	1441 ± 205	0.9 ± 0.03	3947 ± 1888	3.0 ± 1.1
control	7	0.47	**NA**	**0.47**	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	5.0 ± 4.2	1282 ± 457	3.2 ± 2.9	1169 ± 1093	17.0 ± 21.3
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L [c]	4	3.71	NA	3.71	15.46	15.46	1.0 ± 1.4	361 ± 510	0.4	157 ± 222	12.1

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Hermanutz et al (1992).

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

[c] No active nests, embryos, or larvae found in one of the 30 µg/L streams. Therefore, N = 1 for % dead embryos and dead larvae in the 30 µg/L treatment

Table 3. Effects on Progeny - Study II[a]

Egg cup observations												
treatment	stream	No. of trials	% hatch	% survival to 3rd day	% edema	% lordosis	% hemorr	% healthy[b]	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
									Early	Final	Geometric Mean	
control	1	6	93.0	75.2	0	0	0	97.8	1.02	0.78	0.89	3.72
control	5	5	96.4	71.5	0	0	0	97.9	1.09	0.76	0.91	3.79
2.5 µg/L	2	0	NA	NA	NA	NA	NA	NA		1.82	1.82	7.58
2.5 µg/L	7	4	81.4	71.6	0	0	3.6	92.2	2.02	3.36	2.61	10.86
10 µg/L	3	3	83.3	57.7	100	11.1	49.3	0		8.1	8.10	33.75
10 µg/L	8	2	91.1	57.1	100	18.2	41.1	0	6.96	12.6	9.36	39.02
rec 30 µg/L	4	0	NA	NA	NA	NA	NA	NA				
rec 30 µg/L	6	6	92.9	73.0	17.4	0	11.5	70.7	5.87	13.2	8.80	36.68

Nest Observations														
Treatment	Stream	# active Nests	# embryos Collected	% dead embryos	# larvae collected	% dead larvae	#samples w larvae	% edema	% lordosis	% hemorr	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
											Early	Final	Geometric Mean	
control	1	6	2458	0.94	3252	0.03	7	0	0	0	1.02	0.78	0.89	3.72
control	5	9	1329	0	3435	1.05	13	0	0	0	1.09	0.76	0.91	3.79
2.5 µg/L	2	1	0		2497	0.20	3	4.1	25	77.6		1.82	1.82	7.58
2.5 µg/L	7	5	1462	0	4717	0.08	8	0	0	52	2.02	3.36	2.61	10.86
10 µg/L	3	2	672	0	5376	0.50	9	81.4	5.0	55.5		8.1	8.10	33.75
10 µg/L	8	3	931	0.32	750	0.40	4	50	14.7	26.7	6.96	12.6	9.36	39.02
R 30 µg/L	4	0	NA	NA	NA	NA	NA	NA	NA	NA				
R 30 µg/L	6	8	646	0	6782	7.8	16	27.3	0	17.1	5.87	13.2	8.80	36.68

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Tao et al. (1999).

[b] Among live larvae that survived up to third day after first larvae hatched; assumes the observations of multiple abnormality types always co-occurred in the same organism. This may overestimate the actual % healthy when this assumption is violated.

[c] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Table 4. Effects on Progeny - Study III[a]

Egg cup observations									
treatment	**Stream**	**number of trials**	**% hatch**	**% survival to 3rd day**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	92	58.6	0	0	0	1.2	5.0
control	5	3	76.7	69.2	0	0.9	0.8	0.93	3.88
R 2.5 µg/L	2	3	87.3	66	0	0	0	1.84	7.67
R 2.5 µg/L	7	6	87.2	76.5	0	0	0	1.97	8.21
R 10 µg/L	3							6.25	26.04
R 10 µg/L	8	3	75.3	74.5	0	0	0	2.44	10.17
R 30 µg/L	4	5	92	78				3.82	15.92
R 30 µg/L	6								

Nest observations								
treatment	**stream**	**# active nests**	**# samples with larvae**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	5	0	0	0	1.2	5.0
control	5	2	3	0	0	0	0.93	3.88
R 2.5 µg/L	2	5	5	0	0	0	1.84	7.67
R 2.5 µg/L	7	5	2	0	0	0	1.97	8.21
R 10 µg/L	3	2	4	0	0	0	6.25	26.04
R 10 µg/L	8	4	4	0	0	0	2.44	10.17
R 30 µg/L	4	9	13	0	0	0	3.82	15.92
R 30 µg/L	6							

[a] The NOAEC for the study are from recovering 30 µg Se/L treatment.

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Effects Data: Tables 2 through 4 include exposure and effects data for Study I, II, and III, respectively. Study I & II deformity and survival data reported in the tables above from the nest and egg in response to Se concentrations in parental ovaries (mg/kg dw) were compiled in Table 5 for TRAP analysis. Study I effects data were obtained from Hermanutz et al. (1992), and corresponding Study I ovary Se concentrations were obtained from Hermanutz et al. (1996). Study II and III exposure data were obtained from Hermanutz et al. (1996) and effects data from Tao et al. (1999).

In this study ovary concentrations were measured in an aliquot of females taken from each treatment. The exposure and effects data are thus not as directly linked as they would be in field studies of more recent design – where offspring health can be directly linked to measured tissue concentrations of their female parent.

In a change from the analyses published in drafts of this criterion document, ovary, muscle, and whole-body concentrations measured too early in the exposure period (that is, during the month of May, and labeled "early" in Tables 2 and 3) have not been used because they were not sufficiently co-occurrent with the effects measurements. On the other hand, the data for the Study II recovering stream and all Study III recovering streams *are* included in the analyses. For this analysis, the nest data continue not to be used, because they were less consistent than the egg-cup data.

EC_{10}s are based on the combined effects on survival and deformities: that is, reduction in the percentage of individuals surviving and normal. Table 5 shows the exposure and effects data used. Figures 2 and 3 show the ovary, and whole-body concentration-response curves and an explanation of how the EC_{10} values were derived. The same approach was used for the muscle data, that is, an interpolation using a nonlinear regression threshold sigmoid equation. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 11.2 mg/kg muscle and the average background survival/normal of 69.1% and the second point set to the LOEC of 21.0 mg/kg and a survival/normal of 5.8%. The resulting EC_{10} is 13.4 mg/kg muscle dw. The EC_{10} estimates for the three tissues (below) are slightly different than the EC_{10} values in the previous draft document. The reason for the difference is the use of the interpolation method in the current version rather than an inappropriate usage of a TRAP model in the previous document.

Chronic Value: This study's chronic values for bluegill based on percentage surviving and free of deformities are the following EC_{10} values:
Ovary: 14.7 mg Se/kg ovary dw
Muscle: 13.4 mg Se/kg muscle dw
Whole body: 10.6 mg Se/kg WB dw.

Table 5. Final Exposure Concentrations and Egg Cup Survival and Deformity Rates Used for TRAP Analysis (Studies I, II, & III). The percent deformity is the maximum percentage of the individual deformity types for each treatment.

		Tissue concentration at end of exposure (dw)			Effects data from Hermanutz et al. (1996) and Tao et al. (1999)		
Study	**Treatment (µg/L)**	**Se ovary (mg/kg)**	**Se muscle (mg/kg)**	**Se WB (mg/kg)**	**% Survival**	**% Deformity**	**%Normal +Surviving**
I	Control	2.21	2.05	1.546	69.7	1.8	68.4
I	10	16.73	21.03	18.131	28.8	80	5.76
I	30	>25[1]	No data	No data	9.1	50.3	4.52
II	Control	3.25	1.96	1.63	75.2	0	75.2
II	Control	3.17	2.61	1.47	71.5	0	71.5
II	2.5	7.58	6.73	5.40	No data	No data	No data
II	2.5	14	7.13	4.40	71.6	3.6	69
II	10	33.75	36.51	16.47	57.7	100	0
II	10	52.5	55.25	26.79	57.1	100	0
II	R-30	55	39.78	24.29	79	17.4	65.3
III	Control	5.0	3.37	1.27	62.9	0	62.9
III	Control	3.88	3.11	2.66	68	0	68
III	R-2.5	7.67	5.78	4.17	71.3	0	71.3
III	R-2.5	8.21	6.48	4.25	72.2	0	72.2
III	R-10	10.17	11.20	9.29	63.4	0	63.4
III	R-30	15.92	15.12	13.77	81.1	No data	No data

[1] No data were recorded for this treatment, but a value 50% higher than the 10 µg/L treatment was added for inclusion in the analysis.

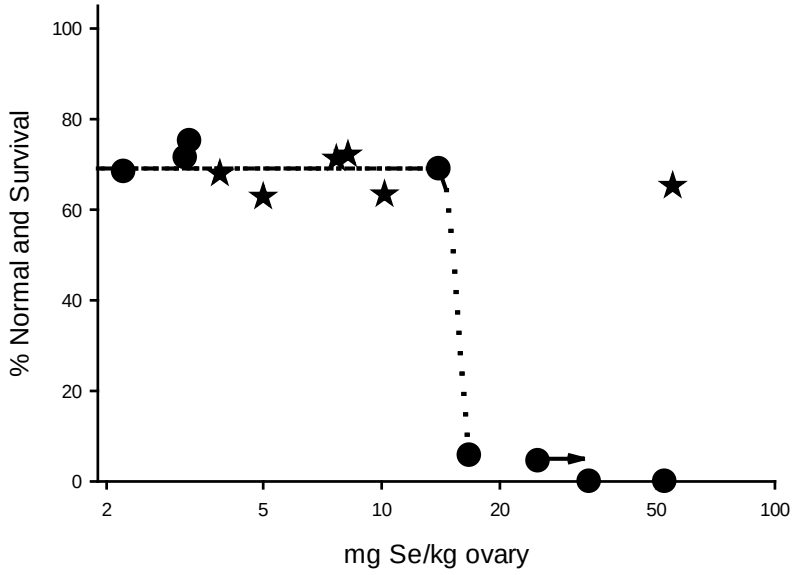


Figure 2. TRAP interpolation curve for the Table 5 ovary data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 14.0 mg/kg and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.7 mg/kg and a survival/normal of 5.76%. The resulting EC_{10} is 14.7 mg/kg ovary dw.

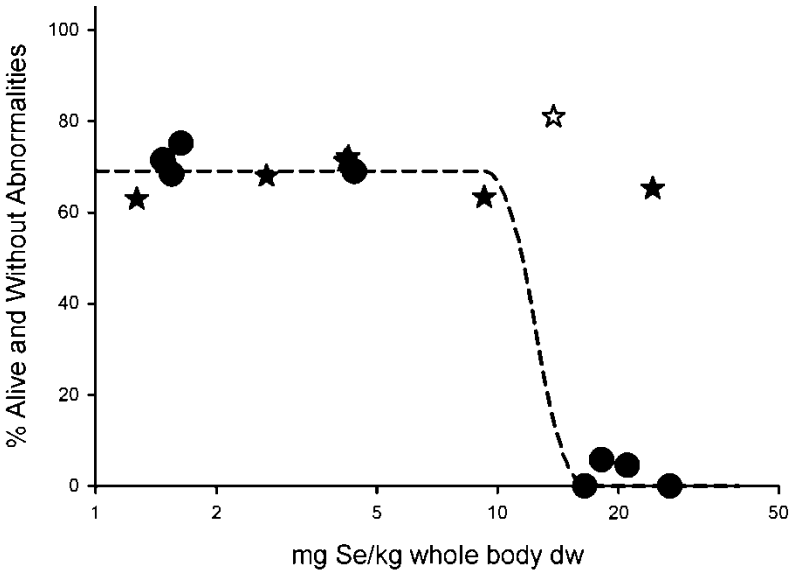


Figure 3. TRAP interpolation curve for the Table 5 whole body data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 9.3 mg/kg whole body and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.5 mg/kg and a survival/normal of 0%. The resulting EC_{10} is 10.6 mg Se/kg whole body dw.

Coyle, J.J., D.R. Buckler and C.G. Ingersoll. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). Environ. Toxicol. Chem. 12:551-565.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; two-year old pond-reared adult fish and resultant fry)

Exposure Route: Dietary and waterborne
Dietary
Seleno-L-methionine added in an aqueous solution to Oregon moist pellets; moisture content of diet was 25 percent.
Waterborne
Flow through 10 µg Se/L nominal, 6:1 ratio of selenate:selenite, 98 percent purity, adjusted to pH 2 with HCl to prevent bacterial growth and change in oxidation states of Se(IV) and Se(VI).

Test Duration: 140 days

Study Design: The experiment consisted of a test control and food control (see Test Treatment table below) with fish (n=28 initially) in the four remaining treatments fed one of the four seleno-methionine diets in combination with 10 µg Se/L in water. Spawning frequency, fecundity, and percentage hatch were monitored during the last 80 days of the exposure period. Survival of resulting fry (n=20) was monitored for 30 days after hatch. Adults and fry were exposed in separate, modified proportional flow-through diluters. Fry were exposed to the same waterborne selenium concentrations as their parents. Adults were fed twice daily *ad libitum*. Whole-body selenium concentrations in adult fish were measured at days 0, 60, and were calculated from individually analyzed carcass and gonadal tissue (ovaries and testes) at day 140. Eggs not used in percentage of hatch determinations were frozen and analyzed for total selenium.

Measured Se in:	**Test Treatments**					
	1 (test control)	2 (food control)	3	4	5	6
water (µg Se/L)	0.56	8.4	10.5	10.5	10.1	11.0
diet (mg Se/kg dw)	0.76	0.76	4.63	8.45	16.8	33.3

Effects Data: There was no effect of the combination of highest dietary selenium concentration (33.3 mg/kg dw) in conjunction with exposure to a waterborne selenium concentration of 11.0 µg/L on adult growth (length and weight), condition factor, gonad weight, gonadal somatic index, or reproductive endpoints (i.e., spawning frequency, number of eggs per spawn, percentage hatch) during the 140-day exposure (Table 1). The mean corresponding whole-body selenium concentration in adults exposed to this waterborne and dietary selenium combination was 19 mg/kg dw. Survival of fry from the exposed adults was affected by 5 days post-hatch. Concentrations of whole-body selenium in adult tissue at day 60 were used to determine effects in the fry because eggs were taken for the larval tests beginning at day 60 of the adult exposure.

Table 1. Effects on Adults

Se in diet, mg/kg dw	Se in water, µg/L	whole-body Se (140 d), mg/kg dw	replicate	total no. spawns	eggs/spawn	hatchability, %
0.8	0.5	0.8	A	15	14,099	94.5
			B	10	5,961	90.5
0.8	7.9	1.0	A	12	9,267	89.5
			B	11	9,255	84.5
4.6	10.5	3.4	A	20	9,782	86.5
			B	12	13,032	96.5
8.4	10.5	6.0	A	2	10,614	96.5
			B	9	7,995	90
16.8	10.1	10	A	13	10,797	83
			B	13	9,147	91.5
33.3	10.1	19	A	14	8,850	80
			B	4	8,850	80

In the 30-d survival after hatch test, there was complete mortality after one week at the highest exposure and no significant differences in survival at lower concentrations. Table 2 provides the survival data at 5 days post hatch used in the analysis of the effect concentration. The day 5 data are given in Table 2 because this was the only day in which control survival was over 90%, with the control and all the treatments showing substantial and increasing toxicity over the next 4 days.

Because the survival in the fifth treatment was about 5% below the average of the lowest four and because the highest treatment still had some survivors, this

provided two partial effects for TRAP to fit a curve. However, the legitimacy of this depends on the lower survival in the fifth treatment actually being a significant Se effect, rather than reflecting random variation of background survival. Because there were multiple spawns with 200-500 total larvae tested for each survival value above, this might be expected to be a real effect, but there is insufficient data reported to test this. However, from day 6 through day 30, survival at the fifth treatment was above that in the first and third treatments, indicating this is not an effect level. These later data establish that the highest treatment is best considered an EC_{100} and the fifth treatment an EC_0. So an interpolation was done using 42 mg/kg as an EC_{100}, resulting in a slope of 7.6 and an EC_{10} of 26.3 mg/kg. The interpolation between the EC_0 and EC_{100} resulted in a slightly higher EC_{10} in the previous draft document (24.15 mg/kg) which used a TRAP model to estimate the EC_{10}. A figure is not provided here because this interpolation represents a synthesis of the data not tied to the data for a specific day.

As for the analysis with egg concentrations, the whole-body analysis recognizes the highest treatment as an EC_{100} (16 mg Se/kg dry wt whole body) and the second highest treatment as an EC_0 (7.2 mg Se/kg dry wt whole body). The interpolation method then results in an EC_{10} of 8.6 mg/kg. As for the egg concentration analysis, no plot is given because the EC_0 is not for a specific day or survival value.

Table 2. Survival of Larvae at Day 5 in the 30-day Post-hatch Test

Se in diet, mg/kg dw	Se in water, µg/L	egg, mg/kg dw	adult whole-body (60 d), mg/kg dw	mean survival, %
0.8	0.5	1.8	0.9	92
0.8	7.9	1.8	0.9	93
4.6	10.5	7.3	2.9	90
8.4	10.5	13	4.9	95
16.8	10.1	23	7.2	87
33.3	10.1	42	16	7

Chronic Value:

effect level	egg, mg Se/kg dw	whole body, mg Se/kg dw
EC_{10}	**26.3**	**8.6**

Cleveland, L. et al. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill sunfish (*Lepomis macrochirus*). Aquatic Toxicol. 27:265-280.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*)

Life Stage: juvenile (5 months - waterborne exposure; 3 months - dietary exposure)

Exposure Route: waterborne (60-d) and dietary (90-d) - separate exposures
waterborne - 6:1 selenate:selenite at 0.17, 0.34, 0.68, 1.38, 2.73 mg/L; dietary - seleno-L-methionine in Oregon moist at 1.63, 3.25, 6.5, 13, 26 mg Se/kg dw)

Study Design: Fish were exposed using a flow-through diluter. Each test consisted of an exposure and a depuration phase. Whole body tissue measurements were made at 31 and 60 days of waterborne exposure and at 31, 59 and 90 days of dietary exposure. Mortality and condition factor, K (weight x 10^5/length^3), were reported at selected intervals.

Effects Data: The waterborne exposure (see table below) was determined to have an EC_{20} = 4.07 mg Se/kg dw (1.96-8.44 mg/kg 95% CL). However, because it was a water-only exposure, it was not considered in the derivation of the FCV. These data nevertheless provide evidence that exposure route influences the tissue concentration toxicity threshold, although the mechanistic explanation for this phenomenon is lacking.

A mortality effect level for the dietary exposure could not be calculated because the highest selenium whole body concentration (13.4 mg Se/kg dw) only had 17.5% mortality. The middle selenium concentration did have 22.5% mortality. Cleveland et al. reported a significant decrease in K between 4.7 and 7.7 mg/kg dw (see table below).

Waterborne Exposure Study

Measured selenium in water (:g/L)	**60-d measured selenium in whole body (mg/kg dw)**	**60-d mortality (%)**	**Condition factor (K)**
20 (control)	1.1	10	1.5
160	2.8	12.5	1.5
330	4	22.5	1.6
640	5.3	52.5	1.5
1120	9.8	70	1.6
2800	14.7*	97.5	NA

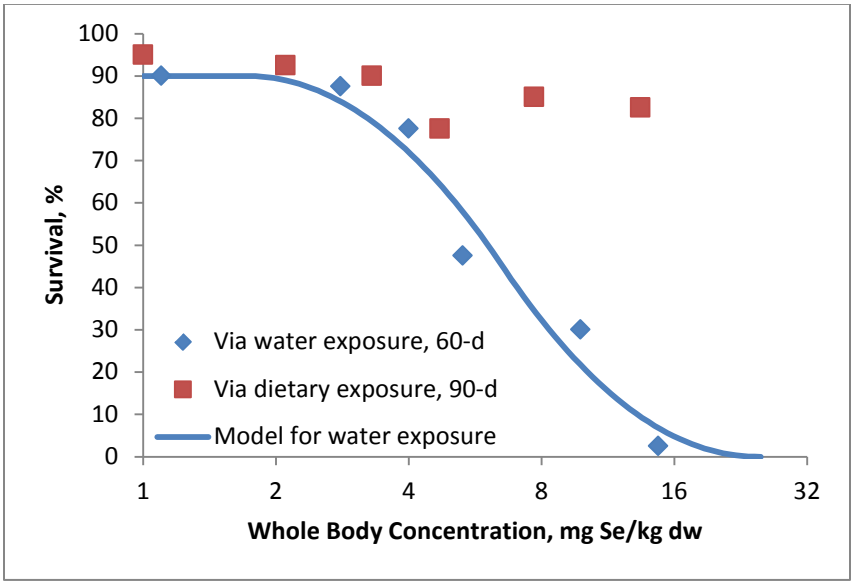
*[a] 30-d measurement because all fish were dead at 60 days in this concentration.

Dietary Exposure Study

Measured selenium in food (mg/kg ww)	90-d measured selenium in whole body (mg/kg dw)	90-d mortality (%)	Condition factor (K)
0.68 (control)	1	5	1.3
2.3	2.1	7.5	1.3
3.5	3.3	10	1.3
6.6	4.7	22.5	1.3
12.7	7.7	15	1.2
25	13.4	17.5	1.2

Discussion

The study demonstrates the influence of exposure route on the potency of a given tissue concentration, as shown in the figure. The TRAP threshold sigmoid concentration-response curve for the water-only exposure yields an EC50 of 6.5 mg Se/kg dw WB. In contrast, higher whole-body concentrations acquired via diet did not yield significant effects and cannot support a TRAP-fitted concentration-response curve or EC estimate. Examination of the graph indicates that the water-only concentration-response curve would need to be shifted to the right a minimum of 4-fold (or possibly more) to be able to fit the (lack of) effects observed in the dietary study. This supports the decision to derive the criteria only from studies relying on the environmentally relevant exposure route, diet.



Survival at 60-days (for water exposure) or 90-days (for dietary exposure) versus whole-body concentration.

Chronic Value: Given (a) the very slight reduction in K (1.3 to 1.2 between 4.7 and 7.7 mg Se/kg dw WB, with no further reduction at 13.4 mg Se/kg dw WB) and uncertain relevance of growth data, and (b) no apparent concentration-related effect on mortality between 4.7 and 13.4 mg Se/kg dw WB, the NOAEC is interpreted to be 13.4 mg Se/kg dw for this study; and the chronic value is >13.4 mg Se/kg dw whole body.

Lemly, A.D. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicol. 27:133-158.

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile 50-70 mm)

Exposure Route: Waterborne and dietary
Water
1:1 selenite:selenate in stock at pH 2; metered in to reach 5 :g/L
Diet
seleno-L-methionine in TetraMin (5 mg/kg dw)

Test Duration: 180 days

Study Design: Fish were exposed (treatment and control) under intermittent flow-through conditions for 180 days. Tests were run at 4° and 20°C with biological (histological, hematological, metabolic and survival) and selenium measurements made at 0, 60, 120 and 180 days. Fish were fed at a rate of 3% body weight per day. All treatments were initiated at 20°C and then decreased in the cold treatment at a rate of 2°C per week for 8 weeks to reach 4°C and then maintained at that temperature for the remainder of the 180 days.

Effects Data : In the 20°C test, fish accumulated 6 mg/kg dw selenium (whole-body) with no significant effect on survival (4.3% and 7.4% mortality in control and treatment, respectively). In the 4°C test, fish exposed to selenium accumulated 7.9 mg/kg dw (whole-body) selenium and had significant mortality after 120 (33.6%) and 180 days (40.4%) relative to control (3.9%). Several hematological measurements were significantly different in both the warm and cold selenium exposures relative to controls. Both warm and cold selenium treatments also had greater O_2 consumption than controls. Fish lipid content in the cold Se treatment decreased more than the cold control; lipid content did not decrease in either the warm control or the warm Se treatment (see summary tables below). The results suggest significant mortality occurs in juvenile bluegill during winter months when tissue concentrations reach 7.91 mg/kg dw and lipid levels decrease to 6 percent.

Chronic Value: 20°C, >6 mg Se/kg whole-body; 4°C, <7.91 mg Se/kg dw whole body

Comments: See “Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies” in this appendix after presentation of the McIntyre et al. (2008) study.

Mean Concentration of Selenium in Tissues, Cumulative Survival*, Percent Lipid Content and Oxygen Consumption in Juvenile Bluegill

day	cold - Se control				cold + Se				warm - Se control				warm + Se			
	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]
0	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98
60	1	97.1	12.5	58	5.8	92.9	10	63	1.2	95.7	13.3	98	5.8	100	13.3	103
120	1.1	97.1	11.5	57	7.9	66.4	6	81	1.1	95.7	13.4	100	6	96.7	13.4	120
180	1.4	97.1	10.5	57	7.9	59.6	6	78	1.2	95.7	13.6	100	6	92.6	13.5	120

[a] whole body Se tissue concentration, mg/kg dw
[b] oxygen consumption, mg/kg/hr

* Cumulative Survival: In this experiment, 240 juvenile bluegill were placed in three 400-L fiberglass tanks, 80 in each, and exposed to each control and treatment for a period of 180 days. Ten fish were removed at random from each treatment replicate on days 0, 60, 120, and 180 for selenium, histological, hematological, and metabolic measurements.

Replicate and Average Whole-body concentrations (mg/kg dry weight) of selenium in juvenile bluegill*

	day 0				day 60				day 120				day 180			
replicate	1	2	3	mean	1	2	3	mean	1	2	3	mean	1	2	3	mean
c+Se	0.87	1.21	0.95	1.01	6.30	5.49	5.76	5.85	8.36	7.31	7.85	7.84	7.53	8.01	8.19	7.91
w+Se	1.17	0.96	0.90	1.01	5.61	6.19	5.43	5.74	6.37	5.92	5.50	5.93	5.48	5.72	6.02	5.74
c-Se	0.89			0.89	0.97			0.97	1.01			1.01	1.10			1.10
w-Se	0.99			0.99	1.12			1.12	0.99			0.99	0.96			0.96

* Each value is for a composite sample made from 5 fish.

The Kaplan-Meier estimator was used to calculate survival at time t

$$\hat{S}(t) = \frac{\prod r(t_i) - d_i}{r(t_i)}$$

where $r(t_i)$ is the number of fish alive just before time t_i, i.e. the number at risk, and d_i is the number of deaths in the interval $I_i = [t_i, t_{i+1}]$. The 95% confidence interval for such estimate (Venables and Ripley 2002) was computed as

$$\exp\left\{-\hat{H}(t)\exp\left[\pm k_\alpha \frac{\text{s.e.}\left(\hat{H}(t)\right)}{\hat{H}(t)}\right]\right\}$$

where

$$\hat{H}(t) = \sum \frac{d_j}{r(t_j)} \qquad \text{and } j \# i$$

The following table lists the estimates of survival in the cold + Se treatment at 60, 120 and 180 days. The term n.event is the number of deaths at a given interval; n.risk is the number of organisms alive at the beginning of the interval; survival is computed by the Kaplan-Meier estimator.

Time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
60	210	15	0.929	0.0178	0.884	0.956
120	165	47	0.664	0.0350	0.590	0.728
180	88	9	0.596	0.0381	0.517	0.666

Hematological Measurements in Juvenile Bluegill Sunfish (*indicates significantly different from control)

Warm Exposure	day 0		day 60		day 120		day 180	
blood parameter	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se
total erythrocyte, 10^6/ml	2.95	2.92	2.96	2.93	2.99	2.95	2.96	2.89
% mature	85	86	86	93*	86	94*	85	94*
nuclear shadows, 10^4/ml	0.95	0.86	0.97	2.05*	0.83	2.38*	0.91	2.30*
total leucocytes, 10^4/ml	17.22	17.41	16.90	17.55	16.73	17.62	17.05	17.36
% lymphocytes	23	25	20	23	19	26	21	22
% neutrophils	15	13	14	15	17	19	17	16
hematocrit, %	37	36	37	29*	36	29*	38	28*
MCHC (mean corpuscular hemoglobin conc.)	23	25	25	19*	25	18*	25	17*
Cold Exposure	day 0		day 60		day 120		day 180	
blood parameter	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se
total erythrocyte, 10^6/ml	2.91	2.93	2.97	2.90	3.01	2.95	3.00	2.99

% mature	84	82	87	95*	85	96*	85	97*
nuclear shadows, 10^4/ml	0.86	0.84	0.83	2.30*	0.89	2.49*	0.90	2.36
total leucocytes, 10^4/ml	16.48	16.88	16.79	16.91	16.80	16.74	16.96	16.63
% lymphocytes	17	16	16	17	19	15	19	18
% neutrophils	13	12	15	11	15	12	12	14
hematocrit, %	39	37	40	30*	41	28*	39	27*
MCHC (mean corpuscular hemoglobin conc.)	26	25	25	18*	22	17*	23	17*
MCV (mean corpuscular volume)	182	171	188	146*	180	135*	185	130*

McIntyre et al. 2008. Effect of Selenium on Juvenile Bluegill Sunfish at Reduced Temperatures. US EPA, Health and Ecological Criteria Division. EPA-822-R-08-020

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); juvenile; average length 47 mm, average weight 1 g

Exposure Route: Waterborne and dietary

Water

1:1 selenite:selenate; For exposure systems (ES) 1 and 3, fish were exposed to a control and a series of 6 nominal concentrations, 1.25, 2.5, 5, 10, 20 and 40 µg Se/L. For ES2, fish were exposed to a control and one nominal concentration, 5 µg Se/L.

Diet

For ES1 and ES3, fish were fed a series of six concentrations of selenium and a background control in *Lumbriculus variegatus*. The measured selenium concentrations in the *L. variegatus* treatments in ES1 were: 2.3 (control), 4.5, 5.3, 7.5, 14.2, 25.7 and 34.9 mg Se/kg dw; in ES3: 2.2 (control), 4.2, 5.0, 7.2, 15.2, 25.4 and 46.7 mg Se/kg dw. Fish were fed worms at a rate of 4% of the current biomass in each fish tank. Selenium was accumulated in *L. variegatus* by feeding the worms in separate tanks a series of six concentrations of selenized-yeast diluted with nutritional yeast: 1.7, 3.3, 6.7, 13.3, 26.7 and 53.5 mg Se/kg dw. Control worms were fed nutritional yeast only. Each tank was additionally exposed to the associated aqueous concentration selenium, e.g., the worms fed the 1.7 mg Se/kg dw selenized yeast were exposed to 1.25 :g Se/L, the worms fed the 3.3 mg Se/kg dw selenized yeast were exposed to 2.5 :g Se/L, and so on. For ES2, fish were fed TetraMin spiked with seleno-L-methionine at a nominal concentration of 5 mg/kg dw and at a rate of 3% of the current biomass in each tank.

Test Duration: 182 days

Study Design: Juvenile bluegill were exposed concurrently to selenium using three separate exposure systems, ES1, ES2 and ES3. In ES1 and ES3, 100 fish were exposed to each of 6 selenium treatments (low through high treatments are referred to as Treatments 1 through 6) and two controls in 200 L carboys under flow-through conditions. Each treatment consisted of an aqueous selenium concentration and an associated dietary selenium concentration, e.g., the fish in the lowest ES1 treatment were exposed to 1.25 :g Se/L and fed worms containing 4.5 mg Se/kg dw (see Exposure Route for other treatment concentrations). Temperature was controlled in each system through the immersion of the carboys in a temperature-controlled water bath and by controlling the temperature of the dilution water being added to the carboys. The temperature in ES1 was maintained at 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The only difference between ES1 and ES3 was temperature was decreased 2°C/week until it reached 9°C (test day 65) at which point temperature was maintained until test termination (test day 182).

The exposure of ES2 was similar to ES1 and ES3 in that 100 juvenile bluegill were exposed to treatment in 200 L carboys under flow-through conditions. The

ES2 selenium treatment consisted of two replicates of 5 µg Se/L waterborne and 5 mg Se/kg dw diet (Tetramin). Two controls were maintained with ES2. The temperature regime for ES2 was identical to ES1.

Observations on fish behavior and mortality were checked daily. Total selenium was measured in each fish tank weekly and selenium speciation was measured monthly in each fish tank. Whole body total selenium was measured in the worms from each tank (2 replicate 5 g samples) on test days 0, 30, 60, 112 and 182 and in the bluegill from each tank (3 replicates of 3-fish composites - total 9 fish) on test days 0, 7, 30, 60, 112 and 182. The standard length and weight of each fish was measured on each sample day. Lipid content was measured in fish at day 0 and from each treatment at test termination.

Effects Data:

Selenium increased in bluegill as the exposure concentrations increased (see following table). No meaningful mortality was observed in ES2. The number of fish that died in ES2 during the 182 day test was two fish in one treatment replicate and none in the other treatment replicate; no deaths were reported in ES2 controls. Significant mortality of juvenile bluegill was observed in ES1 and ES3. After 182 days, a total of 24 and 68 fish died in Treatments 5 and 6, respectively in ES1; and a total of 38 and 61 fish died in Treatments 5 and 6, respectively in ES3. See table below for mortalities in all treatments. Estimates of bluegill survival were adjusted for the removal of individuals from the test population. Individuals were removed from the experiments before test completion, for sampling tissue concentrations or because they suffered accidental deaths unrelated to selenium toxicity. For such data, it was necessary to account for the reduction in number of individuals at risk of death due to selenium over time. If $r(t_i)$ is the number of individuals at risk just before time t_i and d_i is the number of deaths in the interval, $I_i = [t_i, t_{i+1})$, then survival (S) at time t can be estimated as

$$\hat{S}(t) = \prod \frac{r(t_i) - d_i}{r(t_i)}$$

The product (P) was calculated for each period in which one or more deaths occur. The equation is the Kaplan-Meier estimator (Venables and Ripley 2002). This correction was applied to calculate the proportion of survival in treatments with ten or more deaths (10% mortality). The table below provides the adjusted proportion and surviving bluegill in each treatment along with the concentration of selenium in bluegill at test termination. The values in this table were used to calculate the EC_{20} and EC_{10} values using the TEAM software. Growth and lipid content of the bluegill was not negatively affected by the selenium exposures.

Measured total selenium concentrations in bluegill sunfish for all treatments and controls in Exposure System 1, 2 and 3.

Total Selenium in Whole Body Bluegill Tissue, mg/kg dw

ES1	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.43 (0.31)	2.48 (0.11)	2.43 (0.18)	2.64 (0.06)	2.72 (0.07)	3.27 (0.27)	4.27 (0.44)
30	2.10 (0.21)	2.85 (0.10)	3.10 (0.04)	2.94 (0.13)	4.24 (0.22)	6.62 (0.23)	10.21 (0.36)
60	2.11 (0.02)	2.70 (0.20)	3.07 (0.05)	3.69 (0.25)	5.21 (0.30)	8.62 (0.45)	12.66 (0.45)
112	1.98 (0.04)	3.16 (0.11)	3.41 (0.08)	3.99 (0.26)	6.42 (0.05)	11.60 (0.43)	
182	2.08 (0.10)	2.56 (0.21)	3.15 (0.25)	4.02 (0.21)	6.72 (0.09)	10.71 (0.55)	

ES3	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.50 (0.10)	2.60 (0.29)	2.38 (0.10)	2.82 (0.20)	3.19 (0.33)	4.29 (0.20)	6.13 (0.62)
30	2.24 (0.41)	2.44 (0.26)	2.70 (0.16)	3.13 (0.10)	3.95 (0.16)	6.06 (0.36)	11.07 (0.92)
60	2.70 (0.22)	2.88 (0.08)	3.04 (0.39)	3.79 (0.24)	5.54 (0.21)	9.50 (0.91)	15.14 (0.96)
112	2.16 (0.14)	2.49 (0.10)	3.10 (0.12)	3.64 (0.16)	6.54 (0.21)	11.50 (0.25)	17.24 (0.30)
182	1.67 (0.21)	3.20 (0.27)	3.83 (0.47)	5.48 (0.24)	9.38 (0.63)	16.01 (0.30)	

ES2	Control	5A	5B
Test Day	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.19 (0.19)	3.55 (0.25)	3.08 (0.50)
30	2.49 (0.15)	7.05 (0.76)	7.51 (1.18)
60	1.53 (0.03)	8.23 (1.55)	8.09 (0.67)
112	1.57 (0.01)	8.97 (1.28)	9.45 (1.73)
182	1.38 (0.06)	9.41 (1.63)	10.61 (0.38)

Total number of deaths in ES1 and ES3 Treatments throughout the experiment's duration (182 days). Both ES1 and ES3 had two control tanks.

Treatment	ES1	ES3
Control (#1, #2)	0, 7	1, 1
1	5	0
2	1	1
3	0	0
4	3	3
5	24	38
6	68	61

The concentration of selenium in bluegill and the adjusted proportion of surviving fish at the end of the 182 day exposure.

	ES1		ES3	
Treatment	$[Se]_{tissue}$, mg/kg dw	surv	$[Se]_{tissue}$, mg/kg dw	surv
control	2.08	0.962	1.67	0.988
1	2.56	0.988	3.20	1.000
2	3.15	0.984	3.83	0.988
3	4.02	1.000	5.48	1.000
4	6.72	0.962	9.38	0.960
5	10.71	0.497	16.01	0.435
6	12.66	0.075	17.24	0.168

Chronic Value: The NOAEC for bluegill in ES2 was calculated as the geometric mean of the concentration of bluegill in the two replicates at the end of the exposure period, 9.992 mg Se/kg dw whole body. The chronic value for ES2 is therefore >9.992 mg Se/kg dw whole body. The EC_{20} and EC_{10} values for ES1 and ES3 are given in the following table.

	ES1 (4°C)	ES3 (9°C)
	Whole body	Whole body
EC_{20} mg Se/kg dw	9.78	14.64
EC_{10} mg Se/kg dw	9.27	14.00

Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies of Lemly (1993a) and McIntyre et al. (2008)

The Lemly (1993a) and McIntyre et al. (2008) cold-temperature juvenile bluegill studies are summarized on the previous pages. This discussion compares and contrasts these studies.

Both studies indicated that juvenile bluegill are more sensitive to selenium at lower temperature than at higher temperature. For a 4°C temperature regime, the EC_{10} of 9.27 mg Se/kg dw WB obtained with McIntyre's selenized yeast-worm-fish dietary bioaccumulation system is somewhat similar to the threshold of 5.85 mg Se/kg dw WB estimated from the time course of bioaccumulation and mortality in Lemly's single treatment with seleno-L-methionine in TetraMin. These chronic values differ by a factor of 1.58.

The difference in diet does not appear to explain the modest difference in results; however, since McIntyre's other 4°C experiment (Exposure System ES2), which used Lemly's seleno-L-methionine in TetraMin diet, experienced no significant toxicity, whereas Lemly's similarly exposed fish experienced 40 percent mortality by the end of the test. In addition to the difference in observed mortalities, Lemly's bluegill in the 4°C selenium exposure decreased in both lipid content and body condition over the 180 days whereas no decreases in these measurements were observed in the McIntyre et al. study, although the fish used in both studies were of comparable size and body condition at test initiation: 47 mm average standard length (range 44 to 54 mm) and a body condition index (100 x fish weight/standard length) of 3.2 in ES2 compared to 50 to 70 mm total length and a body condition factor of 3.9 in Lemly.

There are several possible reasons why such results could differ between studies. (1) ES2 maintained exposure at 20°C for the first 30 days of exposure before decreasing the temperature compared to 7 days in the Lemly study. (2) Lemly measured O_2 consumption by removing and reintroducing test fish to the test tanks, which was not done by McIntyre et al. (3) The two studies differed in photoperiod – Lemly "began with a 16:10 h light/dark photoperiod which was gradually reversed to 10:16" (sic) whereas McIntyre et al. used a fixed photoperiod of 16:8. (4) Some genetic differences between the tested batches of organisms may be expected, reflecting different origins, despite the similarities in their starting size and condition.

The modification to maintain 20°C for 30 days was to allow a longer period of time for the fish to accumulate selenium during a warmer condition prior to decreasing the temperature. This did result in shortening the exposure in ES2 at 4°C by 19 days (103 days at 4°C) compared to 122 days at 4°C in Lemly's study. However, as the majority of deaths in Lemly's study occurred between in the middle 60 days of the 180-day test, the slightly shorter cold period in the McIntyre study would not explain the differences in mortalities.

As stated above, Lemly removed fish (N = 15) from each treatment for oxygen consumption measurement and then returned these fish to the exposure tanks. There is the possibility that the fish removed from the cold plus selenium treatment were sufficiently stressed by the exposure conditions that the additional handling stress contributed to the mortality observed in this treatment. Between test days 60 and 180, 56 fish died Lemly's cold plus selenium treatment. Even if stress due to handling affected all the fish used in the oxygen consumption measurements (up to 30 fish), it does not explain all the mortality that was observed and therefore does not explain the difference between the two studies.

Both Lemly (1993) and McIntyre et al. (2008) showed reduced survival of juvenile bluegill exposed to elevated selenium under lab-simulated winter conditions, albeit at somewhat different concentrations. But only Lemly, not McIntyre et al., found the decreased survival to be accompanied by loss of lipid and body

condition. It was hypothesized that the decrease in EC_{10} observed by Lemly (1993) in the cold water treatment between 60-180 days was attributed to "winter stress syndrome" (WSS). WSS is hypothesized to occur in warmwater fish species because the presence of a stressor places additional metabolic costs on exposed organisms. These stresses can be better tolerated during periods of warm weather and active feeding. However, during the winter months, feeding and activity levels decrease but the metabolic costs of the stressor remain. As a result, fishes deplete their lipid stores, resulting in lower condition factors and increased susceptibility to mortality (Lemly 1996). Lemly noted three conditions that must be met simultaneously in order for WSS to occur: 1) a significant metabolic stressor must be present, 2) cold water temperatures must be present, and 3) fish must respond by reducing activity and feeding (Lemly 1996).

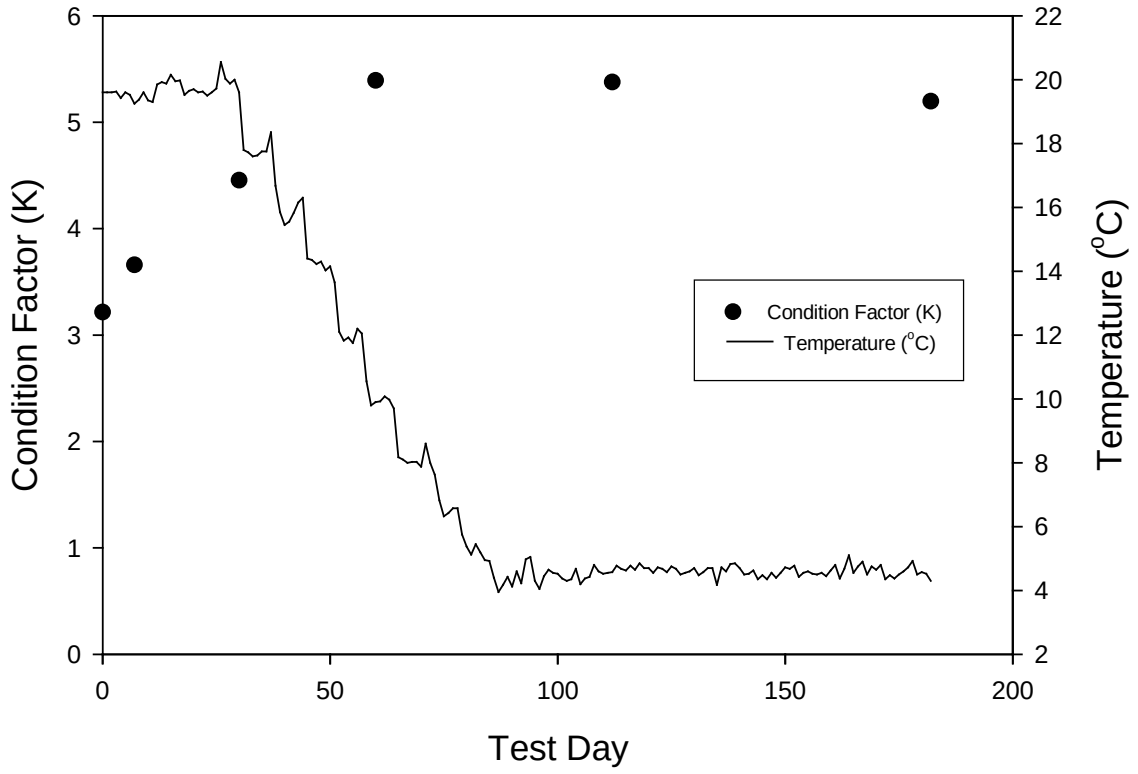
Several other studies have reported decreased feeding and activity levels for several fish species. McCollum et al. (2003) observed decreased overwinter feeding, and subsequent weight loss, of white crappie. Parrish et al. (2004) observed overwinter weight loss among mature, but not juvenile, salmon in a laboratory study in experimental raceways. Current speed, and by extension prey delivery rate, was the most important factor regulating overwinter feeding and growth. Eckmann (2004) observed overwinter reductions in feeding, weight, and lipid levels in yellow perch, but not in ruffe. Sogard and Olla (2000) observed walleye pollock could mitigate the effects of overwinter lipid depletion by moving to colder waters, where reduced metabolism allowed them to conserve energy. In all of these studies, fish continued to feed during the winter, but feeding rates decreased. The increase in weight among ruffe was attributed to its ability to feed on benthos in the dark during the winter months, suggesting that feeding reduction during winter may be more pronounced for species dependent on vision to feed. This was supported by Bennett and Janz (2007a), who observed that burbot, which rely primarily on smell while feeding on benthic invertebrates, experienced significant overwinter increases in weight and lipids in all sites, while northern pike, which rely primarily on vision while feeding on zooplankton, experienced slight but non-significant increases in weight and length.

WSS has not been definitively confirmed or refuted, although it has been investigated in the field. Bennett and Janz (2007a) observed no evidence of WSS for juvenile northern pike or burbot. Lengths, weights, and lipids increased for both species, particularly the olfactory feeding burbots, in the spring compared to the previous fall. Overall weights and lipids were higher in the low and high exposure lakes than the reference lake, possibly because of nitrogen limitation in the reference lake coupled with relatively low stressor concentrations in the exposed lakes. In a separate study, overwinter weights and lipids remained similar or increased in northern pike and burbot at both reference and exposure sites, while overwinter weights and lipids decreased at the exposure site for slimy sculpin (Bennett and Janz 2007b). However, this study neither supports nor refutes the WSS hypothesis, because stressor concentrations at the exposure site were not significantly different than at the reference sites, and the weight decrease in sculpin was attributed to higher turbidity at the exposure site, which inhibited food acquisition. In a final field test of WSS fathead minnow, creek chub, and white suckers were collected from reference and exposure sites (Driedger et al. 2009). Stressor levels at exposure sites were high, as whole body Se concentrations in fathead minnow ranged from 11-42 mg/kg dw. All three species either gained or maintained weight overwinter at all sites, indicating that active feeding occurred overwinter. Overall weights at exposure sites were higher, likely because of nutrient limitation at the reference sites, which confounded the ability to fully test the WSS hypothesis.

These results suggest that fish species responses to cold temperatures vary by species and environment. Many species lose weight, but this can be partially explained by the impact of low light levels on consumption levels, especially in northern latitudes where overwinter light limitation is pronounced. Field tests found no evidence of WSS, but were confounded by low stressor levels, nutrient limitation at reference sites, or both.

It may then be questioned whether the fixed photoperiod alone could account for the differences in the results of the two studies. More explicitly, did the longer light period in McIntyre et al. photoperiod allow the fish to feed more than the fish exposed to the shorter light period in the Lemly study, such that lipid and body condition in the McIntyre et al. fish were maintained and therefore not susceptible to "winter stress syndrome." The effects of photoperiod on fish and other ectotherms are well-documented. Temperature-independent seasonal changes in fish have been reported for growth and food conversion efficiency (Biswas and Takeuchi 2003; Jonassen et al. 2000; Simensen et al. 2000), feeding behavior (Volkoff and Peter 2006), metabolic rate (Evans 1984), and reproduction (Koger et al. 1999; Scott 1979). Some of these studies have found conflicting results on the effect of photoperiod on growth (Fuchs 1978; Jonassen et al. 2000; Simensen et al. 2000). Coupled with temperature being a dominant factor in controlling physiological functions in temperate-zone fish as indicated by a 3 to 4-fold fluctuation in metabolic activities over 10°C (Brett 1970; Fry 1971), it is difficult to use literature findings to explain the difference in the two bluegill studies. In field studies of fish at northern latitudes (Eckmann 2004), reduced light resulted in weight loss not though a bioenergetics interaction with cold temperatures, but by inhibiting feeding ability of visual, but not non-visual predators. If this mechanism applies to bluegill, then photoperiod is less likely to play a major role in the difference in results, as the overwinter light:dark cycle (8:16) should have been sufficiently long for the bluegill in Lemly (1993) to feed.

Observational recordings of the feeding behavior in McIntyre et al. noted that in both control replicates and in both treatment replicates the feeding of the juvenile bluegill went from active to not active on test day 78 when temperatures were decreased from 6.6 to 5.8°C. The feeding observations are reflected in a gradual slight decrease in the body condition factor (K) after test day 60 in the figure below. Although food intake was not quantified during the study, the lack of growth indicated in K suggests feeding markedly decreased as the temperature declined, as shown in the figure. Body condition decreased much more in the Lemly's cold plus selenium exposed fish after test day 60 (approximately 50%) but K in his cold-without-selenium exposure decreased only slightly, similar to McIntyre et al. Therefore it is not possible to determine if the greater decrease in K and in lipid content in Lemly's cold plus selenium treatment was due to decreased feeding because of a shorter photoperiod or because the bluegill fish population used in his study were more sensitive to selenium in cold conditions. McIntyre et al. obtained bluegill from Osage Catfisheries in Missouri whereas Lemly collected fish from ponds (assumed to be near Blacksburg, Virginia, not stated in paper). The fish obtained from Missouri, a location with colder winters than Virginia, may have been better adapted for withstanding colder winter temperatures than Lemly's fish and therefore were less sensitive to "winter stress syndrome" as induced by selenium exposure. Similarly, different populations of a species can have varying sensitivities to stressors. Furthermore, the relative difference in the Lemly and McIntyre et al. results is slightly **less** than Delos (2001) found to be typical when equivalent toxicity tests of the same species are compared. There should thus be no expectation that the two study results should agree more closely than they do.



Relationship between body condition factor (K) and temperature in juvenile bluegill fed a diet of Se-enriched TetraMin in the McIntyre et al. (2008) study.

Carolina Power & Light. 1997. Largemouth Bass Selenium Bioassay- Report. Carolina Power & Light Company, Environmental Services Section, 3932 New Hill, North Carolina. December 1997

Test Organism: Largemouth bass (*Micropterus salmoides*)

Exposure Route: Laboratory; dietary exposure only; DL-selenomethionine added to an artificial diet. Adult largemouth bass obtained from a commercial supplier were fed several months prior to spawning a series of selenium concentrations in the artificial diet.

Test duration: Embryo-larval monitoring through swim-up stage.

Study Design: Dietary exposure studies were conducted in 1995 and in 1996. In 1995, the measured dietary Se concentrations were 0.9 (control), 2.9, 7.5 and 11.2 mg Se/kg dw: in 1996, they were 26.7, 53.1 and 78.4 mg Se/kg dw. Parent fish were fed to satiation twice per day. Approximately 100 eggs from each spawn were transferred to each of 2 to 4 incubation cups. Eggs and larvae were monitored for mortality and deformities up to the larval swim-up stage. Selenium was measured in the liver, muscle and gonad tissues of the parent fish. All live deformed larvae at swim-up stage were considered as mortalities in the analyses.

Effects Data: Over the two year period, 56 successful spawns were obtained across all dietary treatments. Live larval fish with deformities (kyphosis, scoliosis, jaw gap, and lordosis) and edema at swim-up stage were considered mortalities for data analysis. The average concentration of selenium in ovaries ranged from 3.1 mg/kg dw in the control to 77.6 mg/kg dw in the high dietary treatment (Table 1). Larval survival generally decreased as the selenium concentration in the ovary increased (Table 1; Figure 1). A plot of the percent survival of larval largemouth bass as a function of the selenium concentration in the parental female ovary shows two groups of data; one at background survival with considerable variability (mean 90.3%, standard deviation 10.9%) and one with <10% survival, with most of the data being at 0% survival. Due to inadequate partial effects, a TRAP interpolation was used to estimate an EC_{10} value. Based on a risk management decision that the LOEC cannot be any higher than the lowest concentration with 0% survival (32.9 mg/kg) and that any ECx should be below this, this establishes the higher concentration point for the interpolation (an EC_{100} of 32.9 mg/kg) and requires that the highest 4 NOECs not be considered in setting the EC_0. The lower concentration point for the interpolation is therefore set here to 24.6, the next highest NOEC with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg (and a steep slope of 16).

An EC_{10} for the muscle tissue in Table 1 was not determined due to uncertainty in the values. The authors of this report also measured selenium in the ovaries and muscle tissues of largemouth bass collected from Mayo Reservoir (Table 2). There was a considerable difference in the proportion of selenium in the ovaries to the muscle tissues between the largemouth bass collected from the bioassay study and the field collected largemouth bass. The ratio of Se in ovaries to muscle in the laboratory fish was approximately 3.3 whereas it was 1.1 in the field collected fish. With the exception of mountain whitefish, the ovary to muscle ratio observed in the laboratory fish is also considerably higher than other

species (see Appendix B Table B-3). Based on this uncertainty in the muscle concentrations in the laboratory fish, an EC_{10} for this tissue was not calculated. The effect concentration based on the ovary selenium concentrations are not considered uncertain because these concentrations represent the direct exposure of selenium to the larvae from which the effect was observed.

Effect Concentration: **26.3** mg/kg dw in ovaries

Table 1. Selenium concentrations in the diet, ovary and muscle tissues and the percent mortality and deformities.

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
0.9 ± 0.1 (0.7 – 1.3)	6	1.62	5.38	3.1	75.5	95.3
	12	1.77	7.34		99.7	
	13	2.01	3.51		96.2	
	26	2.27	5.74		88.9	
	34	1.18	1.58		99.5	
	35	1.28	1.36		96.8	
	3	1.534	2.09		98.8	
	4	1.583	1.85		100	
	10 (2F)	1.15	2.11		97	
	13	1.181	1.86		97.1	
	14	1.341	1.40		98.4	
2.9 ± 0.5 (2.1 – 3.8)	9	2.075	9.59	8.8	84.9	94.8
	12	1.853	8.03		100	
	15	2.026	9.73		98.5	
	18	3.134	7.66		95.9	
7.5 ± 0.6 (6.3 – 8.4)	1	2.741	8.43	10.8	75	85.8
	2	3.737	25.15		63.9	
	5	5.709	15.31		90.6	
	7	3.468	1.20		79.1	
	8	2.545	6.78		95	
	16	7.302	8.25		96.8	
	19	4.776	10.20		100	
11.2 ± 1.4 (9.3 – 14.1)	6	4.521	35.44	25.0	91.5	88.7
	11	6.044	15.08		77.9	
	17	4.882	24.59		96.7	
26.7 ± 1.7 (23.6 – 29.5)	2	7.52	37.14	40.0	91.2	18.3
	5	12.42	44.67		0	
	11	9.73	34.26		75.9	
	16	10.1	35.58		0	
	17	5.74	33.48		9.9	
	19	11.74	48.24		0	
	36	10.21	35.81		6.3	
	37	14.12	37.88		0	
	51	11.68	32.95		0	

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
	52	11.16	59.89		0	
53.1 ± 4.8 (45.5 – 61.9)	22	18.15	46.22	61.0	0	0
	25	21.07	70.45		0	
	30	25.02	81.62		0	
	31	16.63	54.99		0	
	32	14.3	53.96		0	
	41	17.73	51.48		0	
	48 (2F)	26.25	84.31		0	
	50 (2F)	11.66	32.87		0	
	55	18.36	73.33		0	
78.4 ± 4.3 (73.2 – 87.0)	4 (2F)	12.6	66.81	77.6	66	5.5
	7	17.24	56.98		0	
	8	20.36	86.49		0	
	10	19.59	65.99		0	
	18	22.52	72.35		0	
	21	18.58	71.89		0	
	24	22.08	62.44		0	
	28	29.15	99.02		0	
	38	58.2	52.37		0	
	44	17.7	102.82		0	
	47	24.14	88.15		0	
	49	18.94	105.29		0	

[a] ± standard error; range of concentrations in parentheses.

Table 2. Se concentrations in muscle and ovary of field-collected (Mayo Reservoir) female largemouth bass.

Date	Se Muscle (mg/kg dw)	Se Ovary (mg/kg dw)	Ovary to Muscle Ratio
05/10/95	8.48	14.79	1.74
05/10/95	8.48	14.79	1.74
05/09/95	7.29	8.35	1.15
04/21/94	15	19	1.27
04/20/94	15	15	1.00
04/22/94	12	14	1.17
04/22/94	10	18	1.80
04/25/94	18	15	0.83
04/25/94	18	15	0.83
04/27/94	11	12	1.09
04/27/94	11	9.4	0.85
04/27/94	13	10	0.77
05/04/94	11	11	1.00
			Median Ratio 1.09

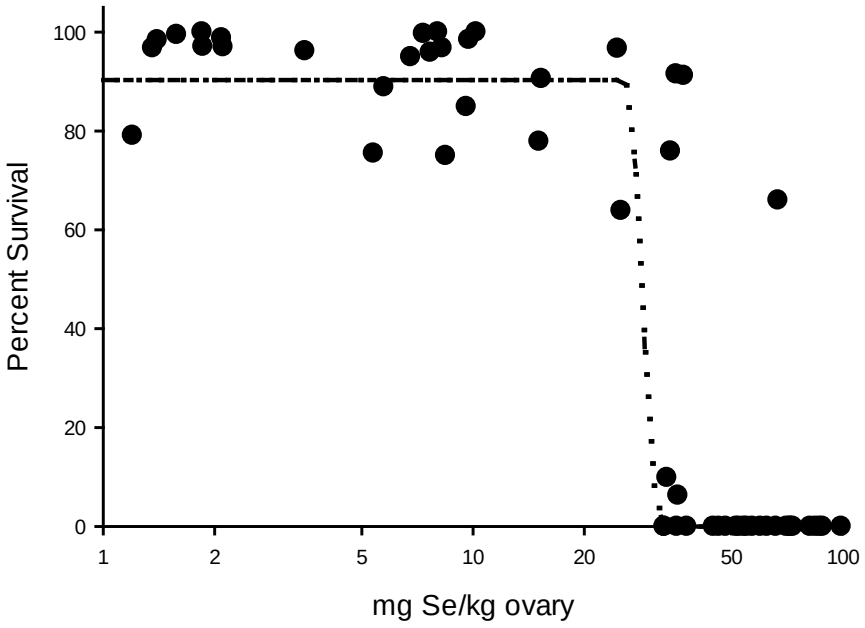


Figure 1. Largemouth bass larval survival relative to Se in ovary. TRAP interpolation was used to estimate the EC_{10} value. The higher concentration point for the interpolation was set at 32.9 mg/kg (EC_{100}) and the lower concentration point for the interpolation was set at 24.6 (NOEC) with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg and a steep slope of 16.

APPENDIX D: SUMMARY STUDIES OF NON-REPRODUCTIVE EFFECTS

1.0 STUDIES OF NON-REPRODUCTIVE EFFECTS

1.1 Acipenseridae

1.1.1 Acipenser transmontanus (white sturgeon)

Juvenile white sturgeon were exposed for 8 weeks to a series of 5 concentrations of seleno-L-methionine added to an artificial diet (Tashjian et al. 2006). Survival was not affected by selenium treatment with a mean survival rate of 99% across all groups. Fish fed the highest three dietary treatments of selenium, 41.7, 89.8 and 191.1 mg Se/kg dw, exhibited significant declines in growth assessed by body weight measurements. The EC_{10} for reduction in body weight is 15.08 mg Se/kg dw in whole body or 27.76 mg Se/kg dw muscle; the EC_{20} is 17.82 mg Se/kg dw in whole body or 32.53 mg Se/kg dw muscle tissue. The criterion values derived in this document that are based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study.

1.2 Cyprinidae

1.2.1 Pogonichthys macrolepidotus (Sacramento splittail)

Teh et al. (2004) exposed juvenile Sacramento splittail (7 months-old) to 8 levels of dietary selenium, 0.4 (no added selenium), 0.7, 1.4, 2.7, 6.6, 12.6, 26.0, and 57.6 mg/kg. Selenium was added to the diet via selenized yeast which was diluted with Torula yeast (inactive) to attain the target levels. Mortality, growth, histopathology, deformities and selenium content in muscle and liver were observed or measured after 5 and 9 months of exposure. The appearance of deformities was the most sensitive endpoint. The authors determined the occurrence of deformities was higher in fish fed 6.6 and 12.6 mg Se/kg in their diet; however, such pathology was examined for only 15 of the 120 individuals per treatment, and a consistent concentration-response relationship did not occur (i.e., no deformities in the high concentration). The lack of a concentration-response relationship for the incidence of deformities has also been observed in another study. Crane et al. (1992) exposed a European species of perch, *Perca fluviatilis* to three aqueous and dietary selenium treatments in experimental ponds for 288 days up through spawning. Crane et al. (1992) found an increased occurrence of deformities in embryos and larvae in the lowest selenium treatment relative to the control, but a decrease in the middle treatment. No hatching occurred in the high treatment. Teh et al. (2004) proposed several physiological mechanisms to explain the lack of a dose-response relationship, but it appears that the underlying mechanism is not understood at this time. Toxicity tests with unusual dose-response relationships are typically not considered for criteria derivation, but since another assay (Crane et al. 1992) observed a similar relationship, the Teh et al. (2004) study with *P. macrolepidotus* is included. Using prevalence of deformities as the endpoint, the NOEC, LOEC and MATC (chronic value) in *muscle tissue* are 10.1, 15.1 and 12.34 mg Se/kg dw, respectively. The critieron value in muscle tissue, based on the reproductive EC_{10}, is 11.8 mg Se/kg dw. Appendix C provides further details on the study results and an approximate estimate of their relationship to egg-ovary and whole-body concentrations. Teh et al. (2004) is the only study in which deformities developed in fish that were not exposed to selenium from their mothers' ovaries. The selenium criterion values derived based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study, considering the non-reproductive muscle MATC of 12.3 mg Se/kg dw is greater than the reproductive muscle criterion of 11.8 mg Se/kg dw.

1.2.2 Pimephales promelas (fathead minnows)

Non-reproductive chronic values for fathead minnows were derived from two laboratory-based studies. These studies (Bennett et al. 1986 and Dobbs et al. 1996) involved exposing algae to selenium (either as

sodium selenite or sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fathead minnows. In the Bennett et al. (1986) study, larval fathead minnows were fed control rotifers (cultured in chambers without selenium containing algae) or selenium-contaminated rotifers (cultured in chambers with selenium containing algae previously exposed to sodium selenite in the water) in three separate experiments lasting 9 to 30 days. The different experiments were distinguished by 1) the day selenium-laden rotifers were first fed; 2) the day selenium-laden rotifers were last fed; and 3) the age of larvae at experiment termination. The results from the three experiments reported by Bennett et al. (1986) were conflicting. Larval growth was significantly reduced at larval whole-body selenium concentrations of 43.0 mg Se/kg dw in the first experiment and 51.7 mg Se/kg dw in the second experiment, but was slightly but not significantly reduced at 61.1 mg Se/kg dw in the third experiment (see Appendix C). Following the approach of Section 7.1.1, the geometric mean of these three values, 51.40 mg Se/kg dw, is the chronic value for this study.

Dobbs et al. (1996) used a test system similar to that of Bennett et al (1986) (described above). Larval fathead minnows were exposed to the same concentrations of sodium selenate in the water as their prey (rotifers), but also received additional selenium from the consumption of the selenium-contaminated rotifers. In this study, the fathead minnows did not grow well at concentrations exceeding 108.1 µg Se/L in water, and they survived only to 11 days at selenium concentrations equal to or greater than 393.0 µg/L in the water (75 mg Se/kg dw in the diet, i.e., rotifers). The LOEC for retarded growth (larval fish dry weight) in this study was <73 mg Se/kg dw tissue.

A third laboratory study, by Ogle and Knight (1989), examined the chronic effects of elevated foodborne selenium on growth and reproduction of fathead minnows. Juvenile fathead minnows were fed a purified diet mix spiked with inorganic and organic selenium in the following percentages: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine. The pre-spawning exposure lasted 105 days using progeny of adult fathead minnows originally obtained from the Columbia National Fishery Research Laboratory, as well as those obtained from a commercial fish supplier. After the 105 day exposure period, a single male and female pair from each of the respective treatment replicates were isolated and inspected for spawning activity for 30 days following the first spawning event of that pair. There was no effect from selenium on any of the reproductive parameters measured, including larval survival, at the dietary concentrations tested (5.2 to 29.5 mg Se/kg dw food). Sub-samples of larvae from each brood were maintained for 14 days post-hatch and exhibited >87.4 percent survival. The pre-spawning adult fish fed a mean dietary level of 20.3 mg Se/kg dw exhibited a significant reduction in growth compared to controls (16 percent reduction), whereas a nonsignificant reduction in growth (7 percent) occurred in the fish fed 15.2 mg Se/kg dw. The chronic value, as determined by the geometric mean of the NOEC and the LOEC measured at 98 days post-test initiation, was 17.57 mg Se/kg expressed as the above dietary concentrations, and 5.961 mg Se/kg dw as fathead minnow whole-body tissue. The concentration-response relationship, as indicated by the study data presented in Appendix E, was uniformly shallow; not resembling the sharp sigmoidal function characteristic of most selenium response curves.

Since Ogle and Knight reported that food in the higher selenium concentrations remained uneaten and fish were observed to reject the food containing the higher selenium concentrations, the authors suggested that the decreased growth was caused by a reduced palatability of the seleniferous food items, which contained unnatural percentages of inorganic selenium (Fan et al. 2002). This is a common observation also noted by Hilton and Hodson (1983) and Hilton et al. (1980) and apparent in Coughlan and Velte (1989). It is here interpreted to be an artifact of unrealistic spiking of the diet with inorganic selenium in this early experimental protocol. That is, in the real world it is not expected that avoidance of food items that were unpalatable because of excessive selenium would be either a mechanism by which selenium causes effects or a mechanism by which organisms can avoid exposure. (See Janz et al. (2010) for a more complete discussion of selenium's mechanism of toxicity.) Given the no observed effect on larval

survival and the apparent non-toxicological effect on growth in the Ogle and Knight study, a chronic value for this study is not included.

1.3 Catostomidae

1.3.1 Xyrauchen texanus (razorback sucker)

Two non-reproductive endpoint studies have been done with the endangered razorback sucker. In the first study, Beyers and Sodergren (2001a) exposed larval razorback suckers for 28 days to a range of aqueous selenate concentrations (6.12, 25.4, 50.6, 98.9, and 190.6 µg/L) and respectively fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw). Reflecting the lack of effects on survival and growth in any exposure, the chronic value for this study, based on selenium measured in the larvae at the end of the test, is >12.9 mg Se/kg dw.

In a second study, Beyers and Sodergren (2001b) exposed larval razorback suckers to a control water and three different site waters containing varying concentrations of selenium for 28 days. Two treatments were tested within each water type: fish fed rotifers cultured in the same water type (site diet) and fish fed rotifers cultured in control water. There were no reductions in survival or growth in fish exposed to both the site water and site diet compared to fish exposed to control water and control diet. There were, however, reductions in growth of fish exposed to site water/site food compared to the same site water and control food. The authors did not attribute the effect on larval growth by the diet to selenium and cited several lines of evidence, including: (1) there was not a dose-response relationship in the concentration of selenium in the food (rotifers) and growth, nor in the concentration of selenium in the fish larvae and growth across the three water types; and (2) water from the De Beque site promoted a significant reduction in the growth of fish exposed to site water/site food relative to site water/control food, but contained low levels of selenium in the water (<1 µg/L) and in food (2.10 mg/kg dw) typically lower than those that have been found to elicit effects. The chronic value for this study is >42 mg Se/kg dw based on the whole body concentration of selenium in the larval razorback suckers exposed to North Pond site water.

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the razorback sucker (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results, of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). Summaries of each of these two studies as well as a third study with razorback suckers (Hamilton et al. 2005d) are presented in Appendix E.

Due to the confounding results, lack of dose-response within and among related studies, and the uncertainty of the effect of other inorganic contaminants on larval response to the various dietary and waterborne treatments, the data from these three studies for razorback sucker (Hamilton et al. 2001a,b; Hamilton et al. 2005d) have not been included. A more detailed explanation of why these studies were not included is given in Appendix E. Because of the vastly different results between the Beyers and Sodergren studies and Hamilton et al. studies and the inability to resolve the differences, SMCV and GMCV were not calculated for the razorback sucker.

1.3.2 Catostomus latipinnis (flannelmouth sucker)

Beyers and Sodergren (2001a) exposed flannelmouth sucker larvae to a range of aqueous selenate concentrations (<1, 25.4, 50.6, 98.9, and 190.6 µg/L) and fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively). There were no survival or growth effects observed after the 28 day exposure. The chronic value based on the concentration of selenium measured in the larvae exposed to the highest test concentration was >10.2 mg Se/kg dw.

1.4 Salmonidae

1.4.1 Oncorhynchus tshawytscha (Chinook salmon)

Hamilton et al. (1990) conducted a 90-day growth and survival study with swim-up larvae fed one of two different diets. The first diet consisted of Oregon moist™ pellets where over half of the salmon meal was replaced with meal from selenium-laden mosquitofish (*Gambusia affinis*) collected from the San Luis Drain, CA (SLD diet). The second diet was prepared by replacing half the salmon meal in the Oregon moist™ pellets with meal from low-selenium mosquitofish (i.e., the same relatively uncontaminated mosquitofish that were used in the control diet) and spiked with seleno-DL-methionine (SeMe diet). Analysis of the trace element composition in the two different diets indicated that while selenium was the most toxic element in the SLD diet, concentrations of boron, chromium, iron and strontium in the high-selenium mosquitofish replacement diet (SLD diet type) were slightly elevated compared to the replacement diet. These trace elements were, however, only 1.2 (e.g., iron) to 2.0 times (e.g., chromium) higher in the SLD diet than the SeMe diet, which contained the following measured concentrations (dry weight basis) in the food: 10 mg boron/kg, 2.8 mg chromium/kg, 776 mg iron/kg, and 48.9 mg strontium/kg.

During the test, survival of control Chinook salmon larvae (consuming food at approximately 3 mg Se/kg dw) was 99 percent up to 60 days post-test initiation. Between 60 and 90 days of exposure, however, the control survival declined to 66.7% in the SLD test and to 72.5% in the test using the SeMe diet, indicating compromised health. Therefore, only data collected up to 60 days post-test initiation were considered for analysis. Nevertheless, there remains the possibility that even at 60 days, the control organisms were not healthy, although overt signs of stress did not appear until later.

For the SeMe diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 7.355 mg Se/kg dw and an EC_{20} of 10.47 mg Se/kg dw. For the SLD diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 11.14 mg Se/kg dw and an EC_{20} of 15.73 mg Se/kg dw. Note: The San Luis Drain mosquitofish (comprising the Chinook salmon's SLD diet) were not tested for contaminants other than certain key elements. Because the San Luis Drain receives irrigation drainage from the greater San Joaquin Valley, there is a possibility that the SLD diet might have contained elevated levels of pesticides, possibly a confounding factor, although the SLD diet was less toxic than the SeMe diet.

1.4.2 Oncorhynchus mykiss (rainbow trout)

Hilton and Hodson (1983) reared juvenile rainbow trout on either a high (25 percent) or low (11 percent) available carbohydrate diet supplemented with sodium selenite for 16 weeks. Body weights, feed: gain ratios, and total mortalities were followed throughout the exposure every 28 days. Tissues (livers and kidneys) were extracted for selenium analysis after 16 weeks. By the end of the exposure, fish fed diets (low carbohydrate and high carbohydrate) with the highest selenium concentrations (11.4 and 11.8 mg Se/kg dw food, respectively) exhibited a 45 to 48 percent reduction in body weight (expressed as kg per 100 fish) compared to control fish. The authors attributed such results to food avoidance. With only two dietary exposure concentrations and a control, these data were not amenable to regression analysis. The MATC for growth of juvenile rainbow trout relative to the concentrations of selenium in liver tissue of trout reared on the high carbohydrate seleniferous dietary type is the geometric mean (GM) of 21.00 mg Se/kg dw liver (NOEC) and 71.7 mg Se/kg dw liver (LOEC), or 38.80 mg Se/kg dw liver. The calculated MATC for the same group of experimental fish exposed to selenium in the low carbohydrate diet is 43.5 mg Se/kg dw liver tissue, which is the same MATC for trout exposed for an additional 4 weeks based on the occurrence of nephrocalcinosis in kidneys (see Hicks et al. 1984; Appendix C).

Hilton et al. (1980) employed a similar test design to that of Hilton and Hodson (1983) to examine the narrow window at which selenium changes from an essential nutrient to a toxicant affecting juvenile rainbow trout. The food consisted of a casein-Torula yeast diet supplemented with selenium as sodium selenite. As discussed previously for the Ogle and Knight (1989) study with fathead minnow, this represents an unrealistic fraction of inorganic selenium in the diet. The experiment lasted for 20 weeks. During this time, the trout were fed to satiation 3 to 4 times per day, 6 days per week, with one feeding on the seventh day. Organs (liver and kidney) and carcasses were analyzed for selenium from fish sacrificed at 4 and 16 weeks. No gross histopathological or physiological effects were detected in the fish, although trout raised on the highest dietary level of selenium (13.06 mg Se/kg dw food) had a significantly lower body weight (wet basis), a higher feed:gain ratio, and higher number of mortalities (10.7; expressed as number per 10,000 fish days). The MATC for growth and survival of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue is the geometric mean of the NOEC (40 mg Se/kg dw liver) and the LOEC (100 mg Se/kg dw liver), or 63.25 mg Se/kg dw, both of which hinge on accepting dietary spiking entirely with inorganic selenium as an acceptable experimental protocol.

The non-reproductive GMCV for *Oncorhynchus* (both rainbow trout and Chinook salmon) is 9.052 mg Se/kg dw whole body based on the EC_{10} value derived from the Hamilton et al. (1990) study with Chinook salmon. The NOEC values for the rainbow trout studies conducted by Hilton and Hodson (1983), Hilton et al. (1980), and Hicks et al. (1984) were not used in the GMCV calculation because of the large difference between the NOEC and the LOEC values. If adult fish contained whole-body selenium concentrations equal to 9.052 mg Se/kg dw, their egg-ovary concentrations would be estimated to be 21.5 mg Se/kg dw when translated using the factor 2.37. The criterion values derived based on reproductive endpoints are protective of the endpoint measured.

1.5 Moronidae

1.5.1 Morone saxitilis (striped bass)

A non-reproductive chronic value for selenium was determined from a laboratory dietary exposure conducted using yearling striped bass (Coughlan and Velte 1989). During the experiment, the bass were fed contaminated red shiners (38.6 mg Se/kg dw whole body) from Belews Lake, NC (treated fish) or golden shiners with low levels of selenium (1.3 mg/kg dw whole body) purchased from a commercial supplier (control fish). The test was conducted in soft well water and lasted up to 80 days. During the experiment, all fish were fed to satiation 3 times per day. Control fish grew well and behaved normally.

Treated fish behaved lethargically, grew poorly due to a significant reduction in appetite, and showed histological damage, all eventually leading to the death of animals. The final selenium concentration in muscle of treated striped bass averaged from 16.2 to 18.5 mg/kg dw tissue (assuming 78.4 percent moisture content), which was 3.4 to 3.6 times higher than the final selenium concentrations in control striped bass, which averaged 5.10 mg/kg dw tissue. The chronic value for this species was determined to be <16.2 mg Se/kg dw in muscle tissue.

1.6 Centrarchidae

1.6.1 Lepomis macrochirus (bluegill)

Bryson et al. (1985b) conducted juvenile survival toxicity tests using hatchery bluegill and various forms of selenium spiked to an artificial diet as well as a diet consisting of zooplankton collected from Hyco Reservoir. There was no effect on length or weight of the juvenile bluegill after 60 days of exposure. The highest concentration of selenium measured in whole body of the juveniles in these tests was in the seleno-DL-cysteine-2X treatment (3.74 mg Se/kg dw).

Cleveland et al. (1993) performed a 90-day diet-only laboratory exposure in which juvenile bluegill were fed a range of selenomethionine concentrations added to Oregon moist™ pellets. The authors observed no significant effects on survival, but did report a very small but apparently statistically significant decrease in the condition factor, K, from 1.3 at four concentrations between 1.0 and 4.7 mg Se/kg dw whole body, to 1.2 at the two concentrations 7.7 and 13.4 mg Se/kg dw whole body. The condition factor (weight x 10^5/length^3) is intended to reflect a fish's reserves. In contrast to the studies of Ogle and Knight (1989), Hilton and Hodson (1983), and Hilton et al. (1989), which appear to have involved an inorganic selenium food palatability problem, this study did not use inorganic selenium in the diet. Nevertheless, given that the reduction in K (1.3 to 1.2) is slight and shows no increasing effect between 7.7 and 13.4 mg Se/kg dw, thus not yielding a sigmoidal concentration-response curve to support an EC_{10} calculation, the chronic value for this study was estimated at >13.4 mg Se/kg dw in whole body tissue.

Data from Lemly (1993a) indicate that over-wintering fish may be more susceptible to the effects of waterborne and dietary selenium due to increased sensitivity at low temperature. The author exposed juvenile bluegill in the laboratory to a single elevated exposure level, waterborne (1:1 selenite:selenate; nominal 5 µg Se/L) and foodborne (seleno-L-methionine in TetraMin; nominal 5 mg Se/kg dw food) selenium for 180 days. Tests with a control and the treated fish were run at 4°C and 20°C with biological and selenium measurements made every 60 days. Survival and whole-body lipid content were unaffected at 20°C (whole-body selenium concentrations equal to 6 mg/kg dw, the sole treatment exposure) when compared to control fish. Thus, at 20°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium based on survival was >6 mg/kg dw in whole-body tissue. Fish exposed to the combination low-level waterborne and dietary selenium at 4°C exhibited significantly elevated mortality (40.4 percent) relative to controls (2.9 percent), and exhibited significantly greater oxygen consumption and reduced lipid content, which are indicative of stress. At 4°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium was <7.91 mg Se/kg dw in whole body based on mortality and tissue measurements at the end of the test (180 days), and 5.85 mg Se/kg dw in whole body based on mortality at 180 days and tissue measurements at 60 days. The increase in the concentration of whole-body selenium between Day 60 and 180 at 4°C was apparently due to reductions in body weight caused by loss of lipid (comparatively low in selenium) while body burden in other tissues remained relatively constant. If this concentration of selenium in tissues occurs in sensitive overwintering fish in nature, a concentration of 5.85 mg/kg dw (the selenium tissue concentration in the 4ºC exposure after 60 days) in fish collected during the summer or fall months could be considered a threshold concentration for the selenium-sensitive fish during the winter months. Therefore, this study's chronic value for the threshold concentration prior to winter stress is 5.85 mg Se/kg dw in whole body tissue.

McIntyre et al. (2008) also investigated the toxicity of selenium to juvenile bluegill under cold temperature conditions in the laboratory. Whereas relative to the control, Lemly (1993a) tested only one exposure level, 5 mg Se/kg in the diet and 5 µg Se/L and one low temperature regime, 4°C, McIntyre et al. (2008) evaluated a range of diet and water concentrations, two types of diet, and two low-temperature regimes. The goal of the study was to determine EC_{10} and EC_{20} values for selenium exposure to juvenile bluegill in 4°C and 9°C low-temperature regimes. Three separate exposure systems were run concurrently for 182 days. Two systems exposed juvenile bluegill to a series of six aqueous and dietary selenium treatments and a control; one exposure system (ES1) with a cold temperature regime (4°C), and one (ES3) with a cool temperature regime (9°C), both using a yeast-worm-fish food chain bioaccumulation system. That is, graded levels of selenized-yeast in ES1 and ES3 were fed to the oligochaete, *Lumbriculus variegatus*, which in turn was fed to bluegill. The third exposure system (ES2) used diet and exposure conditions similar to Lemly's 4°C treatment, i.e., nominal 5 µg Se/L in the water and nominal 5 mg Se/kg dw food (seleno-L-methionine in TetraMin). The cold temperature regime for ES1 and ES2 was 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The cool temperature regime (ES3) was similar except when the temperature reached 9°C (test day 65), it was maintained until test termination (test day 182).

At the end of the 182 day exposure in the ES2 (with Lemly's diet and temperature), the bluegill accumulated an average (geometric mean) whole body concentration of 9.99 mg/kg dw with no meaningful mortality in the treatment or control. Significant mortality of juvenile bluegill was observed in the two highest treatments in the cold (ES1) and cool (ES3) *Lumbriculus*-fed tests. No effects on body weight or condition factor were observed. The EC_{10} and EC_{20} values for the cold treatment (ES1) are 9.27 and 9.78 mg Se/kg dw in whole body, respectively. The EC_{10} and EC_{20} values for the cool treatment (ES3) are slightly higher at 14.00 and 14.64 mg Se/kg dw in whole body, respectively.

The design and the results of the McIntyre et al. (2008) study have similarities and differences with Lemly (1993a), as presented in detail with comparisons and contrasts in Appendix C. Both studies found juvenile bluegill were more sensitive in a cold-temperature regime than in a cool (McIntyre et al.) or a warm regime (Lemly). The effect levels determined for the cold temperature regime differed by a factor of 1.58 (ES1 of McIntyre et al., 9.27 mg Se/kg; Lemly, 5.85 mg Se/kg), a difference rather typical of chronic studies conducted in different laboratories using different fish populations (Delos 2001) and similar to the 1.51 factor difference between two EC_{10}s of Hamilton et al. (1990) for chinook salmon.

The difference in the effect levels of the McIntyre ES2 exposure (>9.99 mg/kg) and the Lemly study (5.85 mg/kg) could have been due to the fitness of the fish entering the cold regime. The condition factor, K, in the ES2 selenium-exposed bluegill increased from 3.2 at the start of the exposure to 5.2 at day 60 (approximately 10°C at day 60) and decreased only slightly through over 100 days of 4°C exposure (see figure in bluegill summary in Appendix C). In contrast, K in the Lemly selenium-exposed fish decreased approximately 50% after 120 days of exposure. Shoup and Wahl (2011) conducted an overwinter exposure study with bluegill in which they fed and starved young of year bluegill (the larger size similar to the McIntyre and Lemly fish) under two temperature regimes, 4°C (harsh winter) and 9°C (mild winter) for 140 days and a 10 h light:14 h dark photoperiod. The juvenile bluegill in the Shoup and Wahl study ate in both temperature regimes. The 4°C exposed fish consumed 0.4-0.8% of their body weight/day and their K was not significantly different at the end of the test compared to the start. The Shoup and Wahl results only provide an indication that cold-exposed fish under a winter photoperiod feed and can maintain K.

The mortality observed in the Lemly laboratory study does not appear to be consistent with field observations. The occurrence of mortality in the field at the concentrations Lemly (1993a) reported to cause mortality in his lab was not observed in the Lemly (1993b) field study of centrarchid deformities in Belews Lake. In that field study, Lemly (1993b) found larval centrarchid deformities at concentrations ranging from 12-80 mg Se/kg dw WB. If juvenile mortality occurred at concentrations lower than those found to induce larval deformities and at concentrations as low as Lemly (1993a) reported in the lab (EC_{40} = 7.91 mg Se/kg WB), then centrarchids would likely not have been present in Belews Lake. The observations of Lemly (1993b) are evidence that larval deformity, not juvenile mortality, is the more sensitive endpoint.

The Crutchfield and Ferson (2000) predictions and field observations of recovery of bluegill at Hyco Reservoir likewise suggest that significant mortality was unlikely to be occurring at the concentrations Lemly (1993a) reported to cause substantial mortality. During a time period over which Crutchfield (2000) indicated dietary invertebrate concentrations exceeded 20 mg Se/kg dw, Crutchfield and Ferson (2000) indicated that bluegill population growth occurred at rates predicted to be natural for the unimpaired species. In contrast, if the Lemly (1993a) lab EC_{40} of 7.91 mg Se/kg dw whole-body were applicable to this field situation, the mortality associated with the resulting bluegill whole-body concentrations (25 mg Se/kg dw whole-body, assuming a trophic transfer factor of 1.27) would have prevented any recovery.

Selenium-induced cold temperature loss of lipid and body condition, a non-reproductive sublethal effect that Lemly (1993a) observed to accompany juvenile mortality in the laboratory (but which McIntyre et al. (2008) did not observe in a similar study) has also not generally been corroborated by field evidence (Janz 2008). Several studies have measured growth and energy storage indicators in juvenile fish just prior to and just after winter at reference sites and sites with elevated selenium in northern Canada (Bennett and Janz 2007a, b; Kelly and Janz 2008; Driedger et al 2009; Weber et al. 2008). The growth (length, weight, condition factor, muscle RNA:DNA ratio, muscle protein) and energy storage (whole body lipids, whole body triglycerides, liver triglycerides, liver glycogen) indicators for five fish species (northern pike, burbot, fathead minnow, creek chub, white sucker) measured just after winter were similar or greater than those measured just before winter at the selenium exposed sites. The slimy sculpin did show a decrease in whole body triglycerides, but the reduction was similar at exposed and reference sites.

Given the uncertainty in the occurrence of winter stress, the results of all four cold-temperature (4°C and 9°C) juvenile-survival lab studies were combined per the standard procedure described in the U.S.EPA Ambient Water Quality Criteria Guidelines, to determine the non-reproductive SMCV for bluegill. The SMCV for the combined 4°C and 9°C tests is 9.33 mg Se/kg dw whole body, based on the four chronic values: (a) the Lemly (1993a) concentration prior to winter stress (5.85 mg Se/kg dw whole body), (b) the McIntyre et al. (2008) ES1 EC_{10} (9.27 mg Se/kg dw whole body), (c) the McIntyre et al. (2008) ES2 NOEC (>9.992 mg Se/kg dw whole body), and the McIntyre et al. (2008) ES3 EC_{10} of 14.00 mg Se/kg dw whole body. This value is not less than the reproductive endpoint-based whole-body criterion concentration of 8.5 mg Se/kg dw. The studies of Bryson et al (1985b) and Cleveland et al. (1993) were not conducted at cold temperatures and were thus not used for these SMCV calculations.

Table D-1. Freshwater Chronic Values from Acceptable Tests - Non-Reproductive Endpoints (Parental Females Not Exposed).
(Same as Table 6.2 in the main document).

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Acipenser transmontanus white sturgeon	Tashjian et al. 2006	dietary (lab) 8 weeks	seleno-L-methionine in artificial diet seleno-L-methionine in artificial diet	EC_{10} juvenile growth	15.08 WB 27.76 M	EC_{10} 15.1 WB 27.8 M	15.1 WB 27.8 M
				EC_{20} juvenile growth	17.82 WB 32.53 M	EC_{20} 17.8 WB 32.5 M	
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	dietary (lab) 9 months	selenized-yeast	NOEC	10.1 M	10.1 M 15.1 M 12.3 M	10.1 M 15.1 M 12.3 M
				LOEC	15.1 M		
				MATC juvenile deformities (juvenile exposure only)	12.34 M		
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab) 9 to 19 days	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40 WB	51.40 WB 69.83 M	51.40 WB 69.83 M
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab) 8 days	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOEC for larval fish dry weight after 8 d	<73 WBb		
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>12.9 WBb	see text	see text
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001b	dietary and waterborne (lab) 28 days	water: site waters; diet: algae exposed to site water then fed to rotifers which were fed to fish	NOEC for survival and growth	>42 WBb		
Catostomus latipinnis flannelmouth sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>10.2 WB	>10.2 WB	>10.2 WB

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab) 60 days	mosquitofish spiked with seleno-DL-methionine	EC_{10} for juvenile growth	7.355 WB	EC_{10} 9.052 WB EC_{20} 12.83 WB	EC_{10} 9.052 WB
				EC_{20} for juvenile growth	10.47 WB		
			mosquitofish spiked with SLD diet	EC_{10} for juvenile growth	11.14 WB		
				EC_{20} for juvenile growth	15.73 WB		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab) 16 weeks	sodium selenite in food preparation	juvenile growth NOEC	21 Liver	NOAEC 28.98 L LOAEC 84.68 L MATC 49.52 L	
				LOEC	71.7 Liver		
				MATC	38.80 Liver		
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juvenile survival and growth NOEC	40 Liver		
				LOEC	100 Liver		
				MATC	63.25 Liver		
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab) 80 days	Se-laden shiners from Belews Lake, NC	LOEC for survival of yearling bass	<16.2 M[c]	<16.2 M	<16.2 M
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab) 180 days 20 to 4°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOEC for juvenile mortality at 4oC	<7.91 WB	4°C EC_{10}-NOAEC 8.15 WB 4°C EC_{20}-LOAEC 8.80 WB 9°C EC_{10} 14.0 WB 9°C EC_{20} 14.6 WB	4°C & 9°C 9.33 WB
				Threshold prior to "winter stress"	5.85 WB		
		dietary and waterborne (lab) 180 days 20°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC for juvenile mortality at 20oC	>6.0 WB		
Lepomis macrochirus bluegill	McIntyre et al. 2008	dietary and waterborne (lab) 182 days 20 to 4°C (ES1)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES1	9.27 WB		
				EC_{20} juv. survival ES1	9.78 WB		
		dietary and waterborne (lab) 182 days 20 to 9°C (ES3)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES3	14.00 WB		
				EC_{20} juv. survival ES3	14.64 WB		

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
		dietary and waterborne (lab) 182 days 20 to 4°C (ES2)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC juv. surv. ES2	>9.992 WB		
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary (lab) 60 days	seleno-DL-cysteine	NOEC for juvenile growth	>3.74 WBb		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary (lab) 90 days	seleno-L-methionine	NOEC for juvenile survival	>13.4 WBb		

[a] All chronic values reported in this table are based on the measured concentration of selenium in whole body (WB), muscle (M) or liver (L) tissues.

[b] Chronic value not used in SMCV calculation (see text).

[c] Tissue value converted from ww to dw. See Appendix C for conversion.

APPENDIX E: OTHER DATA

1.0 SELENITE

Additional data on the lethal and sublethal effects of selenium on aquatic species are presented in Table E-1. Bringmann and Kuhn (1959a,b, 1976, 1977a, 1979, 1980b, 1981), Jakubczak et al. (1981), and Patrick et al. (1975) reported the concentrations of selenite that caused incipient inhibition (defined variously, such as the concentration resulting in a 3% reduction in growth) for algae, bacteria, and protozoans (Table E-1). Although incipient inhibition might be statistically significant, its ecological importance is unknown. Albertano and Pinto (1986) found the growth of three red algal species was inhibited at selenite concentrations that ranged from 790 to 3,958 µg/L.

2.0 SELENATE

Dunbar et al. (1983) exposed fed *D. magna* to selenate for seven days and obtained an LC_{50} of 1,870 µg/L. This value is in the range of the 48-hr EC_{50}s in Table E-1.

Watenpaugh and Beitinger (1985a) found that fathead minnows did not avoid 11,200 µg/L selenate during 30-minute exposures (Table E-1). These authors also reported (1985b) a 24-hr LC_{50} of 82,000 µg/L for the same species and they found (1985c) that the thermal tolerance of the species was reduced by 22,200 µg/L. Westerman and Birge (1978) exposed channel catfish embryos and newly hatched fry for 8.5 to 9 days to an unspecified concentration of selenate. Albinism was observed in 12.1 to 36.9% of the fry during the five years of such exposures. Pyron and Beitinger (1989) also investigated fathead minnows, and after a 24-hr exposure, no effect on reproductive behavior was found at 36,000 µg/L, but when adults were exposed to 20,000 µg/L selenate for 24-hr, edema was observed for their larvae.

The respiratory rate of the eastern oyster, *Crassostrea virginica*, was unaffected by exposure to selenate at 400 µg/L for 14 days (Fowler et al. 1981). Embryos of the striped bass were quite tolerant to selenate in dilute salt water (Klauda 1985a, b). There was a 93% successful hatch of embryos at 200,000 µg/L, but 50% of 72-day-old juveniles died after four days at 87,000 µg/L. Exposure of juvenile fish for up to 65 days to concentrations of selenate between 39 and 1,360 µg/L caused developmental anomalies and pathological lesions.

Table E-1. Other Data on Effects of Selenium on Aquatic Organisms

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	96 hr	Incipient inhibition (river water)	2,500	Bringmann and Kuhn 1959a,b
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	Decreased dry weight and chlorophyll a	75	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 12-21[b]	10-100	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 11,164[c]	150	Foe and Knight, Manuscript
Alga, *Chrysochromulina breviturrita*	Selenious acid	-	30 days	Increased growth	320	Wehr and Brown 1985
Red alga, *Cyanidium caldarium*	Selenious acid	-	20 days	Inhibited growth	3,958	Albertano and Pinto 1986
Red alga, *Cyanidioschyzon merolae*	Seleniousacid	-	20 days	Inhibited growth	3,140	Albertano and Pinto 1986
Red alga, *Galdieria sulphuraria*	Seleniousacid	-	20 days	Inhibited growth	790	Albertano and Pinto 1986
Algae (diatoms), Mixed population	Sodium selenite	-	18 days	Inhibited growth	11,000	Patrick et al. 1975
Bacterium, *Escherichia coli*	Sodium selenite	-	-	Incipient inhibition	90,000	Bringmann and Kuhn 1959a
Bacterium, *Pseudomonus putida*	Sodium selenite	-	16 hr	Incipient inhibition	11,400 (11,200)	Bringmann and Kuhn 1976; 1977a; 1979; 1980b
Protozoan, *Entosiphon sulcatum*	Sodium selenite	-	72 hr	Incipient inhibition	1.8 (1.9)	Bringmann 1978; Bringmann and Kuhn 1979; 1980b; 1981
Protozoan, *Microreqma heterostoma*	Sodium selenite	-	28 hr	Incipient inhibition	183,000	Bringmann and Kuhn 1959b
Protozoan, *Chilomonas paramecium*	Sodium selenite	-	48 hr	Incipient inhibition	62	Bringmann and Kuhn 1981; Bringmann et al. 1980

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Protozoan, *Uronema parduezi*	Sodium selenite	-	20 hr	Incipient inhibition	118	Bringmann and Kuhn 1980a; 1981
Snail, *Lymnaea stagnalis*	Sodium selenite	-	7.5 days	LT50	3,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenite	-	48 hr	EC50 (river water)	2,500	Bringmann and Kuhn 1959a,b
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	LC50	16,000	Bringmann and Kuhn 1977a
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	EC50 (swimming)	9.9	Bringmann and Kuhn 1977b
Cladoceran, *Daphnia magna*	Sodium selenite	329	48 hr 96 hr 14 days	EC50 (fed)	710 430 430	Halter et al. 1980
Cladoceran (<24 hr), *Daphnia magna*	Sodium selenite	-	48 hr 21 days	EC50 (fed)	685 160	Adams and Heidolph 1985
Cladoceran (5th instar), *Daphnia magna*	Sodium selenite	-	48 hr	LC50 (fed)	680	Johnston 1987
Cladoceran, *Daphnia magna*	Selenious acid	220[d]	48 hr	LC50 (fed)	1,200	Kimball, Manuscript
Cladoceran (preadult), *Daphnia pulex*	Sodium selenite	42	24 hr	Did not reduce oxygen consumption or filtering rate	>498	Reading and Buikema 1980
Ostracod, *Cyclocypris* sp.	Sodium selenite	100.8	48 hr	LC50	130,000	Owsley 1984
Amphipod, *Hyalella azteca*	Sodium selenite	329	14 days	LC50 (fed)	70	Halter et al. 1980
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	48 hr	LC50	623	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodiumselenite	133	10 days	LC50 (fed)	312	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	24 days	LOEC reproduction (static-renewal)	200	Brasher and Ogle 1993
Midge (first instar), *Chironomus riparius*	Sodium selenite	134	48 h	LC50	7,950	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenite	40-48	48 h	LC50	14,600	Ingersoll et al. 1990
Coho salmon (fry), *Oncorhynchus kisutch*	Sodium selenite	325	43 days	LC50	160	Adams 1976
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	LC50	460	Adams 1976

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	Reduced growth	250	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	330	5 days	LC50	2,700 2,750	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	48 days	LC50	500	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	96 days	LC50	280	Adams 1976
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	200	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	4.7 µg/g dw (whole-body)	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	BCF = 23	100	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC growth (dietary only exposure)	>9.96 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC survival (dietary only exposure)	5.34 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	9 days	LC50	7,020	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	7,200 5,410	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	8,200 6,920	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	41 days	LOAEC (Reduced hatch of eyed embryos)	26	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	50 wk	Decreased iron in blood and red cell volume	53	Hodson et al. 1980
Rainbow trout (fertilized egg), *Oncorhynchus mykiss*	Sodium selenite	135	44 wk	BCF = 33.2 BCF = 21.1	53	Hodson et al. 1980
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	120 hr	Did not reduce survival or time to hatch	10,000	Klaverkamp et al. 1983b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	-	90 days	Chronic value for survival	14	Mayer et al. 1986
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	LC50	55.2[e]	Hunn et al. 1987
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	MATC survival	31.48	Hunn et al. 1987
Rainbow trout (egg), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 17.5 BCF = 3.5	0.4 45.6	Hodson et al. 1986
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 3.1 BCF = 3.0	0.4 45.6	Hodson et al. 1986
Rainbow trout (sac-fry), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 13.1 BCF = 1.6	0.4 45.6	Hodson et al. 1986
Rainbow trout (swim-up fry) *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 80.3 BCF = 20.2	0.4 45.6	Hodson et al. 1986
Northern pike, *Esox lucius*	Sodium selenite	10.2	76 hr	LC50	11,100	Klaverkamp et al. 1983a
Goldfish, *Carassius auratus*	Selenium dioxide	157	14 days	LC50	6,300	Cardwell et al. 1976a,b
Goldfish, *Carassius auratus*	Sodium selenite	-	10 days	Mortality	5,000	Ellis 1937; Ellis et al. 1937
Goldfish, *Carassius auratus*	Sodium selenite	-	46 days	Gradual anorexia and mortality	2,000	Ellis et al. 1937
Goldfish, *Carassius auratus*	Selenium dioxide	-	7 days	LC50	12,000	Weir and Hine 1970
Goldfish, *Carassius auratus*	Selenium dioxide	-	48 hr	Conditional avoidance	250	Weir and Hine 1970
Fathead minnow, *Pimephales promelas*	Selenium dioxide	157	9 days	LC50	2,100	Cardwell et al. 1976a,b
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	96 hr	LC50 (fed)	1,000	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	14 days	LC50 (fed)	600	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Selenious acid	220[d]	8 days	LC50 (fed)	420	Kimball, Manuscript
Creek chub, *Semotilus atromaculatus*	Selenium dioxide	-	48 hr	Mortality	∃12,000	Kim et al. 1977
Bluegill, *Lepomis macrochirus*	Sodium selenite	318	48 days	LC50	400	Adams 1976
Bluegill, *Lepomis macrochirus*	Selenium dioxide	157	14 days	LC50	12,500	Cardwell et al. 1976a,b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	16	323 days	MATC larval survival (dietary only exposure)	19.75 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	25 and 200	120 days	No mortality	>10	Lemly 1982
Largemouth bass (juvenile), *Micropterus salmoides*	Sodium selenite	25 and 200	120 days	No mortality	10	Lemly 1982
Yellow perch, *Perca flavescens*	Sodium selenite	10.2	10 days	LC50	4,800	Klaverkamp et al. 1983a,b
African clawed frog, *Xenopus laevis*	Sodium selenite	-	7 days	LC50	1,520	Browne and Dumont 1980
African clawed frog, *Xenopus laevis*	Sodium selenite	-	1-7 days	Cellular damage	2,000	Browne and Dumont 1980
Selenium (VI)						
Alga, *Chrysochromulina breviturrita*	-	-	30 days	Increased growth	50	Wehr and Brown 1985
Rotifer, *Brachionus calyciflorus*	Sodium selenate	120	96 hr	EC20 Growth (dry weight)	42.36 (µg/g dw)	Dobbs et al. 1996
Snail, *Lymnaea stagnalis*	Sodium selenate	-	6 days	LT50	15,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenate	129.5	7 days	LC50 (fed)	1,870	Dunbar et al. 1983
Cladoceran (juvenile), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	550	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	750	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	90 hr	42% of organisms had visible changes in gut morphology	250	Johnston 1989
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	48 hr	LC50	2378	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	10 days	LC50 (fed)	627	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	24 days	LOEC reproduction (static renewal)	>700	Brasher and Ogle 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	18 (SO_4=3.4)	10 days	LC50 (fed)	43	Borgmann et al. 2005
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	124 (SO_4=32)	10 days	LC50 (fed)	371	Borgmann et al. 2005
Midge (first instar), *Chironomus riparius*	Sodium selenate	134	48 h	LC50	16,200	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenate	40-48	48 h	LC50	10,500	Ingersoll et al. 1990
Rainbow trout (embryo, larva), *Oncorhynchus mykiss*	Sodium selenate	104 (92-110)	28 days	EC50 (death and deformity)	5,000 (4,180) (5,170)	Birge 1978; Birge and Black 1977; Birge et al. 1980
Goldfish (embryo, larva), *Carrassius auratus*	Sodium selenate	195	7 days	EC50 (death and deformity)	8,780	Birge 1978
Goldfish, *Carassius auratus*	Sodium selenate	-	24 hr	BCF = 1.42 BCF = 1.15 BCF = 1.47 BCF = 0.88 BCF = 1.54	0.45 0.9 1.35 2.25 4.5	Sharma and Davis 1980
Fathead minnow, *Pimephales promelas*	Sodium selenate	337.9	48 days	LC50	2,000	Adams 1976
Fathead minnow, *Pimephales promelas*	Sodium selenate	338	48 days	LC50	1,100	Adams 1976
Fathead minnow, *Pimephales promelas*	-	51	30 min	No avoidance	11,200	Watenpaugh and Beitinger 1985a
Fathead minnow, *Pimephales promelas*	-	-	24 hr	LC50	82,000	Watenpaugh and Beitinger 1985b
Fathead minnow, *Pimephales promelas*	-	-	24 hr	Reduced thermal tolerance	22,200	Watenpaugh and Beitinger 1985c
Fathead minnow, *Pimephales promelas*	Sodium selenate	44-49	7 days	Chronic value - growth Chronic value-growth Chronic value-survival	1,739 561 2,000	Norberg-King 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	No effect on reproductive behavior	36,000	Pyron and Beitinger 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	Edema in larvae produced from adults exposed to Selenium VI	20,000	Pyron and Beitinger 1989

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Channel catfish (embryo, fry), *Ictalurus punctatus*	Sodium selenate	90	8.5-9 days	Induced albinism	-	Westerman and Birge 1978
Narrow-mouthed toad (embryo, larva), *Gastrophryne carolinensis*	Sodium selenate	195	7 days	EC50 (death and deformity)	90	Birge 1978; Birge and Black 1977; Birge et al. 1979a
Organo-selenium						
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	16	323 days	MATC larval survival (dietary only exposure)	20.83 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	283	90 days	EC20 survival (dietary only exposure)	>13.4 µg/g dw (food)	Cleveland et al. 1993
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	53.83 µg Se/g dw (liver)	Reash et al. 1999
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	23.38 µg Se/g dw (ovaries)	Reash et al. 1999
Redear sunfish (adult), *Lepomis microlophus*	Selenium	-	field	LOEC Adverse histopathological alterations	<38.15 µg Se/g dw	Sorensen 1988
Selenium Mixtures						
Phytoplankton, Mixed population	Selenium	-	field	Reduced growth rates	18	Riedel et al. 1991
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC growth	115.2 µg Se/L	Ingersoll et al. 1990
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC productivity	21.59 µg/g dw (whole-body)	Ingersoll et al. 1990
Midge (<24-hr), *Chironomus riparius*	Selenite-Selenate mixture	138	30 days	MATC emergence	503.6	Ingersoll et al. 1990
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	NOEC survival	340	Cleveland et al. 1993
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	EC20 survival	4.07 µg/g dw (whole body)	Cleveland et al. 1993

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Anaerobic bacterium, *Methanococcus vannielli*	Sodium selenite	-	110 hr	Stimulated growth	79.01	Jones and Stadtman 1977
Bacterium, *Vibrio fisheri*	Sodium selenite	-	5 min	50% decrease in light output (Microtox7)	68,420	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenite	32	14 days	5-12% increase in growth	10-10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenite	32	14 days	23% increase in growth	100-10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenite	32	20 days	Increased growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b
Diatom, *Skeletonema costatum*	Selenium dioxide	-	5 days	BCF = 18,000 BCF = 16,000 BCF = 10,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Chaetoceros muelleri*	Selenium dioxide	-	6 days	BCF = 337,000 BCF = 65,000 BCF = 5,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Phaeodactylum tricornutum*	Selenium dioxide	-	8 days	BCF = 109,000 BCF = 27,000 BCF = 7,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Thallassiosira aestivalis*	Selenium oxide	29-30	72 hr	No effect on cell morphology	78.96	Thomas et al. 1980a
Brown alga, *Fucus spiralis*	Sodium selenite	-	60 days	1355% increase in growth of thalli	2.605	Fries 1982
Red alga, *Porphyridium cruentum*	Sodium selenite	32	27 days	Increase growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Selenium (VI)						
Bacterium, *Vibrio fisheri*	Sodium selenate	-	15 min	50% decrease in light output (Microtox7)	3,129,288	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenate	32	14 days	No effect on rate of cell	10-1,000	Wheeler et al. 1982
Green alga, *Chlorella* sp.	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10-100	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	71% reduction in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	16% decrease in rate of cell population growth	100	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	50% decrease in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Brown alga, *Fucus spiralis*	Sodium selenate	-	60 days	160% increase in growth rate of thalli	2.605	Fries 1982
Red alga, *Porphridium cruentum*	Sodium selenate	32	14 days	23-35% reduction in rate of cell population growth	10-1,000	Wheeler et al. 1982
Red alga, *Porphyridium cruentum*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Eastern oyster (adult), *Crassostrea virginica*	Sodium selenate	34	14 days	No significant effect on respiration rate of gill tissue	400	Fowler et al. 1981
Striped bass (embryo), *Morone saxatilis*	Sodium selenate	7.2-7.5	4 days	93% successful hatch and survive	200,000	Klauda 1985a,b
Striped bass (larva), *Morone saxatilis*	Sodium selenate	4.0-5.0	4 days	LC50 (control survival= 77%)	13,020	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	9-65 days	Significant incidence of development anomalies of lower jaw	39-1,360	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	45 days	Significant incidence of severe blood cytopathology	1,290	Klauda 1985a,b

[a] Concentration of selenium, not the chemical. Units are µg selenium/L of water unless noted otherwise.
[b] Converted from dry weight to wet weight basis (see Guidelines).
[c] Growth of algae was inhibited.
[d] From Smith et al. (1976).
[e] Calculated from the published data using probit analysis and allowing for 8.9% spontaneous mortality.

3.0 OTHER DATA - ENDANGERED SPECIES

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the endangered species, razorback sucker, *Xyrauchen texanus* (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results in the context of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). A summary of each of these two studies is presented below.

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1996 (Hamilton et al. 2001a; also Hamilton et al. 2005 a,b,c)

This study was initiated with 5-day old razorback sucker larvae spawned from adults (first time spawners) which were previously held (9 months) in three different locations along the Colorado River that contained varying levels of selenium: Horsethief (the designated reference site which receives water pumped directly from the Colorado River near Fruita, CO, and where dissolved selenium concentrations in water ranged from <1.6 to 3.9 µg/L during the period of exposure), Adobe Creek (low level selenium contamination - dissolved selenium concentrations in water ranged from 1.5 to 11.6 µg/L; avg. = 3.8 µg/L), and North Pond (high level selenium contamination - dissolved selenium concentrations in water ranged from 3.8 to 19.6 µg/L; avg. = 9.5 µg/L). The selenium content in eggs from three Horsethief females ranged from 5.8 to 6.6 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 3.4 to 5.0 mg Se/kg dw. The selenium content in the eggs from three Adobe Creek females ranged from 38.0 to 54.5 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 11.5 to 12.9 mg Se/kg dw. The selenium content in the eggs from three North Pond females ranged from 34.3 to 37.2 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 14.1 to 17.3 mg Se/kg dw. The selenium content in eggs from one of three hatchery brood stock females was 7.1 mg Se/kg dw, and the selenium content in muscle plugs of two of three hatchery brood stock females at spawning ranged from 2.6 to 13.8 mg Se/kg dw. The razorback sucker larvae spawned from fish hatchery brood stock (older, previously spawned females) and held in Colorado River (Horsethief) water were used as an additional reference group of test fish.

The experimental groups were subdivided into those receiving reference water (hatchery water; 24-Road Fish Hatchery) or site water (Table E-2). They were further subdivided into those receiving a daily ration of reference food (brine shrimp) or zooplankton (predominantly cladocerans and copepods) collected from each site where their parents were exposed for the previous 9 months. A total of 60 larvae from each of the four adult sources (Horsethief, Adobe Creek, North Pond, Brood Stock held in different ponds at Horsethief) were exposed to each treatment (2 replicates x 3 spawns x 10 fish/beaker). The larvae were held in beakers containing 800 ml of test water. Fifty percent of the test water was renewed daily.

Table E-2. Treatment conditions during the 30-day larval study.

Source of Larvae	Treatments	Se in food (mg/kg dw)	Dissolved Se in water (µg/L)
Horsethief Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9
Adobe Creek Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	5.5
	Site food: Reference water	20	< 1.6
	Site food: Site water	20	5.5
North Pond Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	10.7
	Site food: Reference water	39	<1.6
	Site food: Site water	39	10.7
Hatchery raised Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9

Growth, survival and development were evaluated amongst treatment groups for up to 30 days in the treatment conditions. Each treatment group was fed once daily after renewal. Test waters were collected every day from each site as grab samples for the renewal. A small portion of this water was retained at 3- and 7-day intervals for an analysis of total and dissolved selenium concentrations. At approximately 2-day intervals, aquatic invertebrates and brine shrimp not used for feeding were sieved from the media for selenium analysis. The number of live fish was recorded daily. After the 30-day exposure period, the surviving fish were sacrificed and measured for total length. At this same time,

approximately four fish from each treatment, when available, were collected as a composite sample and analyzed for total selenium.

After 30 days of exposure in the reference food-reference water treatment, survival of razorback sucker larvae from brood stock and Horsethief adults (89 and 87 percent, respectively) was slightly higher than those from Adobe Creek adults (84 percent) and North Pond adults (75 percent). Corresponding selenium concentrations in larval whole-body tissue were 3.6, 3.3, 7.7 and 9.7 mg Se/kg dw, respectively. Survival was similar or slightly reduced in larvae from all four sources after 30 days of exposure in the reference food-site water treatments; corresponding selenium concentrations in larval whole-body tissue were 5.2, 5.1, 12.7 and 15.2 mg Se/kg dw, respectively. In contrast, none of the larvae spawned from parents from Horsethief, Adobe Creek, or North Pond survived to 30 days when fed zooplankton collected from the three sites, irrespective of the water type they were exposed to (i.e., reference or site). Only the larvae from brood stock adults, which were fed zooplankton from the Horsethief site for this treatment, survived, and even these larvae suffered substantial mortality (40 and 60 percent respectively). The mean selenium concentrations in whole-body tissue of larvae from brood stock adults after the 30-day exposures were 5.4 mg Se/kg dw (site food-reference water treatment) and 6.9 mg Se/kg dw (site food-site water treatment).

Several inconsistencies were observed that indicate selenium may not be solely responsible for the effect on larval survival. Larval survival in the Adobe Creek treatment group exposed to reference water (<1.6 µg/L) and reference food (2.7 mg Se/kg dw) was 84 percent, similar to survival of larvae from brood stock (89 percent). The selenium concentration in the larvae from this Adobe Creek treatment group after 30 days was higher (7.7 mg/kg dw) than that of the brood stock fish (5.4 mg Se/kg dw) in the reference water (<1.6 µg/L) and site food (5.6 mg Se/kg dw) treatment, which had a 30-day survival of 62 percent. Also, the time to 50 percent mortality between the site food treatments, where most mortality occurred, was not related to selenium concentration in the diet or in the larvae.

Although the larvae from brood stock held at Horsethief and the larvae from the first-time spawning adults held at Horsethief that were used for the 9 month exposure received the same site food, no larvae from the latter group survived the 30 day exposure. Concentrations of selenium in the larvae of these two treatment groups were essentially the same between days 6 and 12 of the exposure (8.1 to 8.9 mg Se/kg dw). During this same general time frame (6 to 7 days of exposure), larvae from Adobe Creek and North Pond adults apparently tolerated up to 32 and 39 mg Se/kg dw in tissue, respectively, without any increase in mortality when exposed to reference food and reference water. Larvae grown out under hatchery conditions from adults in the Horsethief and Adobe Creek treatments also did not differ in total deformities compared to larvae from brood stock. There was also no difference between treatments (brood stock, Horsethief, Adobe Creek, and North pond) in percent egg viability, percent hatchability,

percent embryos with deformities, and percent mortality of deformed embryos and larvae from a separate test initiated with eggs in the same study (Hamilton et al. 2005b).

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1997 (Hamilton et al. 2001b)

In a similar 30-day larval study conducted by the authors in the following year (1997), razorback sucker larvae from a single hatchery brood stock female (11 mg Se/kg dw muscle) were subjected to the sixteen different combined water and dietary exposure conditions described in the earlier (1996) study. The female parent was held at Horsethief Canyon State Wildlife Area before spawning. The larvae were held in beakers containing 800 ml of test water as before; fifty percent of the test water was renewed daily. Specific treatment conditions for the 1997 30-day larval study are listed in Table E-3.

Table E-3. Treatment conditions during the 30-day larval study.

Water Treatments	Se in food (mg/kg dw)	Se in water (µg/L)
Reference food (brine shrimp): Reference water (24-Road Hatchery)	3.2	< 1
Reference food: Site water (Horsethief)	6.0	1.6
Reference food: Site water (Adobe Creek)	32.4	3.4
Reference food: Site water (North Pond)	52.5	13.3
Horsethief food: Reference water	3.2	< 1
Horsethief food: Site water (Horsethief)	6.0	1.6
Horsethief food: Site water (Adobe Creek)	32.4	3.4
Horsethief food: Site water (North Pond)	52.5	13.3
Adobe Creek food: Reference water	3.2	< 1
Adobe Creek food: Site water (Horsethief)	6.0	1.6
Adobe Creek food: Site water (Adobe Creek)	32.4	3.4
Adobe Creek food: Site water (North Pond)	52.5	13.3
North Pond food: Reference water	3.2	< 1
North Pond food: Site water (Horsethief)	6.0	1.6
North Pond food: Site water (Adobe Creek)	32.4	3.4
North Pond food: Site water (North Pond)	52.5	13.3

After 30 days of exposure in this study, there was also good survival of razorback sucker larvae fed reference food (brine shrimp) and held in reference water or water from Horsethief (83 and 81 percent, respectively). The survival of these larvae was significantly greater than survival of larvae fed brine shrimp and held in water from North Pond (52 percent). Corresponding selenium concentrations in larval whole-body tissue after 10 days were 6.3, 6.7, and 11 mg Se/kg dw, respectively. The average concentrations of selenium in the water for the three treatments were <1, 1.6, and 13.3 µg Se/L. After 30

days the mean selenium concentrations in these larvae were 5.2, 5.2, and 16 mg Se/kg dw, respectively. Survival was markedly reduced (0 to 30 percent survival) in the remaining treatments where larvae were fed zooplankton from the various sites. Complete mortality was experienced by larvae exposed to Horsethief food and reference water treatment after 30 days.

Similar to the previous study, several inconsistencies in results suggested that selenium may not have been solely responsible for the effect on larval survival. The most notable inconsistency was that the greatest effect on larval survival (percent survival or time to 50 percent mortality) was from exposure to Horsethief food, the food with the lowest selenium contamination.

The authors of the above two studies (Hamilton et al. 2001a,b) make a strong argument that some of the inconsistency in response observed in their studies between larvae fed reference and site diets may be related to the difference in arsenic concentration between the two diets. The arsenic concentration measured in the brine shrimp used in the reference diet was 24 mg total As/kg dw (measured in the second larval study) versus between 6 and 7.5 mg total As/kg dw measured in the zooplankton from the various sites. In their publication (Hamilton et al. 2005c), the authors cite several studies reporting an ameliorating effect of arsenic against the toxicity of a variety of forms of selenium in various animals (Dubois et al. 1940, Hoffman et al. 1992, Klug et al. 1949, Levander 1977, Moxon 1938, Thapar et al. 1969). In terms of the survival of larvae from Horsethief, Adobe Creek and North Pond adults when fed the reference diet, the authors propose that the arsenic concentrations in the brine shrimp diet may have resulted in an antagonistic interaction with selenium and reduced adverse effects in larvae. Such hypothesis is questionable, because their studies included diets spiked with inorganic arsenic salts, whereas the arsenic in brine shrimp (and other natural diets), is most likely predominantly organic arsenic (US EPA 2003). Additionally, in a separate but related study by the same authors (Hamilton et al. 2005d), larval razorback sucker spawned from one female at the Ouray Native Fish Facility were fed zooplankton from six sites (S1, S3, S4, S5, SR, and NR) adjacent to the Green River, Utah at four different initial ages (5, 10, 24, and 28 day old larvae) for 20 to 25 days. The selenium concentrations in zooplankton from the S1 reference site ranged from 2.3 to 3.5 mg Se/kg dw (dissolved Se in water <0.6 to <1.1 µg/L). The concentrations in zooplankton from sites S3 and S4 were slightly higher (range 2.4 to 6.7 mg Se/kg dw; water, 0.3-0.8 µg/L), substantially elevated at S5 (12- 26 mg Se/kg dw; water, 0.6-3.1 µg/L), and highest at SR and NR (44-94 mg Se/kg dw; water, 14-107 µg/L). All larvae in the test initiated when they were 5 days old (study 1) died after 25 days of exposure. Median time to death was shortest in fish fed zooplankton from the reference site (S1) and longest for SR and NR. Interestingly, the concentration of arsenic measured in zooplankton collected from S1 was 12 mg As/kg dw, half that of the brine shrimp used in the above study (Hamilton et al. 2001b), which did not appear to antagonize the toxicity of the

selenium in the diet in this test. In this and the previous two studies, additional inorganic contaminants such as vanadium and strontium were elevated in the zooplankton fed to the larval razorback sucker.

De Riu, D., L. Jang-Won, Huang, S., Monielloa, G., and Hung, S. 2014. Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon. Aquat. Toxicol. 148:65-73.

Test Organisms: Green sturgeon (*Acipenser medirostris*)
White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Three different concentrations of L-selenomethionine were added to an artificial diet mixture: nominal concentrations of 0 (control), 50, 100, and 200 mg SeMet/kg (measured: 2.2 mg/kg Se in control diet (no added Se) and 19.7, 40.1 and 77.7 mg/kg Se in the three treatment diets).

Test Duration: 8 weeks

Study Design: Daily rations of the treatment diets (3% BW/d for first 4 weeks and 2% BW/d for second 4 weeks) were fed to the juvenile sturgeon (approximately 30 g). Each of the four dietary treatment consisted of 3 replicate 90 L tanks with 25 juveniles in each tank. Several endpoints were monitored over the 8 week exposure period including survival, percent body weight increase (% BWI), and hepatosomatic index (HSI).

Effects Data: White sturgeon had no mortalities through the highest dietary treatment. Green sturgeon juveniles had 0%, 7.7% and 23.1% mortality with the three dietary treatments (see table below). %BWI had a greater response to selenium concentration in juvenile tissues than HSI (see table below). Of note is the relatively high concentration of Se in the whole body and muscle tissues of the juvenile sturgeon in the control treatment (both species). The reason for the relatively high Se control concentrations was not due to accumulation of Se from the artificial diet because the concentration of Se remained relatively constant over the 8 week exposure.

Chronic Value: TRAP analysis (threshold sigmoid nonlinear regression) of the green sturgeon survival data resulted in a whole body EC_{10} value of 28.93 mg/kg dw. EC_{10} values were lower for % BWI and HSI using TRAP. For % BWI, the whole body EC_{10} value for green sturgeon was 16.36 mg/kg dw, and for white sturgeon, 23.94 mg/kg dw. For HSI, the whole body EC_{10} value for green sturgeon was 10.86 mg/kg dw (with a very wide 95% confidence interval, 1.842-64.08 mg/kg dw), and for white sturgeon there were no discernible effects.

Selenium in Juvenile Sturgeon Tissues and Endpoints Measured at end of Eight Week Exposure

Green Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	7.1	8.4	100	6.6	2
19.7	22.8	31.1	100	2.6	1.3
40.1	27.8	37	92.3	0.8	0.8
77.7	34.3	36.8	76.9	-1	0.9

White Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	5.6	9.2	100	4.2	2.6
19.7	20.1	27	100	4.2	3.6
40.1	31.8	41.3	100	2.8	3
77.7	47.1	57.9	100	1	2.2

4.0 OTHER DATA – CHRONIC STUDIES WITH FISH SPECIES

Some chronic studies met the requirements of an acceptable chronic test but were excluded from being included in the data set used for criterion derivation for a variety of reasons. Summaries of these studies are provided below.

Vidal, D., S.M. Bay and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol.49:71-75.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*)

Exposure Route: Dietary only
Selenomethionine was added to dry fish food; the measured dietary concentrations were 4.6, 12 and 18 µg Se/g dw. The measured selenium in the control diet was 0.23 µg Se/g dw.

Test Duration: 90 days

Study Design: Each of the three dietary treatments and control had 5 replicates, each replicate contained 12 to 16 larval rainbow trout that were 27 days old at initiation. Each fish was fed an average of 10 mg/d for 30 days; 25 mg/d on days 30-60; and 40 mg/d thereafter. Fish were sampled on days 30, 60 and 90 for length, weight, selenium, hepatic GSH and thiobarbituric acid-reactive substances (TBARS) measurements.

Effects Data: The authors reported significant decreases in weight and length after the 90-day exposure (Table E-4). There were no significant differences in the hepatic lipid peroxidation and hepatic GSH to GSSH ratios among the treatments. The authors found significant differences in weight and length in the 4.6 and 12 µg Se/g dw dietary treatments, but not the 18 µg Se/g dw treatment. Based on larval trout body burden, the authors reported an LOEC of 1.20 µg/g ww, the concentration of Se in fish fed the 12 µg Se/g dw dietary treatment. The Se concentration in larval rainbow trout associated with the lowest dietary treatment that showed significant decreases in larval weight and length was 0.58 µg Se/g ww or 2.06 µg Se/g dw based on 71.8% moisture in whole body rainbow trout (NCBP).

Chronic Value: The data from this study was not used to calculate a chronic value for selenium due to several inconsistencies. The significant decreases in length and weight observed in the two lowest concentrations were not observed in the highest dietary treatment. The Se concentrations in the larval rainbow trout were irregular with the 60-day concentrations being considerably higher than the 90-day concentrations. The authors explain this observation to rapid growth in the fish causing dilution of the Se body burden. However, the increase in fish weight from 30 to 60 days was similar to the 60 to 90 day increase and the 60 day Se concentrations increased from day 30. Also, the Se concentration in the control fish went from below detection on day 0 to 0.46 µg/g ww on day 30; to 1.24 µg/g ww on day 60; and to 0.31 µg/g ww on day 90. The 60-day measured Se in the control fish (1.24 µg/g ww) was more than twice the concentration of Se in the fish with lowest concentration showing effects (0.58 µg/g ww).

Table E-4. Mean (SD) rainbow trout growth after four SeMet dietary treatments.

test day	Treatment, µg/g dw	weight, g	fork length, cm	[Se] whole body, µg/g ww	[Se] whole body, µg/g dw**
0	control	0.37 (0.30)	3.14 (0.41)	ND	ND
30	control	1.33 (0.92)	4.66 (0.41)	0.46 (0.20)	1.63
	4.6	1.25 (0.21)	4.84 (0.29)	1.05 (0.77)	3.72
	12	1.33 (0.30)	5.09 (0.46)	1.81 (1.04)	6.42
	18	1.31 (0.37)	4.97 (0.50)	1.60 (0.93)	5.67
60	control	2.96 (0.92)	6.91 (0.56)	1.24 (0.54)	4.40
	4.6	2.33 (0.63)	6.69 (0.67)	1.70 (0.72)	6.03
	12	2.52 (0.38)	6.88 (0.35)	1.83 (0.94)	6.49
	18	2.59 (0.24)	6.92 (0.24)	2.62 (1.22)	9.29
90	control	5.17 (1.09)	7.70 (0.33)	0.31 (0.20)	1.09
	4.6	3.45 (0.35)*	6.93 (0.19)*	0.58 (0.21)	2.06
	12	3.45 (0.35)*	6.84 (0.68)*	1.20 (0.21)*	4.25
	18	3.82 (0.62)	7.37 (0.62)	1.41 (0.27)*	5.00

* Significantly different than the control.
** ww converted to dw using 71.8% moisture for whole body rainbow trout (NCBP).

Pilgrim, N. 2009. Multigenerational Effects of Selenium in Rainbow Trout, Brook Trout, and Cutthroat Trout. Master's Thesis. University of Lethbridge.

Test Organisms: Rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*) and brook trout (*Salvelinus fontinalis*)

Exposure Route: Dietary only
Selenomethionine added to trout chow and gelatin. Two dietary treatment levels, nominal Se concentrations, 15 (low) and 40 (high) mg/kg.

Test Duration: Rainbow trout were fed the experimental diets from August - December 2009, brook trout July - November 2010, and cutthroat trout December 2010 - April 2011.

Study Design: Fish were obtained from a fish hatchery brood stock. Mature females and were fed the experimental diets in 710 L tanks. Spawning was stimulated by injecting Ovaprim® into the females. Eggs were fertilized and incubated at the fish hatchery until the eye spots were visible. A portion of the eyed stage larvae from each treatment was shipped to the University of Lethbridge Aquatic Research Facility for the swim-up stage of the experiment conducted in gravel bed flumes. Endpoints measured included percent survival in the first (spawned eggs to eyed eggs) and second (eyed eggs to yolk-absorbed fry) stages of development, swim-up success, and malformations (spinal, craniofacial and finfold deformities and edema).

Effects Data: Selenium affected larval survival, swim-up success and the percent of malformations in larvae in one or more of the three species tested (see table below). Visual inspection of plots of the replicate data in Pilgrim (2009) showed considerable variation between the endpoints and selenium in eggs. The distribution of selenium among the tissues was markedly inconsistent with other studies that have used these species. For example, the amount of selenium in the eggs was 8 and 18 times greater than the concentration in the respective muscle tissues in cutthroat and rainbow trout. Median ratios (egg Se:muscle Se) calculated for rainbow trout (Casey and Siwik 2000; Holm et al. 2005) and cutthroat trout (Golder 2005; Kennedy et al. 2000; Rudolph et al. 2007) were 1.9 and 1.8, respectively. Due to the considerable variation in the concentration response of the replicate data and anomalous selenium distribution, these data were not included in the data set to derive the criterion.

Table E-5. Mean selenium concentrations in the diet and selected tissues and selected endpoints measured in rainbow trout (RN), brook trout (BK) and cutthroat trout (CT).
Adapted from Table 3.1 in Pilgrim (2009).

		Tissue, mg/kg ww			Survival, %			
Species	**Diet ww**	**Muscle**	**Liver**	**Egg**	**Stage 1**	**Stage 2**	**Swim-up success**	**Total malformations, %**
	1.47	0.21	3.77	1.17	82.36	61.56	57.18	10
	12.7	0.51	6.53	4.30	77.86	48.64	73.83	9.86
RBT	35.2	0.74	17.21	13.0	54.72	30.33	27.45	29.63
	1.47	0.23	0.72	0.81	86.3	82.68	84	21.3
	12.7	1.14	7.23	5.01	71.37	88.72	83.42	23.93
BK	35.2	3.41	20.4	8.15	71.37	44.63	50.11	24.23
	1.47	0.31	1.00	2.02	61.41	61.87	55.3	6.13
	12.7	0.93	6.00	9.80	30.65	14.75	21.71	48.06
CT	35.2	2.05	14.4	18.0	21.99	0	0.08	NA

Formation Environmental. 2012. Appendix E – Yellowstone Cutthroat Trout Adult Laboratory Reproduction Studies. Technical Support Document: Proposed Site-Specific Selenium Criterion, Sage and Crow Creeks, Idaho. Prepared for J.R. Simplot Company. January 2012.

Test Organism: Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*)

Exposure Route: Field collected. Adult female and male Yellowstone cutthroat trout were collected at five field sites from four streams near the Smokey Canyon mine. In addition Yellowstone cutthroat trout eggs were obtained from a hatchery as method controls.

Test Duration: Test duration was from hatch through 15 days post swim up, and averaged 55-56 days for larvae hatched from field collected fish and 64 days for larvae hatched from laboratory collected fish.

Study Design: Eggs were collected from 15 ripe females at five sites from four streams upstream and downstream of the Smokey Canyon mine. This included one selenium impacted stream downstream of the mine, Sage Creek (LSV), one site along Crow Creek upstream of Sage Creek (CC-150) and one site along Crow Creek downstream of Sage Creek (CC-350), and in sites within the reference streams Deer Creek (DC), and South Fork Tincup Creek (SFTC). Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. In addition, eggs were collected from 16 ripe females obtained from Henry's Lake hatchery (HL) to serve as method controls. Hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. For field and hatchery fish, Se was measured in adult fish (whole body) and in eggs of field collected females.

A target of approximately 600 fertilized eggs from each female (were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15 day post swim up feeding trial.

Endpoints measured in the laboratory were hatch, survival (hatch to swim up, and hatch through 15 days post swim up), and deformities. Deformities were combined as assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Eggs failed to hatch for one of the field treatments (SFTC-1), and six of the hatchery treatments, resulting in a final dataset of eggs fertilized from 14 field collected fish and 10 hatchery fish. Se concentrations in eggs obtained from field collected females ranged from 11.4 mg/kg in Deer Creek through 47.6 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in eggs obtained from Henry's Lake hatchery fish ranged from 0.83 mg/kg – 3.23 mg/kg (Table E-6). Se concentrations in whole body tissue samples obtained from field collected females ranged from 8.17 mg/kg in Deer Creek through 25.7 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in whole body tissue samples obtained from Henry's Lake hatchery fish ranged from 0.23-0.91 mg/kg (Table E-6).

Table E-6. Yellowstone cutthroat trout selenium concentrations, survival, and deformity data from hatch to test end.

Sample ID[a]	Egg Se mg/kg	WB[b] Se mg/kg	# Free From Deformities	# Assessed For Deformities	# Died	# Survived	# Assessed + # Died
CC-150/001	17.6	16.3	22	182	33	182	215
CC-350/001	27.9	20.7	14	138	120	138	258
CC-350/002	29.7	19.4	143	602	83	602	685
CC-350/003	22.3	17.0	73	330	36	330	366
CC-350/004	14.6	16.7	149	480	19	480	499
CC-350/005	47.6	25.7	91	392	71	392	463
DC/001	22	8.17	95	275	30	275	305
DC/002	15.4	9.07	133	465	26	465	491
DC/003	11.4	8.63	59	380	39	380	419
DC/004	12.7	16.6	7	38	23	38	61
HL/002	2.03	0.45	5	39	10	39	49
HL/003	2.48	0.44	121	302	19	302	321
HL/004	1.36	0.36	154	416	20	416	436
HL/006	0.83	0.36	21	244	103	244	347
HL/007	2.26	0.44	120	404	18	404	422
HL/008	1.87	0.28	147	412	37	412	449
HL/011	3.23	0.31	69	296	22	296	318
HL/012	1.58	0.23	112	454	27	454	481
HL/013	1.93	0.72	148	483	24	483	507
HL/015	2.06	0.91	0	36	6	36	42
LSV2C/001	40.1	19.4	2	200	536	0	536[c]
LSV2C/002	30.0	21.0	40	319	105	319	424
LSV2C/003	35.6	18.6	92	487	138	487	625
LSV2C/004	30.5	22.5	107	476	75	476	551

a – CC – Crow Creek; DC – Deer Creek; LSV2C – Sage Creek; HL – Henry's Lake (Hatchery)
b – whole body
c – does not include the 200 fish assessed that were dead prior to assessment, as all fish for that treatment died during the swim up stage in this sample.

Figure E-1 is a plot of % free from deformities versus egg concentration. The previous draft used TRAP to estimate an effect level for these data but after further review it was concluded these data just do not demonstrate any clear effect of Se and therefore inappropriate for analysis by TRAP. There is no obvious trend, especially one that is substantial relative to the data variability. The correlation coefficient for these data is not significant and a t-test of the two data clusters is likewise not significant. The survival data also do not show a useful trend, especially one suitable for EC10 estimation. Although no effect concentration was determined for this test, the data do not contradict the other cutthroat trout datasets in that there are no effects up to 30 mg/kg and of the three points in excess of 30 mg/kg, one did show 100% mortality. The data are consistent with *Oncorhynchus* not being one of the four most sensitive genera.

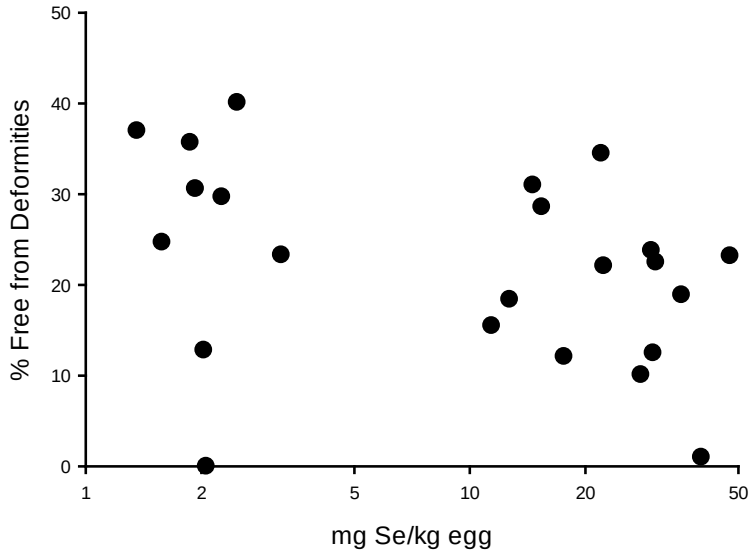


Figure E-1. Plot of percent free from deformities relative to the concentration of selenium in cutthroat trout eggs.

Effect Concentration: NA

Deng, X. 2005. Early life stages of Sacramento splittail (*Pogonichthys macrolepidotus*) and selenium toxicity to splittail embryos, juveniles and adults. Doctoral dissertation, University of California, Davis.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*)

Exposure Route: Dietary only
Four concentrations of selenium in the fish diet (0.6, 17.3, 33.0, and 70.1 mg/g) were created by mixing different proportions of selenized and Torula yeast. A different batch of selenized yeast was used in the adult exposure.

Test duration: 24 weeks

Study Design: Fourteen adult fishes were placed in each circular tank (92 cm diameter, 33 cm height) and fed one of the four diets. Each diet was provided to fishes in three tanks. The twelve tanks were arranged in three rows. Each row had all four treatment concentrations with randomly assigned positions. Thus, the experiment had a randomized block design. Adult splittail fishes were obtained from the Tracy Pump Station (U.S. Bureau of Reclamation, Tracy, CA). After 12 and 24 weeks of exposure, blood samples were collected, the liver, gonad, kidney and white muscle were dissected, and liver and gonad were weighed to calculate hepatosomatic and gonadosomatic indices. Stages of ovarian and testicular development were determined from histological studies.

Effects Data: No mortality occurred throughout the experiment. Fish in control, 17.3, and 33.0 mg/g treatments exhibited normal behavior. Fish exposed to 70.1 mg/g in did not consume as much food as fishes exposed to lower selenium concentrations, and displayed abnormal behaviors. Splittail adults were less sensitive to dietary selenium than juveniles. Relative to control, no changes in body weight, total length, GSI, and condition factor were observed in fishes exposed to selenium concentrations in food up to 33 mg/g. In general, tissue concentrations in fishes exposed to selenium were higher than in the control, but differences in selenium concentrations among them were often small and not significant (Table E-7). Percentages of ovaries with atretic follicles increased with higher concentrations of selenium in their diet: 30% in control, 45.5% in the 17.3 mg Se/g, and 100% in the 33.0, and 70.1 mg/g treatments. The average concentration of selenium in ovaries of fish exposed to 17.3 mg/g in their diet was 6.5 mg/g. This low effect level, though, is disputable because of the very low number of ovaries analyzed, the occurrence of atresia in 30% of ovaries in control, and the lack of significant differences in concentrations of selenium in ovaries among treatments exposed to elevated levels of this element.

Table E-7. Mean concentration of selenium in ovaries (SE).[‡]

	Diet Concentration (mg Se/g)			
	0.6	17.3	33.0	70.1
[Se] in ovary (mg/g dw)	4.4 (0.57)	6.5 (1.0)	8.3 (0.14)	8.9 (0.46)

[‡] Values estimated from Figure 4 in Deng (2005) (pg. 111)

de Rosemond, K. Liber and A. Rosaasen. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker. Bull. Environ. Contam. Toxicol. 74: 1134-1142.

Test Organism: White Sucker (*Catostomus commersoni*)

Exposure Route: Field collected.
In June, 2002, eggs were collected from 4 females from Island Lake (exposed site); milt was obtained from 2 males. Island Lake is downstream from Cluff Lake uranium mine located in northern Saskatchewan. Selenium concentrations in Island lake range from 1 to 11 µg/L and in recent years have been typically 4-5 µg/L. No fish/eggs were collected from a reference site.

Test duration: Through the end of yolk absorption by the larvae; 33 days post-fertilization.

Study Design: Individual batches of eggs were fertilized in the field with milt and water-hardened. Eggs were air transported to the laboratory in Saskatoon for testing. 200 eggs were randomly selected from each clutch and then separated into groups of 100 which were placed into individual test chambers (n = 8).

On test day 30 (3 days prior to test termination), all fish larvae that exhibited macroscopic deformities (e.g., kyphosis, lordosis, scoliosis and edema) were removed, photographed and preserved. At test termination, (day 33), 40 larvae from each female whites sucker were evaluated for deformities using a microscope.

Effects Data: Although all four females were collected from the exposed site, selenium concentrations in eggs were grouped into two low (Fish 2 and 3 in Table E-8) and two high (Fish 1 and 4 in Table E-8). Larval mortality and developmental deformities were not related to selenium concentrations in eggs (Table E-8). The data suggest that embryo/larval effects are not observed at concentrations in eggs reaching 40.3 mg/kg dw (geometric mean of the two high selenium concentrations in eggs). However, because a reference condition with low selenium exposure was not established, it is not appropriate to estimate an effect concentration for this study. Note: the average percent moisture for the four clutches of eggs was 92.6%.

Effect Concentration: NA

Table E-8. Embryo/larval endpoints for eggs from four female white sucker collected from Island Lake in June 2002.

Measurement	Fish 1	Fish 2	Fish 3	Fish 4
Successfully hatched larvae[a]	161	140	176	141
Deformed larvae[b]	21	25	16	13
Dead larvae[c]	6	14	6	4
Macroscopic deformities , %				
Embryological[d]	6.8	6.4	5.7	1.4
Developmental[e]	6.2	11.4	3.4	7.8
Microscopic deformities, %				
Developmental[f]	7.5	5	2.5	7.5
Total developmental deformities, %[g]	13.7	16.4	5.9	15.3
[Se] eggs mg/kg ww[h]	2.7	0.7	0.6	3.2
[Se] eggs mg/kg dw[h]	33.6	9.4	8.4	48.3

[a] Initial number was 200 per fish

[b] Total number of deformed larvae throughout study; includes embryological and macroscopic deformities

[c] Total number of larvae that died throughout study.

[d] Percent of curled deformities that appeared in embryonic fish; deformities were evident immediately after embryos hatched.

[e] Percent of deformities that were designated developmental; deformities became evident as larvae grew and absorbed yolk sac (after experimental day 15).

[f] Percent of microscopic developmental deformities that were evident in the 40 fish examined per female white sucker.

[g] The estimated percentage of offspring that had microscopic and macroscopic developmental deformities combined.

[h] Selenium concentration measured in a subsample of embryos collected on test day 0.

Ogle, R.S. and A.W. Knight. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). Arch. Environ. Contam. Toxicol. 18:795-803.

Test Organism: Fathead minnows (*Pimephales promelas*; juvenile, 59 to 61 d old)

Exposure Route: Dietary only
Purified diet mix spiked with inorganic and organic selenium: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine, homogenized in dextrin.

Test Treatments: Completely randomized block design (2 blocks); 4 replicates per block (n = 8 replicates total per treatment). Actual mean total selenium levels in each exposure treatment were: 0.4 (control), 5.2, 10.2, 15.2, 20.3, and 29.5 mg/kg dw. Fish used in the first randomized block (F_2 generation fish) were progeny from F_1 generation originally obtained from the Columbia National Fishery Research Laboratory, some of which were used in an initial range-finding experiment. Fish obtained from a commercial supplier were used in the second randomized block. The prepared diet was extruded into 1.5 mm pellets which were air-blown dried to 5 percent moisture content and crushed and sieved so that only particles retained by an 11.8 mesh/cm sieve were used in the study. The amount of selenium in water that leached from the food during the experiment averaged only 0.8 µg/L.

Test Duration: 105 days, F_2 generation (block one) and commercial fish (block two);
14 days F_3 generation

Study Design: Ten fish were randomly placed in each cell per block (n = 8x10, or 80 fish total per treatment). Fish were fed twice daily at 6 percent body weight per day, with wastes and uneaten food removed 30 min. after each feeding. Test tanks were flushed with two tank volumes of fresh test water after each feeding (solution renewal). Growth (as wet weight) was determined every two weeks by bulk weighing, and one fish from two of the cells per treatment in a given block (n = 4 total per treatment) was removed for selenium (whole-body) analysis. After 105 days of exposure, a single male and female fish from each treatment replicate (n = 4 breeding pairs per treatment in a given block, or 8 breeding pairs per treatment total) were placed in 250 ml beakers and inspected for spawning activity for 30 days following the first spawning event for that pair (each pair being one replicate). Gonads and muscle tissue were dissected for selenium analysis from these fish at the end of the 30 days spawning period. The spawning substrates were inspected daily for eggs to determine fertility and viability. Samples of not more than 50 eggs from each spawn were incubated in flowing, aerated water and inspected for percent hatch determination. Ten larvae from each incubated brood were transferred to separate glass test chambers and maintained (48 h renewal; fed brine shrimp twice daily) for 14 days to determine percent larval survival.

Effects Data: There was no effect of selenium on any of the reproductive parameters measured at the dietary concentrations tested. Percent hatch and percent larval survival were very high (>87.4 percent) and essentially equal for all of the treatments. Growth of pre-spawning adults was affected by the selenium exposure (Table E-9).

Table E-9. Effects on fathead minnow growth after 98 days of exposure to dietary selenium.

Measured mean selenium in diet, mg/kg dw	Whole-body selenium, mg/kg dw	Mean fish weight, g ww
0.4	1.76	1.30
5.2	2.78	1.24
10.2	3.42	1.20
15.2	5.40	1.21
20.3	6.58	1.09
29.5	7.46	0.94

Chronic Value: An EC value could not be calculated for these data because the data did not meet the minimum requirements for analysis.

GEI Consultants. 2008. Maternal Transfer of Selenium in Fathead Minnows, with Modeling of Ovary Tissue to Whole Body Concentrations.

Test Organism: Fathead Minnow (*Pimephales promelas*)

Exposure Route: Field collected.
Gravid adult fathead minnows were collected from creeks with a wide range of surface water selenium concentrations near the city of Denver, CO during the 2006 summer breeding season.

Sites
Low selenium exposure:

- Sand Creek at Colfax. In 2002, aqueous selenium averaged 0.9 µg/L.

Moderate to high selenium exposure:

- Sand Creek downstream of refinery
- East Tollgate Creek
- Mainstem Tollgate Creek

Control fish – no field exposure

- Laboratory-reared fish from Aquatic BioSystems

Test duration: Embryo-larval test was 48 hours post hatch.

Study Design: Field collected adult fish were either field dissected for selenium measurement in paired tissues or transported live back to the laboratory in coolers with site water. Fish were transported to the laboratory where mating pairs were bred in individual chambers containing spawning substrates. Eggs were removed from the spawning substrate and reared in a standard Falcon dish with lab water. Eggs were screened under a dissecting microscope for viability. Dead eggs were removed and numbers recorded on a datasheet. Three separate breeding experiments were conducted.

Upon hatching, larvae were moved to standard bioassay cups containing lab water and maintained in the laboratory incubator at 25ºC. Larvae were maintained via static conditions in exposure cups for 48 hours post-hatch without food to ensure full absorption of the yolk sac before they were fixed in formalin. Deformity assessment was performed on fixed embryos using a dissection microscope. Test endpoints consisted of egg production, fertilization success, mortality, and deformities (includes edema and skeletal, craniofacial and finfold malformations). The authors used a graduated severity index (GSI) for deformities in which larvae were scored 0 (normal), 1 (slight), 2 (moderate), and 3 (severe) based on the level of defect.

Effects Data: All fish successfully spawned except those collected from Sand Creek downstream from the refinery. These fish had visible parasites and were only used in the ovary-to-whole body selenium analysis. A suite of metal and metalloids were measured in fish samples from each location. Fish collected from East Tollgate Creek had higher concentrations of 9 of the 15 metals that were

measured in fish from at least one site. Aluminum and iron showed the highest difference with an approximate 10-fold increase in the East Tollgate Creek fish.

Only the first brood of each mating pair was used for the analysis because effects appeared to be muted in subsequent broods. The lower response in the second brood was thought to be due to clearing of selenium in the oocytes. There was poor correlation between egg fertilization ($R^2 = 0.13$) and embryo mortality ($R^2 = 0.18$) data with whole body selenium concentrations in the adult fish (see Table E-10 for summary data; see Table E-11 for individual brood data). Neither the fraction of embryos surviving nor fertilization rate as a function of the concentration of selenium in maternal fathead minnows was suitable for estimating EC values. Although there were low survival and fertilization rates at some higher selenium concentrations, these responses were quite varied and did not follow a defined concentration-response relationship (Figure E-2).

Of the 9 broods from fish collected at the three exposed sites only one brood (from East Tollgate Creek) had deformities greater than 10%. The fathead minnow females that produced the brood with the greatest number of deformities and highest GSI also had the second highest concentration of whole body selenium, 46.4 mg/kg dw (Table E-12; Figures E-3 and E-4). Approximately half of the larvae from this brood exhibited some sort of malformation. Similar to the embryo parameters, EC values were not able to be estimated for any of the 4 malformation parameters.

The authors used probit analysis and TRAP to determine effect levels for each of the embryonic and larval endpoints (Table E-13). Although there is an indication of effect due to selenium exposure in both the embryonic and larval endpoints, there is too much variation in the responses observed with the embryos and insufficient response observed with the larvae to derive a reasonable estimate of effect levels. Therefore, no effect level was determined for this study.

Effect Concentration: Unable to determine due to high variability or insufficient response.

Table E-10. Mean fathead minnow first brood embryo and larval parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

Parameter	Site			
	Con	SCC	TGC	ETC
n (number of breeding pairs)	10	3	3	4
WB Se concentration (mg/kg dw)	2.86 ± 0.18	9.17 ± 0.46	35.87 ± 3.73	44.53 ± 2.41
Egg fertilization (%)	84.75 ± 3.32	23.99 ± 22.45	63.42 ± 31.82	59.6 ± 22.26
Embryo mortality (%)	22.03 ± 3.34	89.04 ± 9.70	46.40 ± 26.86	50.76 ± 23.63
Mean spawn size (# of eggs per spawn)	129 ± 23	318 ± 63	162 ± 61	317 ± 158
Total larva evaluated (total # of broods)	957	89	281	254
Mean brood GSI score	4.85 ± 1.22	8.88 ± 8.88	14.88 ± 4.63	21.75 ± 9.53
Larval craniofacial defects (%)	2.64 ± 0.90	4.65 ± 4.65	6.26 ± 3.63	18.48 ± 13.84
Larval skeletal defects (%)	4.74 ± 0.89	9.30 ± 9.30	6.21 ± 1.48	19.62 ± 12.11
Larval finfold defects (%)	2.19 ± 0.78	4.07 ± 4.07	5.71 ± 3.08	17.23 ± 14.48
Larval edema (%)	3.89 ± 1.01	5.23 ± 5.23	6.26 ± 3.63	20.32 ± 12.93
Larval length (mm)	4.90 ± 0.05	4.97 ± 0.12	4.83 ± 0.14	4.90 ± 0.07

Table E-11. Fathead minnow first brood embryo parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); for site acronyms see Table E-9.

Brood	Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-1a-1	CON		2.90	19	0.79	0.96
T-1f-1	CON		3.24	238	0.77	0.88
T-1f-1	CON		1.94	19	0.63	0.73
T-2a-1	CON		2.25	135	0.98	0.98
T-3a-1	CON		2.71	154	0.68	0.72
T-3b-1	CON		2.64	90	0.90	0.95
T-3d-1	CON		3.67	76	0.70	0.71
T-4d-1	CON		3.43	199	0.85	0.91
T-5d-1	CON		3.33	149	0.73	0.87
T-6d-1	CON		2.52	183	0.76	0.78
T-2b-1	SCC		9.92	395	0.00	0.00
T-4a-1	SCC		8.35	193	0.03	0.03
T-6a-1	SCC		9.25	340	0.30	0.69
T-2a-1	TGC		32.29	132	0.83	0.91
T-3a-1	TGC		43.33	79	0.00	0.00
T-4a-1	TGC		31.99	262	0.77	1.00
T-1f-1	ETC		39.76	141	0.52	0.70

Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-3b-1	ETC	47.47	208	0.88	0.92
T-5a-1	ETC	46.37	634	0.07	0.17

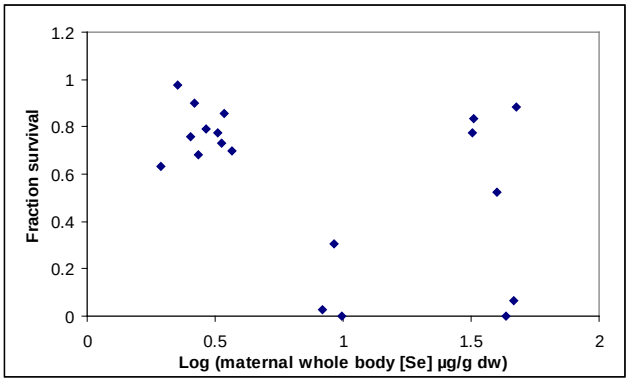
Table E-12. Fathead minnow first brood larval malformations and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

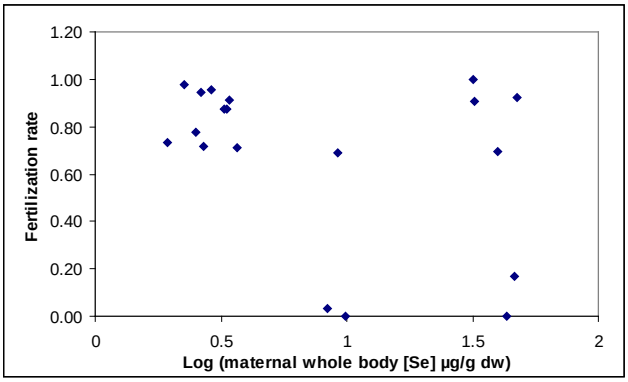
Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total Larvae	Spinal Incidence	%larvae w/o spinal deformity	%larvae w/o craniofacial deformity	%larvae w/o finfold deformity	%larvae w/o edema	Total GSI Score
T-1f-1	CON	1.94	11	9	91	100	100	100	1
T-2a-1	CON	2.25	141	3	97	99	98	96	24
T-6d-1	CON	2.52	117	2	98	99	99	97	16
T-3b-1	CON	2.64	81	4	96	98	99	98	12
T-3a-1	CON	2.71	96	1	99	100	100	100	1
T-1a-1	CON	2.90	14	7	93	93	93	93	10
T-1f-1	CON	3.24	189	8	92	98	98	94	53
T-5d-1	CON	3.33	95	4	96	97	99	98	20
T-4d-1	CON	3.43	164	3	97	98	99	96	28
T-3d-1	CON	3.67	49	6	94	92	94	90	29
T-4a-1	SCC	8.35	3	0	100	100	100	100	0
T-6a-1	SCC	9.25	86	19	81	91	92	90	71
T-4a-1	TGC	31.99	190	5	95	97	97	97	41
T-2a-1	TGC	32.29	91	8	92	90	91	90	78
T-1f-1	ETC	39.76	65	5	95	95	98	94	20
T-5a-1	ETC	46.37	39	44	56	54	54	54	152
T-3b-1	ETC	47.47	150	11	89	95	96	91	89

Table E-13. Authors calculation and comparison of fathead minnow larval deformity EC_{10} estimates using probit analysis and TRAP.

Effect	Endpoint	Probit Results WB [Se] mg/kg, dw (±SE)	TRAP Results WB [Se] mg/kg, dw (95% CL)	Probit Results Ovary [Se] mg/kg, dw (±SE)	TRAP Results Ovary [Se] mg/kg, dw (95% CL)
Edema	EC_{10}	39.48 ± 16.21	45.78 (40.95 - 51.20)	52.99 ± 19.99	61.43 (55.04 – 68.55)
Finfold	EC_{10}	68.55 ± 27.26	48.31 (39.41 - 59.21)	87.95 ± 32.16	64.81 (53.01 – 79.24)
Skeletal	EC_{10}	27.80 ± 9.53	46.08 (41.94 - 50.62)	38.67 ± 12.32	61.82 (56.36 – 67.80)
Craniofacial	EC_{10}	53.86 ± 18.77	47.41 (38.92 - 57.76)	70.83 ± 22.84	63.56 (52.37 – 77.16)
All abnormalities	EC_{10}	16.98 ± 5.38	45.50 (41.10 - 50.37)	24.23 ± 7.06	61.06 (55.26 – 67.48)
All abnormalities except edema	EC_{10}	21.35 ± 6.45	45.69 (41.10 - 50.79)	30.32 ± 8.51	61.27 (55.23 – 67.97)

Figure E-2. The fraction total survival of embryos (top left), fraction of embryos successfully fertilized (right), survival adjusted for fertilization (bottom) versus maternal whole body selenium concentration. Bottom figure EC10=35.2 mg/kg Se dw WB.





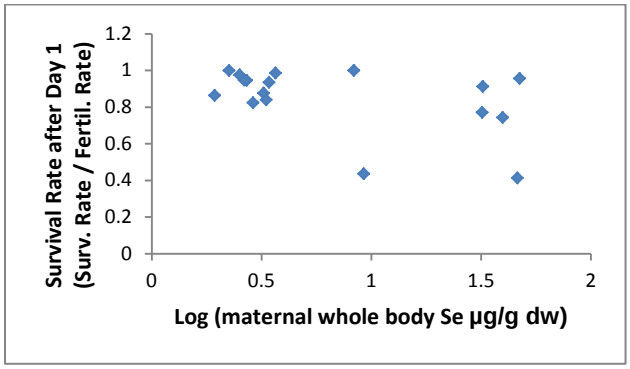


Figure E-3. Percent 2-day post-hatch larvae without edema (A), finfold deformity (B), craniofacial deformity (C), and spinal deformity (D) relative to maternal whole body selenium concentration. EC10s: 61.4 – 64.8 mg/kg dw WB.

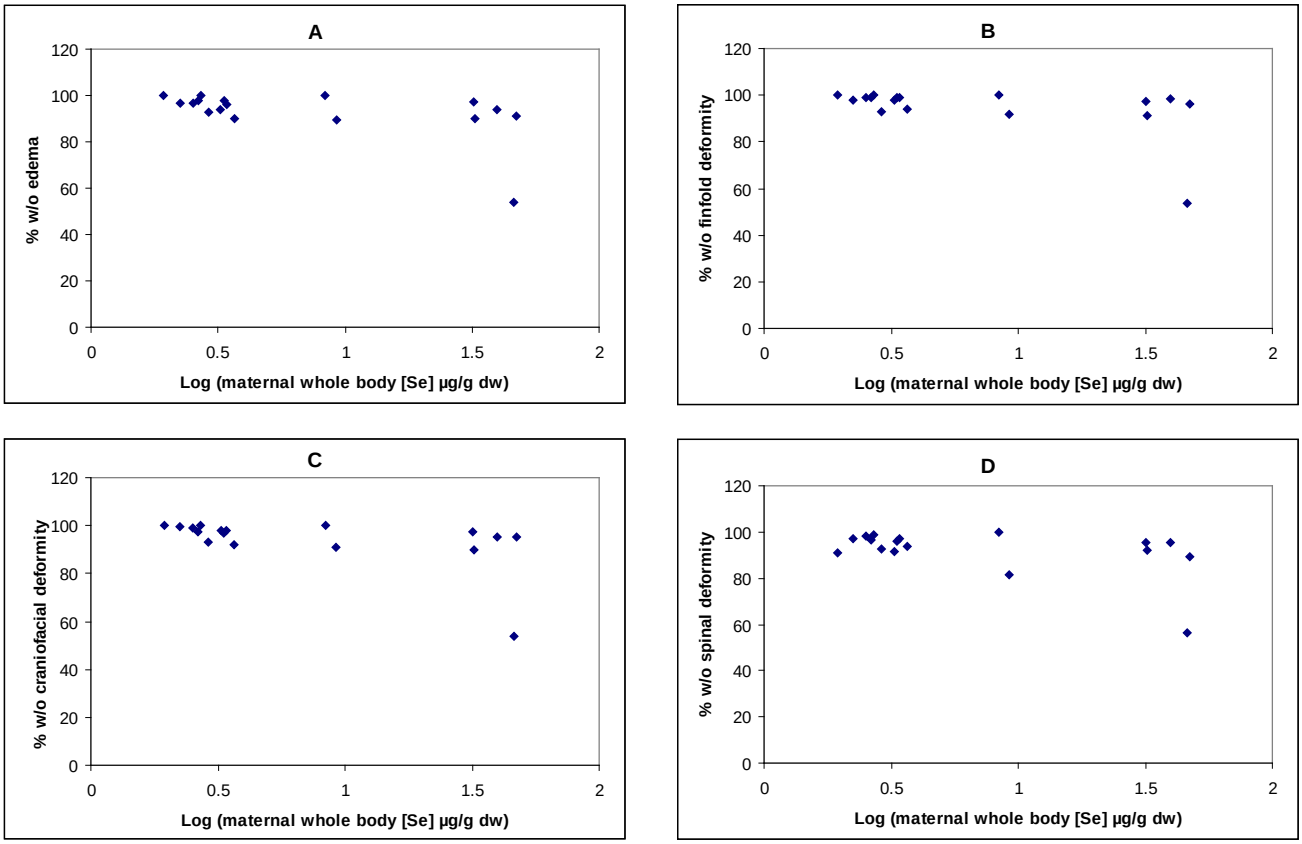
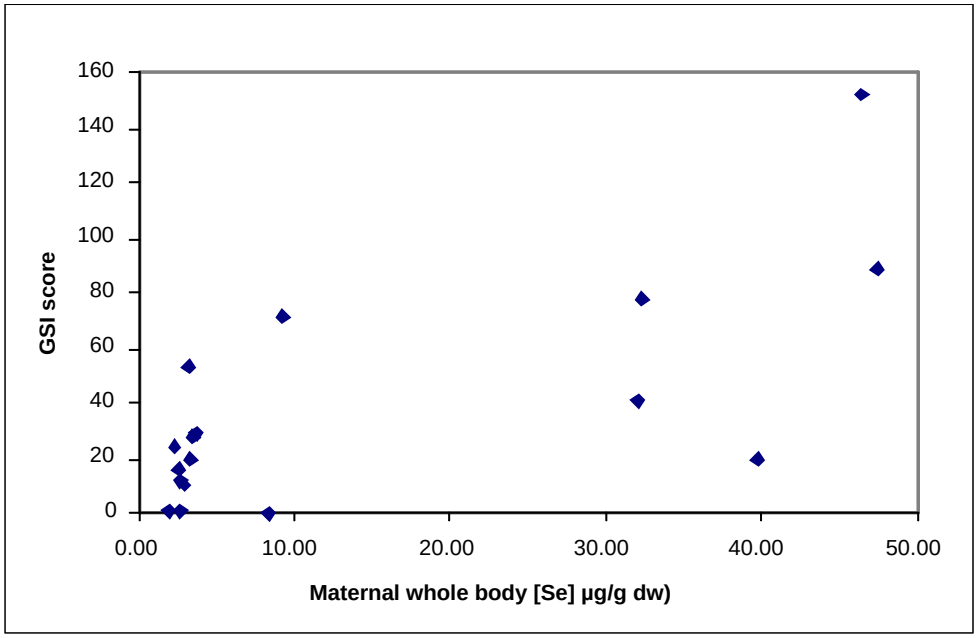


Figure E-4. Percent 2-day post-hatch larvae Graduated Severity Index (GSI) relative to maternal whole body selenium concentration



4.1 Evaluation of zebrafish (Danio rerio) and native cyprinid sensitivity to selenium

Overview:

Two new studies on zebrafish (*Danio rerio*), Thomas and Janz (2014), Thomas (2014), and Penglase et al. (2014), were made available to EPA by David Janz, one of the external peer reviewers. Thomas (2014) and Thomas and Janz (2014) were the original dissertation and peer reviewed paper, respectively, of the same body of work. The apparent sensitivity of the zebrafish to selenium relative to other species in the EPA selenium criteria document was the subject of several public commenters, as well as Dr. Janz in the comments received by EPA.

EPA calculated an EC10 of 7.004 mg Se/kg egg dw, or approximately 3.5 mg/kg whole body) from the Thomas (2014) and Thomas and Janz (2014) study. EPA was not able to calculate an EC10 from Pengalese et al. (2014). The Thomas (2014) and Thomas and Janz (2014) study is summarized in the following section (Part I). Penglase et al. (2014) is summarized in section 7.1.5 of the main document.

EPA noted that the concentration-response curves for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species. The shallow slope indicates partial effects across the range of test doses, with some individuals being very sensitive, and others being less sensitive than other test species. A typical test signature of the nutritionally essential element selenium is that above a particular concentration there is a precipitous increase in adverse effects, with most test organisms affected within a narrow dose range. Additional issues discovered during the analysis of available information in the literature and supplied by the investigator raised questions of test quality that introduced uncertainty in the results reported. This uncertainty, and the fact that zebrafish may not represent the sensitivity range for cyprinids native to the US (discussed in Part II), led to the decision to include this study qualitatively in the effects characterization.

The paucity and relative insensitivity of the available data for cyprinids (fathead minnow EC10 = < 23.9 mg/kg dw; based on LOEC in ovary) relative to other fish families like centrarchids (sunfish), and salmonids (trout and salmon) caused additional concern. This led EPA to investigate the field significance of the zebrafish EC10 (7.004 mg/kg egg) compared to what we know about cyprinid occurrence in selenium impacted waters. The available studies with native cyprinids indicate that a variety of native cyprinid genera (e.g. chubs, shiners, dace) have stable, diverse populations and are reproducing successfully (based on length frequency data) in selenium impacted waters at whole body concentrations far exceeding our proposed whole body criterion element of 8.0 mg/kg dw. Taken together, the available studies (Hamilton et al. (1998), NAMC (2008), Presser (2013), USGS (2012)), indicate that native cyprinids as a family are not expected to be overly sensitive to selenium when compared with other families of freshwater fish. This is important because zebrafish are non-native, and have only been recently discovered in U.S. waters due to accidental introduction.

EPA believes there is significant uncertainty regarding the actual sensitivity to zebrafish, and therefore proposes inclusion of the zebrafish studies in the effects characterization section, as well as inclusion of a comprehensive analysis of the studies as well as the studies on sensitivity of selenium to native cyprinids (below) in its own technical appendix, and issuing an FRN soliciting additional studies or information on zebrafish, as well as native cyprinids.

4.1.1 Part I. Chronic summary of Thomas (2014) and Thomas and Janz (2014)

Thomas, J.K. 2014. Effects of Dietary and in ovo Selenomethionine Exposure in Zebrafish (*Danio rerio*). Dissertation. University of Saskatchewan, Saskatoon, Canada.

Thomas and Janz, D.M. 2014. *In ovo* exposure to selenomethionine via maternal transfer increases developmental toxicities and impairs swim performance in F1 generation zebrafish (*Danio rerio*). Aquatic Toxicol. 152:20-29.

Test Organism: Zebrafish (*Danio rerio*)

Exposure Route: Dietary only
Selenomethionine spiked into Nutrafin® basic flake food

Test Treatments: Control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw).

Test Duration: 90 days

Study Design: Adult zebrafish were fed a control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw) for 60 days, followed by an additional 30-40 days with equal rations (2.5%) of control or SeMet-spiked diets and clean chironomids. After 90 days of feeding exposure, adult fish from each exposure group were bred 3-4 times and embryos were collected and used to assess a number of different effects including larval survival and deformities. Eggs from each treatment were pooled from which replicate samples were collected for selenium measurement, larval survival and deformity assessment

Effects Data: The authors presented mortality and deformities in the F1 generation graphically for days up to 6 days post fertilization (dpf). The bar graphics were initially converted to numeric values using a length measuring tool in GIMP (GNU Image Manipulation Program). EC10 values for both mortality and deformities were very low with deformities being slightly lower. Upon request, the authors provided a table of the number of deformities in observed in 2-6 days post fertilization (dpf) fish larvae for each replicate pool of eggs (Table E-14) (David Janz, pers. comm.). TRAP analysis of these data produced a very low EC10 of 7.0 mg/kg egg Se dw. The concentration-response curve in Figure E-5 is extremely shallow compared to similar tests on other species, such that the apparent sensitivity of zebrafish relative to other species depends on what level of effect is considered. A comparison of egg-ovary zebrafish concentration-response curves for survival and deformities with well-founded concentration-response curves for other species is presented in Figure E-6. The shallow survival and deformity slopes for the zebrafish stand out as atypical for a selenium response. Note the EC50 values for the zebrafish are very similar to the EC50 values for the majority of other fish species and the zebrafish EC90 is similar to the EC90 of the least sensitive fish, Dolly Varden.

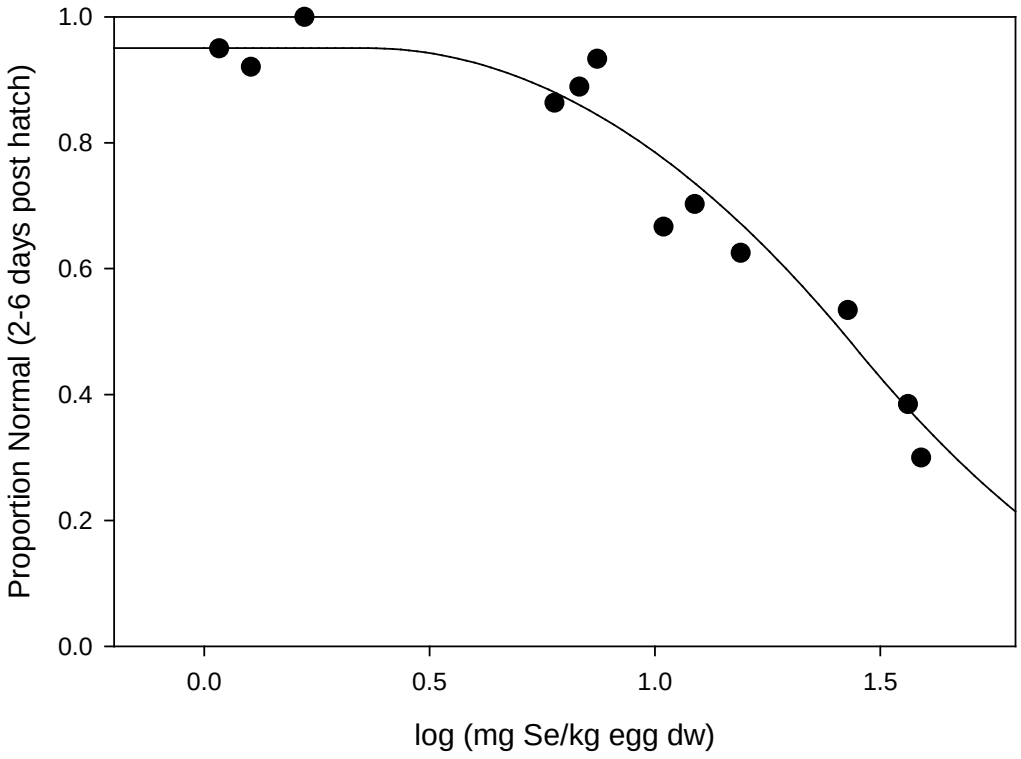
A GMCV based on this test has not been included in the Sensitivity Distribution for several reasons. Although the deformity and survival EC50s are within the range observed for a number of other species, the concentration-response curves

for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species (Figure E-6). Furthermore, if the concentration-response curves are log-symmetrical, as generally has been assumed in estimating EC10s, the projected EC90s for zebrafish would place it among the least sensitive known species**,** indicating greater variability among individuals within this one species than among individuals across the entire class of other fishes represented in the figure. The implication of such a shallow concentration-response curve is that this species has exceptional genetic diversity with respect to selenium tolerance, such that populations could adapt to very high or very low selenium concentrations. The field significance of its exceptionally low EC10 is thus uncertain. The low EC10 might or might not have some relationship to the selenium deficiency reported by Hook (2008) in substantial portions of its home range in the Ganges and Brahmaputra basins in India and Bangladesh.

An assessment of the relative sensitivity of cyprinids using both field and laboratory data is provided in the following section (Part II).

Table E-14. Selenium concentrations in zebrafish eggs and deformities in 2-6 dpf larvae.

Se in eggs, mg.kg dw	Total	Deformed	% Deformity
1.67	35	0	0.00
1.27	63	5	7.94
1.08	40	2	5.00
5.99	44	6	13.64
7.45	45	3	6.67
6.80	36	4	11.11
12.26	37	11	29.73
10.46	39	13	33.33
15.51	48	18	37.50
38.98	30	21	70.00
36.44	65	40	61.54
26.81	88	41	46.59



Parameter Summary:					
Parameter	**Initial**	**Final**	**Std. Error**	**95%LCL**	**95%UCL**
LogX50	1.45	1.4421	0.0408	1.3632	1.5247
Standard Deviation	0.44	0.4421	0.0586	0.3514	0.5964
Y0	0.95	0.9503	0.0184	0.9	0.9799

Effect Concentration Summary:			
%Effect	**ECx**	**95%LCL**	**95%UCL**
90	65.15	45.28	93.73
50	27.79	23.08	33.47
20	11.12	8.647	14.29
10	7.004	4.884	10.04
5	5.053	3.208	7.958

Figure E-5. Tolerance distribution model (triangular distribution model shape) of the proportion of normal zebrafish larvae (1-fraction with deformities) vs. the logarithm of concentration of selenium in zebrafish eggs.

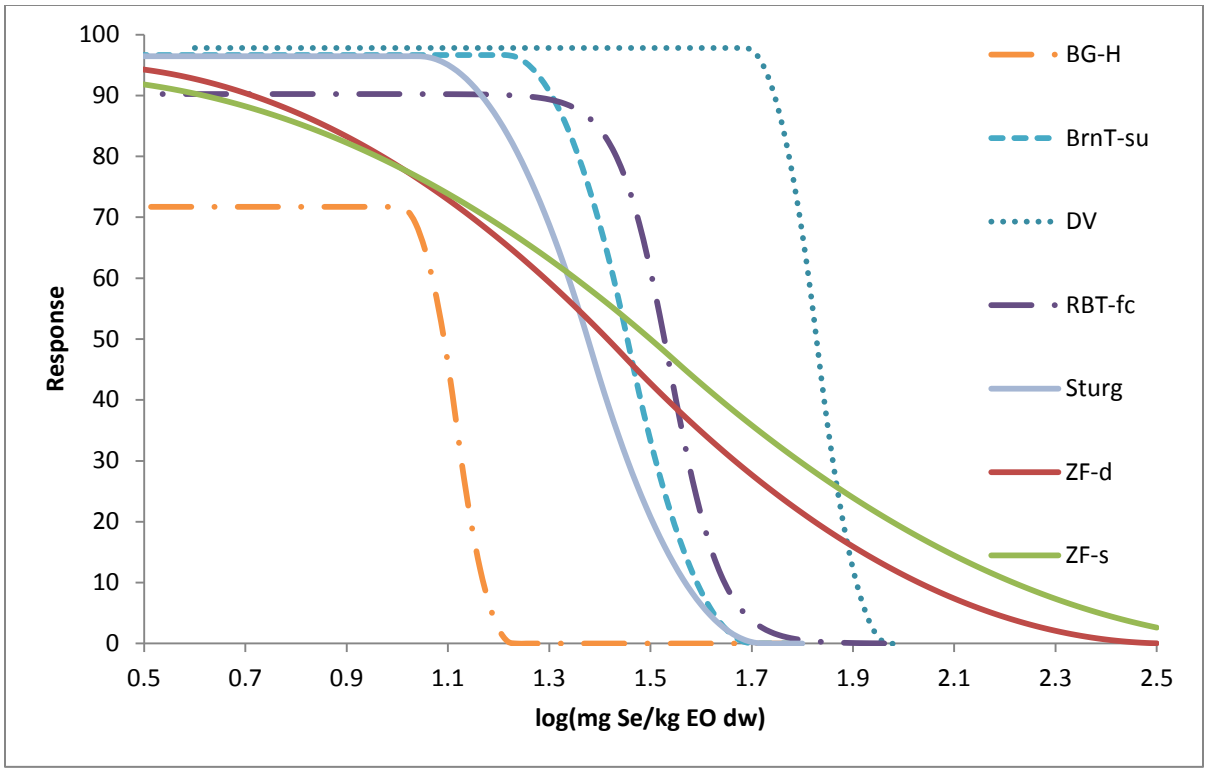


Figure E-6. Thomas and Janz (2014) zebrafish concentration-response curves for deformities and survival, ZF-d and ZF-s, compared with representative concentration-response curves for other species spanning the full range of EC10s.
BG-H: bluegill, Hermanutz et al. (1992, 1996); BrnT-su: brown trout survival to swim-up (Formation 2011); DV: Dolly Varden, Golder (2009; RBT-fc: rainbow trout facial-cranial deformities, Holm (2002) and Holm et al. (2003, 2005); Sturg: sturgeon deformities, Linville (2006).

4.1.2 Part II - Evaluating Sensitivity of Cyprinids (Cyprinidae) to Selenium from Field and Laboratory Data

Background:

The draft selenium criteria document is based on reproductive effects (mortality deformities) to larval fish following maternal exposure. These chronic tests are based primarily on species from the families salmonidae and centrarchidae. There is a paucity of data for a number of fish families used for development of selenium criteria. This limitation in data is particularly notable for the family cyprinidae ("minnows"), because it is comprised of approximately 180 general and is one of the most diverse families in North America. A recent toxicity test with zebrafish (*Danio rerio*), discussed above in Part 1, indicated that some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. This study was very different than all previous studies

examining larval effects in that the slope was very shallow, whereas the slopes for all other species were steep (see Figure E-6).

This analysis considers the results of the zebrafish laboratory survival study and several field collection studies, which evaluated cyprinid abundance and diversity in watersheds impacted by selenium, to compare the sensitivity of the zebrafish evaluated by Thomas (2014) and Thomas and Janz (2014) to native cyprinid populations. Available water and whole body tissue selenium concentrations (> 8.0 mg/kg dw), were compared to the translated egg-ovary to whole body zebrafish EC10 values (~ 3.5 mg/kg dw) to evaluate the relative sensitivity of native cyprinids to the non-native zebrafish test outcome.

Executive Summary:

The occurrence and effect of selenium on native cyprinids were evaluated based on the results of field studies conducted in four aquatic systems (CO, NC, UT, and WV) having elevated selenium concentrations. The objective of this evaluation was to compare the sensitivity of native cyprinid populations with the results of a recent toxicity test with zebrafish (*Danio rerio*) (Thomas (2014), Thomas and Janz (2014)) that suggests some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. The following set of analyses evaluated studies of widely-distributed native cyprinid species occurring in waters impacted by selenium from various sources and the relationships between whole body tissue levels, (and water concentrations where available) and impacts from selenium via toxicity or population metrics.

Cyprinid genera representing many species native to the US were found to be present in waters with selenium concentrations exceeding the current national criteria value (5µg/L). Cyprinid species present in the four studies examined represent 169 of the approximately 180 species present (at the genus level) in the United States. Abundance and diversity at sites impacted by selenium (water concentrations > 5.0 µg/L) were found to be no different than at sites in the Arkansas River, Colorado with low selenium concentrations (3.0-3.5 µg/L) watershed, with the exception of one location where extremely high selenium concentrations (Wildhorse Creek, CO; approximately 413 µg Se/L) were detected.
Whole body tissue concentrations within several widely distributed cyprinid genera exceeded the proposed whole body tissue element of 8.0 mg/kg dw and had sustainable reproducing populations, as indicated by length frequency analysis and occurrence data for the four studies. When evaluated by itself, the influence of selenium whole-body concentration in reducing family Cyprinidae densities was not statistically significant ($R^2 = 0.02$; $p = 0.51$). Rather, substrate characteristics of the waterbodies sampled had the strongest influence. In contrast, when evaluated by itself, the influence of selenium whole-body concentration in reducing family Centrarchidae densities was significant ($R^2 = 0.53$; $p = 0.02$).

In spite of the potential for confounding factors, GEI (2008) obtained parallel results at a different location, Dixon Creek and Canadian River in Texas, affected by refiner effluent selenium. Again, selenium whole-body selenium had no relationship to cyprinid density ($R^2 = 0.00$) but was a significant negative factor for centrarchid density ($R^2 = 0.41$, $p = 0.003$). And in the Sand Creek Drainage, CO, GEI found no negative association between fathead minnow densities and selenium concentrations of 3-26 mg Se/kg whole-body dw and 8-45 mg Se/kg ovary dw.

These findings suggest that native cyprinids are less sensitive than centrarchids, and are thus likely to be protected by a national criterion based heavily on centrarchid and salmonid sensitivity. Based on these available data, native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species, and much greater than indicated by the non-native zebrafish test outcome. It is therefore expected that the proposed selenium criterion will be protective of native cyprinids occurring throughout the United States.

Laboratory Exposures:

1. Chronic Toxicity and Hazard Assessment of an Inorganic Mixture Simulating Irrigation Drainwater to Razorback Sucker and Bonytail. Hamilton et al. (2000). USGS CERC Laboratory

Toxic effects from inorganics associated with irrigation activities, and possibly contributing to the decline of endangered fish in the middle Green River, Utah were investigated. Two 90-day chronic toxicity studies were conducted with two endangered fish, razorback sucker (*Xyrauchen texanus*) and bonytail chub (*Gila elegans*). Swim-up larvae were exposed in a reconstituted water simulating the middle Green River. The inorganic mixtures were tested at 1X, 2X, 4X, 8X, and 16X the measured environmental concentrations of the evaluated inorganic constituents (2 ug/L arsenic, 630 ug/L boron, 10 ug/L copper, 5 ug/L molybdenum, 51 ug/L selenate, 8 ug/L selenite, 33 mg/L uranium, 2 ug/L vanadium, and 20 ug/L zinc).

Bonytail chub survival was 95% or greater at 30, 60, and 90 days except for the 16X treatment (1232 ug/L Se), whereas growth was reduced after 30, 60, and 90 days at the 8X treatment (532 ug/L Se). Swimming performance of bonytail chub was reduced after 90 days of exposure at the 8X treatment. Whole-body residues of copper, selenium, and zinc increased in a concentration-response manner, but did not increase at 90 days of exposure at the 8X treatment for most species tested, and at lower treatment concentrations for the bonytail chub. Mean whole body selenium residues at the 8X treatment were 23.3, 16.7, and 9.4 mg/kg Se dw at 30, 60 and 90 days respectively. Hamilton et al. (2000) concluded that adverse effects in bonytail chub were associated with whole-body concentrations of 9.4 to 10.8 mg/kg Se dw in this study. One key uncertainty is the effect that the combination of toxic elements, in contrast to selenium alone, had on outcomes measured in this study. However, basing the selenium toxicity

evaluation on exposure to multiple contaminants is expected to provide a more conservative estimate of effect on the bonytail chub (*Gila elegans*) than if selenium is tested alone.

Field Collection Studies

2. Selenium Tissue Thresholds: Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field. Part III: Field Application of Tissue Thresholds: Potential to Predict Population or Community Effects in the Field. NAMC Report (2008).

Field studies were conducted by GEI in the Arkansas River, CO mainstem and selected tributaries between 2005 and 2006 to examine the relationship between selenium concentrations as well as habitat characteristics in surface waters and cyprinid abundance and diversity in the Arkansas River. The data collected for the study included:

1) Seasonal fish and macroinvertebrate (not shown) sampling to determine species composition and the relative abundance of aquatic organisms);
2) Whole-body fish tissue, composite macroinvertebrate tissue (not shown), and water and sediment (not presented) sample collection for the evaluation of Se concentrations in these tissues and the evaluation of bioaccumulation pathways; and
3) Physical habitat measurements (not presented), to determine relationships between the occurrence of biota and their physical environment. Data were collected from fall 2004 to fall 2006 from the Arkansas River, Fountain and Wildhorse Creeks, and the St. Charles River.

Total selenium (dissolved) was measured at 4 sites mainstem and 6 sites on three tributaries of the Arkansas River watershed near Pueblo Colorado (Table E-15). Multiple site visits (6 to 17) to collect water for selenium determination were conducted at the 10 sampling stations between 2005 and 2006.

Table E-15. Selenium water column data: Total Selenium (µg/L, dissolved).

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
AR (Arkansas River)				
AR1 (ARM) Mainstem, in Pueblo below Whitlock WWTP	8 months	15	7.05	3.69
AR2 (ARE) Mainstem below Pueblo WW Reclamation Center and Fountain Creek	12 months	9	10.6	4.06
AR3 (ARB) Mainstem, downstream of Pueblo	10 months	7	8.72	4.0
AR4 (ARN) Mainstem, downstream of St. Charles River	10 months	8	8.81	2.85

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
Arkansas River Tributaries				
WHC (Wildhorse Creek)	6 months	17	418	115
FC (Fountain Creek)				
FCP (Upstream)	12 months	9	3.43 (4.9)*	1.05
FC4 (Downstream)	6 months	12	12.1	4.34
SC (St. Charles River)				
SC1 (Upstream)	6 months	6	3.09 (4.8)*	1.37
SC2 (Mid-Point)	6 months	11	11.7	6.22
SC5 (Downstream)	8 months	13	20.3	13

* Maximum [Se] in FCP and SC1 < 5.0 ug/L, current selenium criterion

Summary of Selenium Concentrations in Water:

1) Total selenium concentrations exceeded the EPA chronic selenium standard of 5 µg/L in surface water samples collected from most locations, with only the upper reaches of the St. Charles River and Fountain Creek having mean selenium concentrations below the EPA chronic selenium standard.
2) Selenium concentrations in water samples from Wildhorse Creek were more than 20X greater than in water samples collected from all other sample locations, with a mean selenium concentration of 418 ± 115 µg/L.
3) The minimum concentration measured in water samples from Wildhorse Creek (315 µg/L) was approximately 7X greater than the maximum selenium concentration measured at other study sites (43.6 µg/L at St. Charles River, SC5).

Selenium in Fish Tissue:

Selenium concentrations in fish tissue (whole body) were measured for three representative cyprinid species (central stoneroller, sand shiner, red shiner), one catostomid (white sucker), and three centrarchids (green sunfish, smallmouth bass, and largemouth bass) (Table E-16).

Table E-16. Mean fish tissue concentrations.
[Average whole body mg/kg dw estimated by eye from graphs in NAMC (2008)].

Sample Site	ARM	ARN	ARE	ARB	WHC	FCP	FC4	SC1	SC2	SC5
Mean water [Se] ug/L	7.0	8.8	10.6	8.7	418	3.43	12.1	3.1	11.7	20.3
Cyprinids										
Sand Shiner	10	10-21	25			10-17	15-21			
Red Shiner		23	42				25			30
Central Stoneroller	8	10-20			18-47	12	14	5	45	33
Centrarchids										
Green Sunfish								12	30	
Largemouth Bass	11-15	14-36	22	26						40
Smallmouth Bass	7		20	20						
Catostomids										
White sucker	8-11	10-24	16-18	14-21	32-33	6-10	24	6-14		47

Summary of selenium in fish tissue:

1) The mean concentrations in all cyprinids across all sites was 21.06 mg/kg dwt; SE = 1.38).
2) For comparison, the mean concentration in all centrarchids across all sites was 19.73 mg/kg dw; SE = 1.32; and the mean concentration in white sucker (catostomids) across all sites was 17.52 mg/kg dw; SE = 1.52.
3) Most mean whole-body Se concentrations were well above the U.S. EPA (2014) proposed chronic tissue criterion element for whole body of 8.13 mg/kg dry weight.

Comparison to national draft fish tissue criteria:

Given that these are waters known to be impacted by selenium there were only a few fish samples (Tables E-17, E-18) that were at or below the proposed whole body criteria element of 8.1:

1) The Arkansas River mainstem (mean water [Se] = 7.05 ug/L), had samples from three species that met the criteria in 2006, central stoneroller, smallmouth bass and white sucker.
2) In the tributaries to the Arkansas River that were sampled, white sucker in both Fountain Creek (mean water [Se] = 3.43 ug/L) and St. Charles River met the whole body criteria in 2004 and 2005, whereas the only cyprinid to meet the proposed whole body criterion was the central stoneroller in 2005.

Cyprinid Abundance and Diversity:

Table E-17. Cyprinid Diversity (native spp. present– excludes carp): NAMC 2008 Study.

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	1/6	3/6
ARB	8.72	6/6	5/6
ARN	8.81	5/6	3/6
ARE	10.6	5/6	4/6
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	5/6	4/6
FC4	12.1	4/6	6/6
Whitehorse Creek (WHC)	413	1/6	1/6
St. Charles River			
SC1	3.09	5/6	5/6
SC2[1]	11.7	4/6	NS
SC5	20.3	6/6	5/6

[1]SC2 only sampled in 2005

Table E-18. Cyprinid Abundance (native spp. present– excludes carp): NAMC 2008 Study

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	8	460
ARE	8.72	643	950
ARB	8.81	697	521
ARN	10.6	446	116
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	746	2352
FC4	12.1	1978	1825
Whitehorse Creek (WHC)[1]	413	926	81
St. Charles River			
SC1	3.09	2920	14583
SC2[2]	11.7	2757	NS
SC5	20.3	3102	2568

[1]Whitehorse Creek comprised 1 species, central stoneroller
[2] SC2 not sampled in 2006

Summary of cyprinid abundance and diversity:

1) Diversity as well as abundance of cyprinids in the tributaries vs the Arkansas River mainstem more likely a function of habitat and/or predator density rather than influence of selenium.
2) Several sites on Wildhorse Creek, Fountain Creek, and the St. Charles River, had substantial changes in the populations of some fish species between sample years 2005 and 2006, with fish that were present in one year in high numbers and with a variety of age classes, either

absent or present in low numbers the other year. These changes are likely to be linked to higher stream flows present in 2006 and significant habitat changes due to beaver activity at some sites. Variable population compositions and numbers of cyprinids are not uncommon in plains streams with highly variable flow regimes and habitat conditions (Schlosser 1987).

3) Based on an evaluation of age class distribution (indicated by length-frequency distribution data), it was concluded that the following sites had viable and reproducing cyprinid populations (NAMC 2008:

> Arkansas River mainstem: The length-frequency data collected for the fish species at these sites indicates multiple age groups present for most of the species at the sites.
> Fountain Creek - Length-frequency analysis of the flathead chubs indicated that the populations are reproducing, with juvenile and older adult fish present in relatively high numbers at both sites and years.
> St. Charles River - Length-frequency analysis of the fish populations indicated that sites had reproducing populations of central stonerollers, fathead minnows, and sand shiners, with juvenile and adult fish collected during both years (GEI 2007a).
> Wildhorse Creek - the age class distribution of central stonerollers was similar between years, indicating a reproducing population that includes both juvenile and adult fish in both years, despite the extremely high [Se] in water.

Relevance/Surrogacy of Arkansas River Cyprinids to all Cyprinid Species in US

Cyprinids captured from the Arkansas River are representative of cyprinid species occurring throughout the US. This conclusion is based on the following lines of evidence:

- Six of the seven cyprinid species (central stoneroller, fathead minnow, flathead chub, longnose dace, red shiner, and sand shiner) captured from the Arkansas River during this investigation are native to the United States;
- Four of the six cyprinid species found in the Arkansas River basin (central stoneroller, fathead minnow, sand shiner and red shiner) are widely distributed throughout the United States (see species specific distribution maps Attachment 1); and,
- Six of the native species present in the Arkansas River Basin are direct surrogates at the genus level for the 142 native cyprinids in North America (Table E-19).

Table E-19. Cyprinid species surrogacy and occurrence in water for native species inhabiting the Arkansas River and select tributaries.

Species	Cyprinid group	# of species represented by genus	[Se] in waterbodies where species occurred	Average tissue concentration or range
Campostoma anomalum Central stoneroller	stonerollers	5 species	3.1-418 ug/L	5-47 mg/kg dw
Pimephales promelas Fathead minnow	Blunthead minnows	4 species	3.1 - 20.3 ug/L	No tissue
Platygabio gracilis Flathead chub	Flathead chub	1 species	3.1 - 20.3 ug/L	No tissue
Rhynichthys cataractae Longnoise dace	dace	9 species	3.1 - 20.3 ug/L	No tissue
Cyprinella lutrensis Red shiner	Satinfin shiners	32 species	3.1 - 20.3 ug/L	23-42 mg/kg dw
Notropis stramineus Sand shiner	Eastern shiners	91 species	3.1 - 20.3 ug/L	10-25 mg/kg dw

Summary cyprinid surrogacy:

Cyprinid species collected from the Arkansas River watershed are representative (at the genus level) of the 142 cyprinid species native to North America. With the exception of one sample location (Whitehorse Creek), the abundance and diversity of cyprinid species present and the occurrence of multiple age classes indicates that cyprinids are successfully surviving and reproducing in the Arkansas River watershed, even with selenium concentrations exceeding 5ug/L in water and 8 mg/kg bw in whole body fish tissue. North American species not represented at the genera level comprise 54 species (mostly chubs – 40 species), many of which are geographically isolated.

3. Observations of cyprinids in NC Reservoirs (Hyco Reservoir and Belews Lake) – (located at end of NAMC 2008 report).

Crutchfield et al. (2000) evaluated long-term water quality data, selenium chemical concentration data collected for sediment, invertebrate and fish tissues, and invertebrate and fish population data collected from the Hyco Reservoir to document the recovery of the aquatic community following the 1990 installation of a dry fly ash pollution abatement system. Since 1973, data have been collected from six locations in the Hyco Reservoir, with varying fly ash exposure. Gamefish including bluegill sunfish and largemouth bass were reproductively extirpated due to high selenium concentrations prior to installation of the pollution abatement system. The fish community was dominated by green sunfish (*Lepomis cyanellus*), eastern mosquitofish (*Gambusia holbrooki*), gizzard shad (*Dorosoma cepedianum*), and satinfin shiner (*Cyprinella analostana*). Their main observation was that satinfin shiner was a dominant cyprinid in the Se limited fish community prior to selenium reduction.

Barwick and Harrell (1997) evaluated fish population monitoring and tissue selenium concentration data to document the recovery of fish populations in Belews Lake for the ten years following installation of a dry fly ash pollution abatement system. Fish diversity and biomass data were collected from 1977 to 1994 (with the exception of 1978-1979 and 1982-1983) at two sites on the lake. In 1980 and 1981, fathead minnows (*Pimephales promelas*) dominated the fish community, representing 62 percent and 81 percent of the biomass, respectively (Barwick and Harrell 1997). By 1984, red shiner (*Cyprinella lutrensis*), common carp (*Cyprinius carpio*), and fathead minnows (*Pimephales promelas*) were the dominant cyprinids in the selenium limited fish community prior to selenium reduction. The authors noted that cyprinid abundance started to decrease as green sunfish, a more Se- tolerant sunfish recovered in 1989-1990, followed by further decreases in 1990-1994, as channel catfish, bluegill, and largemouth bass populations increased (Barwick and Harrell 1997).

Young et al. (2010), reviewing the studies of Belews Lake, NC, note that during the period of maximal selenium inputs, egg and ovary concentrations reached 40-159 mg Se/kg dw. Out of as many as 29 resident species prior to contamination, only catfish and the cyprinids common carp and fathead minnows remained during the period of maximum impact.

4. Presser, T.S., 2013, Selenium in ecosystems within the mountaintop coal mining and valley-fill region of southern West Virginia—assessment and ecosystem-scale modeling: U.S. Geological Survey Professional Paper 1803, 86 p. http://dx.doi.org/10.3133/pp1803.

USGS sampled southern West Virginia ecosystems affected by drainage from mountaintop coal mines and valleys filled with waste rock (valley fills) in the Coal, Gauley, and Lower Guyandotte watersheds during 2010 and 2011. Sampling data from earlier studies in these watersheds (for example, Upper Mud River Reservoir) and other mining-affected watersheds in WV are also are included to assess additional hydrologic settings and food webs for comparison.

1) Site-specific fish abundance and richness data documented the occurrence of various species of chub, shiner, dace, minnow, and central stoneroller (*Campostoma anomalum*) in the sampled watersheds.
2) Model species for streams were limited to creek chub (*Semotilus atromaculatus*) and central stoneroller. Creek chub was present at all sites during USGS sampling in 2010-2011. However, both of these species are considered to have high tolerance for environmental stressors based on results of traditional comparative fish community assessments. Concentrations of Se in water and whole body tissues of creek chub, blacknose dace, and stoneroller are shown in Table E-20.
3) The order of abundance for species with greater than 28 individuals was: creek chub, striped shiner, mottled sculpin, green sunfish, central stoneroller, blacknose dace, bluntnose minnow, and northern hog sucker. Shiners and darters were prevalent, but bluegill sunfish were absent during the 2010 survey.

Table E-20. Se in fish whole body tissue samples: Upper Mud River Basin and Tributaries.
(Compilations of data from different sources presented in (Presser et al. 2013).

Stream Segment	Year	[Se] in water Mean (Range) in ug/L	Creek Chub Mean (Range) in mg/kg dw	Blacknose Dace Mean (Range) in mg/kg dw	Stoneroller Mean (Range) in mg/kg dw
Upper Mud River	2011	10.5, 18.2	9.0 (6.4–11)	Not Sampled	Not Sampled
Upper Mud River 1	2010	Not Sampled	10.3 (9.4–10.9)	Not Sampled	Not Sampled
Lower Mud River	2008	7.9	10.3 (9.4-15.4)	Not Sampled	Not Sampled
	2011	5.2, 7	9 (6.4-11)	Not Sampled	Not Sampled
Upper Mud River 2 (above Upper Mud River 1)	2005	9.8 (4–22)[1]	2.9 (<1-8.7)	Not Sampled	Not Sampled
	2006	Not Sampled	5.6 (2.2-10)	Not Sampled	Not Sampled
	2007	Not Sampled	7.7 (3.7-10)	Not Sampled	Not Sampled
Berry Branch	2009-2010	8.3 (1.7–18)[2]	4.0 (3.3–5.0)	9.6 (7.8–13)	Not Sampled
Stanley Fork	2009-2010	6.0 (3.0–7.4)[3]	10.3 (7.2–13)	Not Sampled	Not Sampled
Lower Kanawha River Watershed					
Little Scary Creek	2006	20	Not Sampled	55	Not Sampled
	2009	31.4 (23-42)	28 (3-80)	Not Sampled	Not Sampled
Connor Run	2009	47.8 (4-90)	(21-36)	Not Sampled	Not Sampled
Upper Kanawha River Watershed					
Jack's Branch Mining Complex					
Bull push fork w/pond	2010	9.0-10.0	Not Sampled	66 (19–113)	Not Sampled
Bull push fork downstream	2010	9.1–10	8.6 (6.2–13)	10.7 (5.5–14)	6.9 (3.1–17)
Hughes Fork	2005 - 2007	5.3 (2–10)	7.8 (4.1–10.9) 2005 7.9 (2.7–12.9) 2007	Not Sampled	12.4 (0.5–34.5) 2005
Hughes Creek	2010-2011	2.1-13	9.9 (3.7–17)	16.9 (6.8–25)	9.0 (3.6–14)
Big Coal River Watershed					
Beech Creek	2005-2007	Not Sampled	(3-18)	Not Sampled	Not Sampled
Seng Creek	2005-2009	27.5 (15–42)	8.2 (4.8–14.7)	Not Sampled	Not Sampled
	2011	23.3	8.1 (5.4-10)	Not Sampled	Not Sampled
White Oak Creek	2005-2007	15.8 (8–27)	5.8 (<1-12.8)	Not Sampled	7.1 (2.5–12.8)

[1] Water samples collected between 2005 and 2008.
[2] Water samples collected in 2009 and 2010.
[3] Water samples collected in 2009 and 2010.

Study Summary:

Samples in various environmental media (water, sediment, algae, macroinvertebrates, fish) were collected by USGS (2010-2011), and others (e.g. WVDEP, Potesta) between 2005 and 2011. The stream segments presented here represent a subset of the stream segments with available data. Only streams with water [Se] > 5.0 ug/L are presented to facilitate comparison with other studies with Se-impacted streams. Overarching observations include:

1) [Se] in water averaged from 5.3 ug/L – 31.4 ug/L with a high of 90 ug/L (Connor Run, 2009).
2) [Se] in fish tissue: creek chub – averaged from 5.8 mg/kg wb to 28 mg/kg wb, with a maximum whole body concentration of 80 mg/kg wb (Little Scary Creek, 2009).
3) [Se] in fish tissue: blacknose dace – averaged from 10.7 mg/kg wb to 66 mg/kg wb, with a maximum whole body concentration of 113 mg/kg wb (Bull push fork w/pond, 2010)
4) [Se] in fish tissue: central stoneroller – averaged from 6.9 mg/kg wb to 12.4 mg/kg wb, with a maximum whole body concentration of 34.5 mg/kg wb (Hughes Fork, 2005). Note also, that central stoneroller, although common through stream segments samples, were not ubiquitous, as was observed in the study conducted by NAMC in the Arkansas River near Pueblo CO.

5. Selenium concentrations in fish tissue collected from the Gunnison River.
http://pubs.usgs.gov/of/2012/1235/of12-1235.pdf

Approach: In sampling conducted in summer 2010, muscle tissue plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and whole body tissue samples were collected from speckled dace (*Rhinichthys osculus)* inhabiting critical habitat in the Gunnison River in Western Colorado (Table E-21). Total selenium in fish muscle plugs (mg/kg dw) for roundtail chub, or in whole body (speckled dace) was calculated for all tissues. In follow-up sampling conducted in the summer of 2011, muscle plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and bonytail chub (*Gila elegans, listed*) inhabiting critical habitat in the Gunnison River in Western Colorado.

This study was intended to document any changes in selenium concentration in fish over the last 20 years based on remediation efforts that have been completed to date.

Table E-21. Fish tissue concentrations observed in Cyprinids.

Species	Year	Mean (Range) [Se]	# > muscle = 11*	# > whole body = 8
Roundtail Chub	2010	9.7 mg/kg dw (5.2-32.4)	2/15	
	2011	7.33 mg/kg dw (5.6-11.2)	1/15	
Speckled Dace	2010	7.46 mg/kg dw (5.7-9.7)		6/15

* Muscle plugs were collected since this species is large enough for non-destructive sampling, and b) a listed species.

5.0 OTHER DATA – CHRONIC STUDIES WITH INVERTEBRATE SPECIES

A limited number of studies have evaluated the effects of selenite on invertebrate species, an important prey item for fish and birds as summarized by Debruyn and Chapman (2007). The following **studies** with a rotifer, and annelid, and an insect (mayfly) were found suitable for establishing species sensitivity.

5.1 Rotifers

Dobbs et al. (1996) exposed *Brachionus calyciflorus* to selenate in natural creek water for 25 days in a three-trophic level food chain test system. This is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In this particular study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but received additional selenium from their diet (i.e., the algae fed to rotifers and the rotifers fed to fish). The overall exposure lasted for 25 days. Rotifers did not grow well at concentrations exceeding 108.1 µg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 µg Se/L in the water (40 µg Se/g dw in the algae). Regression analysis of untransformed growth data (dry weight) determined 4 day post-test initiation resulted in a calculated EC_{10} of 37.84 µg Se/g dw tissue.

5.2 Aquatic Worms

Although not intended to be a definitive toxicity study for this invertebrate, Besser et al. (2006) evaluated the bioaccumulation and toxicity of selenized yeast to the oligochaete, *Lumbriculus variegatus,* which was intended to be used for dietary exposure in subsequent studies with the endangered desert pupfish, *Cyprinidon macularius*. Oligochaetes fed selenized-yeast yeast diets diluted with nutritional yeast (54 to 210 mg Se/kg) had stable or increasing biomass and accumulated Se concentrations as high as 140 mg/kg dw. The oligochaetes fed the undiluted selenized-yeast (826 µg/g Se dry wt.) showed reduced biomass. The effect level is considered >140 mg Se/kg dw.

5.3 Aquatic Insects (Plecoptera: Mayfly)

Conley, J.M., D.H. Funk and D.B. Buchwalter. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ. Sci. Technol. 43:7952-7957.

Conley, J.M., D.H. Funk, N.J. Cariello and D.B. Buchwalter. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicol. 20:1840-1851.

Conley, J.M., D.H. Funk, D.H. Hesterberg, L-C. Hsu, J. Kan, Y-T. Liu and D.B. Buchwalter. 2013. Bioconcentration and biotransformation of selenite versus selenite exposed to periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ. Sci. Technol. 47:7965-7973.

Conley et al. (2009) exposed mayfly larvae (*Centroptilum triangulifer*) to dietary selenium contained in natural periphyton biofilms to eclosion. The periphyton fed to the mayfly larvae were exposed to dissolved selenite (radiolabeled ^{75}Se) in November 2008 (12.6 and 13.9 µg/L) and in January 2009 (2.4, 2.4, 4.9, 10.3, and 10.7 µg/L). Periphyton bioconcentrated Se an average of 1113-fold over the different aqueous Se concentrations (Table E-22). Twenty 4 to 6-day old mayfly larvae were exposed for 4.5 to 6 weeks to each of the periphyton diets until the larvae eclosed to subimagos. The subimagos were allowed to emerge to the adult imago stage which deposited their egg masses in Petri dishes. Selenium was measured in postpartum adults along with their dry weights and clutch size.

Table E-22. Selenium concentrations in water exposed to periphyton, periphyton and mayfly adults.

Treatment	Dissolved [Se] exposed to periphyton, µg/L	[Se] in periphyton, mg/kg dw	[Se] in mayfly adult, mg/kg dw
5A	2.4	2.2	4.2
5B	2.4	2.0	5.7
10A	4.9	4.4	9.7
20C	10.3	8.7	16.2
20D	10.7	11.3	27.5
20A	12.6	25.5	56.7
20B	13.9	17.5	34.8

Selenium increased in concentration from periphyton to the adult mayflies (trophic transfer factor) an average of 2.2-fold (Table E-22). The authors observed a decrease in fecundity as maternal postpartum Se concentrations increased. Fecundity was also related to growth of the mayflies. The authors observed a reduction in fecundity for this mayfly when they were fed diets containing more than 11 mg Se/kg dw. This threshold is considered the effect value for this study. Using the trophic transfer factor of 2.2, the periphyton Se concentration of 11 mg/kg dw translates to an adult mayfly Se concentration of 24.2 mg/kg dw.

Conley et al. (2011) exposed larval *C. triangulifer* similar to Conley et al. (2009) to two different rations of periphyton (1x and 2x) to evaluate the effect of feeding ration on the bioaccumulation and life cycle performance of the mayfly. Periphyton (on plates) was initially exposed to low (1.1 to 3.4 µg/L), medium (5.9 – 8.9 µg/L) and high (19.2 – 23.1 µg/L) selenite. Fifteen 1-2 day-old mayfly larvae were then fed either 1 plate (1x ration) or 2 plates (2x ration) in bottles containing 1.8 L water to eclosion to subimagos (25-29 days). Subimagos were induced to emerge to adults in petri dishes and their clutch size measured through digital imaging. Selenium measurements from this study are given in Table E-23.

Table E-23. Selenium concentrations in water, periphyton and mayfly tissues for two feeding rations.
(Adapted from Table 1 in Conley et al. 2011)

Feeding ration – Se level	Mean dissolved Se exposed to periphyton, µg/L	Mean periphyton, mg Se/kg dw	Mean mayfly tissue, mg Se/kg dw
1x – low	1.1	4.2 ± 0.6 (4)	12.8 ± 3.6 (28)
1x – medium	5.9	11.9 ± 2.1 (4)	31.7 ± 7.5 (15)
1x - high	21.4	27.2 ± 4.2 (4)	68.4 ± 24.0 (9)
2x – low	2.7/3.4[a]	9.5 ± 0.9 (3)	14.1 ± 3.8 (19)
2x – medium	7.1/8.9[a]	19.9 ± 1.6 (3)	21.6 ± 2.8 (22)
2x - high	19.2/23.1[a]	40.9 ± 1.7 (3)	37.3 ± 6.7 (13)

[a] Two values represent two different loading exposures, September and October. The plates were combined for mayfly exposure.

Mayflies fed the 1x ration had 54% and 72% reductions in survival relative to controls in the medium and high Se treatment levels, respectively, both significant ($p<0.05$). The mayflies fed the 1x ration also had significant reductions in fecundity in the low (44% reduction), medium (63% reduction) and high (77% reduction) Se treatment levels. However, for the mayflies fed the 2x ration, there were no significant differences between the controls and any of the three Se treatment levels for any of the endpoints measured including survival and fecundity. The 2x ration mayflies had 60% more biomass than the 1x ration mayflies. This growth difference explains why the 1x ration mayflies had higher concentrations of Se in their tissues. The two different rations resulted in vastly different effect levels for Se, <12.8 mg/kg dw in the 1x ration test and >37.3 mg/kg dw in the 2x ration. It is apparent from this study that if the mayflies do not obtain sufficient nutrition, they are more sensitive to selenium. Although reduced feeding levels occur in nature, it is a confounding variable in this study that cannot be used to set a chronic effect level for selenium.

Conley et al. (2013) evaluated the accumulation of selenite and selenate into periphyton with subsequent feeding exposure to mayfly larvae. As in his previous studies, *C. triangulifer* larvae were fed periphyton previously exposed to different concentrations of selenium. In this study, periphyton plates were first exposed to low (10 µg/L) and high (30 µg/L) concentrations of either selenite or selenate and then fed to mayfly larvae to ecolsion to subimagos. The selenite and selenate treatment exposures resulted in similar levels of selenium in the subimagos. Since no differences in selenium accumulation was observed, the selenite and selenate treatments could be pooled for measuring the endpoints, survival and secondary production (total mayfly biomass produced). Mean selenium concentrations fed the mayflies were 2.2, 12.8 and 37 mg/kg Se dw in the control, low and high treatments, respectively. Mayfly tissue (subimago) concentrations (extrapolated from Figure 4a in Conley et al. 2013) were approximately 4-7, 20-35, and 45-75 mg/kg Se dw, in the control, low and high treatments, respectively. The authors reported significant reductions in survival from the control in the high Se treatment (both pooled data and individual selenite and selenate treatments) but no significant differences were observed in the low Se treatments. Secondary production was significantly reduced relative to the control in the high Se treatment for both selenium species. For the low Se exposure treatment, secondary production was not significantly different than the control for the selenite treated periphyton exposure, but was for the selenate and pooled data suggesting an effect level between 20 and 35 mg/kg Se dw. These results as well as those observed in 2x ration exposures in Conley et al. (2011) where no effects were observed at 37.3 mg/kg Se dw generally support the chronic value determined for Conley et al. (2009) of 24.2 mg/kg Se dw.

The following invertebrate studies were inconclusive for establishing species sensitivity because of limitations in the experimental designs, as explained for each.

5.4 Aquatic Insect (Midge: Chironimids)

Malchow et al. (1995) fed fourth instar *Chironomus decorus* midge larvae a diet of seleniferous algae under laboratory conditions for 96 hours. For algae cultured with selenite, a larval tissue concentration of 4.05 µg Se/g dry weight resulted in a 46% reduction in growth relative to the controls. At a larval tissue concentration of 8.6 µg Se/g dry weight, larval growth was reduced by only 39%. Since the study only reported two exposure concentrations, it is unclear if the tissue effect concentration at 4.05 µg Se/g dry weight is real or an anomaly. Additional exposure concentrations and subsequent effect levels are needed to resolve this issue.

Malchow et al. (1995) also fed fourth instar *Chrionomus decorus* midge larvae a diet of algae cultured with selenate, and the midge larvae were exposed under laboratory conditions for 96 hours. A dietary exposure of 2.11 µg Se/g dry weight significantly reduced larval growth (15% reduction) at tissue concentrations of 2.55 µg Se/g dry weight. At a larval tissue concentration of 6.62 µg Se/g dry weight, growth was reduced 20% relative to the controls. The 15-20% reduced growth at larval tissue concentrations 2.55 µg Se/g dry weight may be statistically significant, but not biologically meaningful. In addition, exposure to only two selenium concentrations precludes confirmation of a dose-response.

Alaimo et al. (1994) also exposed *2010* midge larvae to selenite diet, but the selenium source was from field contaminated widgeongrass (*Ruppia maritima*). *Ruppia* stems and leaves were collected from four selenium contaminated evaporation ponds located in the San Joaquin Valley of California. Three-day old larvae were exposed to each of the four treatment diets (*Ruppia* from each pond) plus a Cerophyll control for 14 days (egg to pupation), with the moderately hard reconstituted water renewed at day 7 and every three days thereafter. The growth (weight) of exposed larvae was significantly reduced in all of the selenium treatments when compared to the controls. The lowest effect level was observed for the Westlake pond (primarily selenite), where growth was reduced 40 percent relative to the controls at a larval tissue concentration below the detection level (1.0 ppm dry weight, or 1.0 µg Se/g dry weight). These results are suspect because the field collected *Ruppia* likely contained contaminants other than selenium, the control organisms were fed a different diet (Cerophyll), and the single concentration exposure is difficult to defend.

6.0 OTHER DATA – FIELD STUDY WEST VIRGINIA IMPOUNDMENTS

In response to the USEPA (2004) draft whole fish tissue criterion for selenium, the West Virginia Department of Environmental Protection (2010) initiated a study to assess selenium bioaccumulation among fishes residing in the State's lakes and streams. A focus of the study was the collection and evaluation of bluegill, *Lepomis macrochirus*, larvae (ichthyoplankton) from selected waterbodies since

2007, based on concerns regarding fish population health at locations subjected to elevated selenium inputs, particularly during the more sensitive developmental life stages of fishes (e.g. yolk-sac larvae). Also, in 2009, WVDEP began acquiring data about selenium concentrations within fish eggs of various species within reference and selenium-impacted waters. WVDEP also conducted deformity surveys of adult fishes in selenium enriched waters as well as at reference locations in 2008-2009.

WVDEP scientists found that larval deformity rates were variable throughout the study duration but were nonetheless correlated with waterborne selenium exposure. Reference locations produced age-based larval bluegill subsamples (24-168 hours) with low deformity rates (0 - 1.27%); whereas, locations with seleniferous inputs exhibited bluegill deformity rates ranging from 0% to 47.56% in developmental stages up to 312 hours. Maximum deformity rates among staged bluegill subsamples as determined through these evaluations were 19.28%, representing specimens collected from selenium-enriched waters. Concentrations of selenium within fish eggs also varied according to study location and ranged from <0.8 mg/kg dry weight among bluegill eggs at the control site to 64.62 mg/kg dry weight among largemouth bass, *Micropterus salmoides*, eggs collected from selenium-enriched waters. Searches for more mature, yet developmentally-deformed fishes revealed increased deformity rates (14%) among largemouth bass residing in a selenium impacted reservoir as compared to deformity rates among largemouth bass found in the reference lake (0%). The data on egg selenium concentrations are not adequate for constructing a concentration-response curve. Nevertheless, the overall deformity rate in the contaminated Upper Mud River Reservoir was 5% among 10,000 individual fish, average egg selenium concentration 9.8 mg/kg dw. The overall deformity rate in the reference Plum Orchard Lake was 0.5% among 13,000 individuals, average egg selenium concentration nondetectable or <0.8 mg/kg dw.

7.0 OTHER DATA - NUTRITIONAL DEFICIENCY/SUFFICIENCY STUDIES CONTAINING MEASURED SELENIUM IN THE DIET AND WHOLE BODY FISH TISSUE

Ingested dietary dose studies in fish designed to identify nutritionally deficient and/or nutritionally sufficient selenium doses in fish food or prey primarily describe selenium effects on growth, with survival reductions and effects on antioxidant enzyme activity also occasionally reported. A number of the dietary studies have measured a range of dietary doses that maximize fish growth, as opposed to a single dietary dose associated with nutritional sufficiency for growth. Regardless of whether nutritionally sufficient dietary doses are reported as a single concentration or as a range of concentrations, reduced growth or survival is observed at both lower dietary doses (nutritional deficiency) and at higher dietary doses (toxicity).

Although dietary doses are normally presented as selenium concentrations in food, expressed in terms of mg/kg Se in the diet, several studies have also concurrently presented nutritionally deficient/sufficient Se levels in terms of the whole body Se concentration in the fish. These studies permit a comparison of nutritionally deficient/sufficient whole body Se residues in fish to the national criterion for Se in whole bodies of fish. When combined with measured whole body fish tissue residues associated with toxicity, a complete picture of the range of Se residues in whole body fish tissue associated with nutritional deficiency, nutritional sufficiency and toxicity emerges.

Eight fish species have information on both nutritionally deficient dietary doses and whole body concentrations of selenium measured in the same study (Table E-24). Six of the eight species are native to North America. Nutritionally deficient dietary doses of Se range between 0.03 mg/kg dw in Atlantic salmon (*Salmo salar*, Poston et al. 1976) associated with reduced survival to 1.4 mg/kg dw in Atlantic cod (*Gadus morhua*, Hamre et al. 2008), also associated with reduced survival. Whole body Se residues identified as nutritionally deficient range between 0.64 mg/kg dw in Malabar grouper (*Epinephelus malabaricus*) associated with suboptimal weight gain and feed efficiency (Lin and Shiau 2005) and 4.72 mg/kg dw in North African catfish (*Clarias gariepinus*), also associated with suboptimal weight gain (Abdel-Tawwab et al. 2007). The whole body Se residues associated with growth and/or survival reductions due to nutritional deficiency of the six North American species (Prussian carp, Han et al. 2011; common carp, Gaber 2007; Atlantic cod, Hamre et al. 2008; Coho salmon, Felton et al. 1990; cobia, Lin et al. 2010; Atlantic salmon, Poston et al. 1976) all range between 1.0 and 2.7 mg/kg dw.

Ten fish species have information on both nutritionally sufficient dietary doses and whole body concentrations of selenium measured in the same study (Table D-23). Eight of the 10 species are native to North America. Nutritionally sufficient dietary doses of Se for the North American resident species, all but one of which are based on maximum growth of fish, range between 0.1 mg/kg dw in hybrid striped

bass (Jaramillo 2006) and 6.6 mg/kg dw in rainbow trout (Hilton and Hodson 1983). Several studies have identified a range of dietary doses and associated whole body residues that maximize growth and survival relative to that of fish fed lower dietary doses and which subsequently contain lower whole body selenium residues. Whole body Se residues associated with nutritional sufficiency based on maximal growth and/or survival of all North American species except for hybrid striped bass (Jaramillo 2006) range between 0.2 – 3.63 mg/kg dw (Table D-23). For hybrid striped bass, Jaramillo (2006) observed that maximum weight gain occurred in selenite supplemented diets containing 1.19 mg/kg dw Se, which resulted in whole body Se residues of 5.13 mg/kg dw. Jaramillo (2006) also exposed hybrid striped bass to seleno-DL-methionine supplemented diets containing 0.90 mg/kg dw, which resulted in the maximum weight gain of all seleno-DL-methionine supplemented diets tested, and a whole body Se residue of 7.2 mg/kg dw.

The nutritional sufficiency study of Rider et al. (2009) with rainbow trout is unique in that it determined dietary and whole body selenium requirements for both stressed and unstressed fish. Rider et al. (2009) observed that rainbow trout stressed by a combination of low water levels in holding tanks and twice daily handling of fish by 30 second aerial exposure in dip nets resulted in a higher nutritional requirement for selenium than was observed in fish not subjected to the stress routine. They concluded that trout exposed to physical stressors could benefit from an additional 0.3 – 2.0 mg/kg dw additional selenium supplementation over and above the Se content of nutritionally Se sufficient diets for fish not undergoing stress.

The fish with the highest known nutritional requirement for selenium is the non-North American resident North African catfish (*Clarias gariepinus*). Abdel-Tawwab et al. (2007) determined in a 12 week study with fingerlings that Se dietary doses of 1.04 mg/kg dw and 3.67 mg/kg dw were associated with suboptimal and maximum weight gains of the catfish, respectively. Catfish survival was 100% in both the Se-deficient and Se-sufficient dietary dose exposures during the 12 week study period. The respective whole body selenium tissue residues at the end of the 12 week study were 4.72 mg/kg dw in the Se-deficient fish and 15.43 mg/kg dw in the fish fed the nutritionally sufficient Se diet. North African catfish (Abdel-Tawwab et al. 2007) is the only known fish species with an identified whole body nutritional requirement for Se higher than the national aquatic life criterion for whole body Se in fish.

Table E-24. Studies with both empirically measured selenium dietary doses and whole body residues associated with nutritional deficiency and sufficiency in fish.

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.21	Basal diet	0.64	Deficiency	Suboptimal weight gain and feed efficiency	Lin and Shiau 2005
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	0.47	Seleno-methionine	1.0	Deficiency	Suboptimal growth, feeding rate and feed conversion rate	Han et al. 2011
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.04	Basal diet	1.04	Deficiency	Reduced growth and survival	Gaber 2007
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	1.4	Basal diet	1.1	Deficiency	Larval survival 32% lower compared to larvae fed selenium-enriched diet	Hamre et al. 2008
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.21 - 0.62	0.21 = Basal diet, 0.62 = seleno-DL-methionine	1.13 - 2.11	Deficiency	Statistically significantly reduced specific growth rate and survival	Liu et al. 2010
Coho salmon (*Oncorhynchus kisutch*)	Smolt 22.7 g	Hatchery reared	0.7 - 0.9	Not given	1.974	Deficiency	Survival of hatchery reared smolts 1.5 - 2.0x lower than wild smolts	Felton et al. 1990
Atlantic salmon (*Salmo salar*)	Fry 0.1 g	4 weeks	0.03 - 0.04	Basal diet	2.7	Deficiency	Decreased survival relative to fry fed diet supplemented with 0.1 µg/g Se and 0.5 IU/g vitamin E	Poston et al. 1976
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	1.04	Organic Se	4.72	Deficiency	Suboptimal weight gain and specific growth rate	Abdel-Tawwab et al. 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 0.6 g	16 weeks	0.6 - 6.6	Selenite $Na_2SeO_3 \cdot 5H_2O$	0.2 - 1.0	Sufficiency	No deficiency or toxicity signs on growth	Hilton and Hodson 1983

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	1.2	Basal diet	0.58 - 0.70	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	0.77	Basal diet	0.9	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.77	Seleno-methionine	0.92	Sufficiency	Maximal weight gain and feed efficiency	Lin and Shiau 2005
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.4	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.13	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.24 - 0.32	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.23 - 1.29	Sufficiency	Maximal growth and survival	Gaber 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.3 - 3.9	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.6 - 2.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	1.23 - 2.77	Seleno-methionine	1.7 - 3.4	Sufficiency	Maximal growth, no effect on survival, no increase in oxidative stress	Han et al. 2011
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	0.10	Basal diet	2.01	Sufficiency	Minimum dietary requirement for acceptable survival and growth	Jaramillo 2006
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.1	Seleno-methionine	2.06	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.85 - 1.36	Seleno-DL-methionine	2.58 - 2.62	Sufficiency	Maximal and statistically identical specific growth rate and survival	Liu et al. 2010

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.4 - 4.1	Organic Se - yeast	2.8 - 4.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	4.8	Selenite $Na_2SeO_3 \cdot 5H_2O$	3.5	Sufficiency	Larval survival increased 32%, growth essentially unchanged relative to survival of larvae fed basal diet	Hamre et al. 2008
Coho salmon (*Oncorhynchus kisutch*)	Smolt 14.28 g	Wild smolts	Se in natural diet unknown	Unknown	3.63	Sufficiency	Survival of wild smolts 1.5 - 2.0x higher than hatchery reared smolts	Felton et al. 1990
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	1.19	Selenite $Na_2SeO_3 \cdot 5H_2O$	5.13	Sufficiency	Highest weight gain of any selenite diet test, significantly higher than basal diet weight gain	Jaramillo 2006
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.92 g	12 weeks	0.90	Seleno-DL-methionine	7.2	Sufficiency	Highest survival and weight gain of any seleno-DL-methionine diet tested	Jaramillo 2006
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	3.67	Organic Se	15.43	Sufficiency	Maximal weight gain, specific growth rate and survival	Abdel-Tawwab et al. 2007

APPENDIX F: TOXICITY OF SELENIUM TO AQUATIC PLANTS

1.0 SELENITE

Data are available on the toxicity of selenite to 13 species of freshwater algae and plants (Table F-1). Results ranged from an LC_{50} of 70,000 µg/L for the green alga, *Chlorella ellipsoidea* (Shabana and El-Attar 1995) to 522 µg/L for incipient inhibition of the green alga, *Scenedesmus quadricauda* (Bringmann and Kuhn 1977a, 1978a,b, 1979, 1980b). Foe and Knight (Manuscript) found that 75 µg/L decreased the dry weight of *Selenastrum capricornutum* (Table F-1). Wehr and Brown (1985) reported that 320 µg/L increased the growth of the alga *Chrysochromulina breviturrita*.

The 96-hr EC_{50} for the saltwater diatom, *Skeletonema costatum*, is 7,930 µg/L, based on reduction in chlorophyll *a* (Table F-1). Growth of *Chlorella* sp., *Platymonas subcordiformis*, and *Fucus spiralis* increased at selenite concentrations from 2.6 to 10,000 µg/L (Table F-1). Other marine algae exposed to selenite from 14 to 60 days had no observed effect concentrations (NOAEC) that ranged from 1,076 to 107,606 µg/L. These data suggest that saltwater plants will not be adversely affected by concentrations of selenite that do not affect saltwater animals.

2.0 SELENATE

Growth of several species of green algae was affected by concentrations ranging from 100 to 40,000 µg/L (Table F-1). Blue-green algae appear to be more tolerant to selenate with 1,866 µg/L being the lowest concentration reported to affect growth (Kiffney and Knight 1990). Kumar (1964) found that a blue-green alga developed and lost resistance to selenate. The difference in the sensitivities of green and blue-green algae to selenate might be of ecological significance, particularly in bodies of water susceptible to nuisance algal blooms. For example, Patrick et al. (1975) reported that a concentration of 1,000 µg/L caused a natural assemblage of algae to shift to a community dominated by blue-green algae.

The saltwater coccolithophore, *Cricosphaera elongata*, had reduced growth when exposed to 41,800 µg/L selenate for 14 days (Boisson et al. 1995). Seven other saltwater algal species investigated by Wong and Oliveira (1991a) exhibited NOEC growth values that ranged from 1,043 to 104,328 µg/L. At 10,000 µg/L, selenate is lethal to four species of saltwater phytoplankton and lower concentrations increase or decrease growth (Table F-1). Wheeler et al. (1982) reported that concentrations as low as 10 µg/L reduced growth of *Porphyridium cruentum* (Table F-1).

Although selenite appears to be more acutely toxic than selenate to most aquatic animals, this does not seem to be true for aquatic plants. Selenite and selenate are about equally toxic to the freshwater algae *Anabaena cylindrica*, *Anabaena flos-aquae*, *Anabaena variabilis*, *Anacystis nidulans*, and *Scenedesmus dimorphus* (Kiffney and Knight 1990; Kumar and Prakash 1971; Moede et al. 1980) and the saltwater algae *Agemenellum quadroplicatum*, *Chaetoceros vixvisibilis* and *Amphidinium carterae* (Wong and Oliveira 1991a). The two oxidation states equally stimulated growth of *Chrysochromulina*

breviturrita (Wehr and Brown 1985). On the other hand, selenate is more toxic than selenite to the freshwater *Selenastrum capricornutum* (Richter 1982; Ibrahim and Spacie 1990) and the saltwater *Chorella* sp., *Platymonas subcordiformis* and *Nannochloropsis oculata* (Wheeler et al. 1982; Wong and Oliveira 1991a). In addition, Fries (1982) found that growth of thalli of the brown macroalga, *Fucus spiralis*, was stimulated more by exposure to selenite at 2.605 µg/L than to the same concentration of selenate.

A Final Plant Value, as defined in the Guidelines, cannot be obtained because no test in which the concentrations of selenite or selenate were measured and the endpoint was biologically relevant has been conducted with an important aquatic plant species.

Table F-1. Toxicity of selenium to aquatic plants.

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Chlorella vulgaris*	Sodium selenite	-	90-120	Reduced growth	5,480	De Jong 1965
Green alga, *Chlorella ellipsoidea*	Sodium selenite	-	7	EC50	70,000	Shabana and El-Attar 1995
Green alga, *Scenedesmus dimorphus*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	522	Bringmann and Kuhn 1977a; 1978a,b; 1979; 1980b
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	2,500	Bringmann and Kuhn 1959a
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	4	EC50	2,900	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	6	EC50	65,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena constricta*	Sodium selenite	-	7	EC50	67,000	Shabana and El-Attar 1995
Blue-green alga, *Anabaena cylindrica*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenite	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anabaena variabilis*	Sodium selenite	-	6-18	LC50	15,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anacystis nidulans*	Sodium selenite	-	10-18	LC50	30,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcystis aeruginisa*	Sodium selenite	-	8	Incipient inhibition	9,400 (9,300)	Bringmann and Kuhn 1976; 1978a,b
Alga, *Euglena gracilis*	-	-	15	Reduced growth	5,920	Bariaud and Mestre 1984
Duckweed, *Lemna minor*	-	-	4	EC50	2,400	Wang 1986
Duckweed, *Lemna minor*	Sodium selenite	-	14	EC50 (mult. rate)	3,500	Jenner and Janssen-Mommen 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Duckweed, *Lemna minor*	Sodium selenite	-	14	NOEC (mult. rate)	800	Jenner and Janssen-Mommen 1993
Selenium (VI)						
Green alga, *Ankistrodesmus falcatus*	Sodium selenate	-	14	Did not reduce growth	10	Vocke et al. 1980
Green alga, *Scenedesmus dimorphus*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Green alga, *Scenedesmus obliquus*	Sodium selenate	-	14	Reduced growth	100	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	14	Reduced growth	300	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	4	EC50	199	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	6	EC50	<40,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena cylindrica*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenate	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anacystis nidulans*	Sodium selenate	-	6-18	EC50	39,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anabaena viriabilis*	Sodium selenate	-	10-18	EC50	17,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcoleus vaginatus*	Sodium selenate	-	14	Reduced growth	10,000	Vocke et al. 1980
Duckweed, *Lemna minor*	Sodium selenate	-	14	EC50 (mult. rate)	11,500	Jenner and Janssen-Mommen 1993
Duckweed, *Lemna minor*	Sodium selenate	-	14	NOEC (mult. rate)	>2,400	Jenner and Janssen-Mommen 1993

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Green alga, *Dunaliella tertiolecta*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Diatom, *Skeletonema costatum*	Selenious acid[c]	-	4	EC50 (reduction in chlorophyll a)	7,930	U.S. EPA 1978
Coccolithophore, *Cricosphaera elongata*	Sodium selenite	-	14	Reduced growth	4,570	Boisson et al. 1995
Dinoflagellate, *Amphidinium carterae*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Dinoflagellate, *Peridinopsis borgei*	Selenium oxide	-	70-75	Maximum growth	0.01-0.05	Lindstrom 1985
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenite	-	60	NOEC growth	107,606	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodiun selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Selenium (VI)						
Green alga, *Dunaliella tertiolecta*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenate	-	60	NOEC growth	1,043	Wong and Oliveira 1991a
Coccolithophore, *Cricosphaera elongate*	Sodium selenate	-	14	Reduced growth	41,800	Boisson et al. 1995
Dinoflagellate,	Sodium	-	60	NOEC	10,433	Wong and

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Amphidinium carterae	selenate			growth		Oliveira 1991a
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a

[a] Concentration of selenium, not the chemical.
[b] Estimated from published graph.
[c] Reported by Barrows et al. (1980) in work performed under the same contract.

APPENDIX G: UNUSED DATA

Based on the requirements set forth in the guidelines (Stephen et al. 1985) the following studies are not acceptable for the following reasons and are classified as unused data. Note the acceptance of chronic toxicity data included diet and field exposures where selenium was the dominant toxicant.

Studies Were Conducted with Species That Are Not Resident in North America

Ahsanullah and Brand (1985)
Ahsanullah and Palmer (1980)
Baker and Davies (1997)
Barghigiani et al. (1993)
Chidambaram and Sastry (1991a,b)
Congiu et al. (1989)
Cuvin and Furness (1988)
Fowler and Benayoun (1976a,b)
Gaikwad (1989)
Gotsis (1982)
Hiraika et al. (1985)
Juhnke and Ludemann (1978)
Kitamura (1990)
Manoharan and Prabakaran (1994)
Minganti et al. (1994, 1995)
Niimi and LaHam (1975, 1976)
Regoli (1998)
Regoli and Principato (1995)
Rhodes et al. (1994)
Ringdal and Julshamn (1985)
Rouleau et al. (1992)
Sastry and Shukla (1994)
Savant and Nilkanth (1991)
Shultz and Ito (1979)
Srivastava and Tyagi (1985)
Takayanagi (2001)
Tomasik et al. (1995b)
Tian and Liu (1993)
Wrench (1978)

Deelstra et al. (1989), Forsythe and Klaine (1994), Okasako and Siegel (1980) and Petrucci et al. (1995) conducted tests with brine shrimp species that are too atypical to be used in derving national criteria.

These Studies or Reviews Contain Relevant Data That Have Been Published Elsewhere

Adams and Johnson (1981)
Biddinger and Gloss (1984)
Bowie et al. (1996)
Brandao et al. (1992)
Brooks (1984)
Burton and Stemmer (1988)
Chapman et al. (1986)
Davies (1978)
Debruyn and Chapman (2007)
Devillers et al. (1988)
Eisler (1985)
Hall and Burton (1982)
Hodson and Hilton (1983)
Hodson et al. (1984)
Jenkins (1980)
Kaiser et al. (1997)
Kay (1984)
LeBlanc (1984)
Lemly (1993c, 1996ab, 1997d)
Lemly and Smith (1987)
McKee and Wolf (1963)
National Research Council (1976) Neuhold (1987)
NCDNR&CD (1986)
Peterson and Nebeker (1992)
Phillips and Russo (1978)
Presser (1994)
Roux et al. (1996)
Swift (2002)
Thompson et al. (1972)
Versar (1975)

Authors Did Not Specify the Oxidation State of Selenium Used in Study

Greenberg and Kopec (1986)
Hutchinson and Stokes (1975)
Kapu and Schaeffer (1991)
Kramer et al. (1989)
Mahan et al. (1989)
Rauscher (1988)
Snell et al. (1991b)

Not Useful Because of No Effects Observed at Exposure Concentrations or Insufficient Number of Treatments

Muscatello and Janz (2009)
Pyle et al. (2005)
Schlenk et al (2003)

Chronic Study with no Dietary Exposure

Hopkins et al. (2002)
Oti (2005)
Rowe (2003)
Teh et al. (2002)

Selenium Was a Component of an Effluent, Fly Ash, Formulation, Mixture, Sediment or Sludge

Apte et al. (1987)
Baer et al. (1995)
Baker et al. (1991)
Berg et al. (1995)
Besser et al. (1989)
Biedlingmaier and Schmidt (1989)
Bjoernberg (1989)
Bjoernberg et al. (1988)
Bleckmann et al. (1995)
Boisson et al. (1989)
Bondavalli et al. (1996)
Bowmer et al. (1994)
Brieger et al. (1992)
Burton and Pinkney (1984)
Burton et al. (1983, 1987a)
Cherry et al. (1987)
Cieminski and Flake (1995)
Clark et al. (1989)
Cooke and Lee (1993)
Cossu et al. (1997)
Coyle et al. (1993)
Crane et al. (1992)
Crock et al. (1992)
Cushman et al. (1977)
Davies and Russell (1988)
de Peyster et al. (1993)
Dickman and Rygiel (1996)
Dierenfeld et al. (1993)
Doebel et al. (2004)
Drndarski et al. (1990)
Eriksson and Forsberg (1992)
Eriksson and Pedros-Alio (1990)
Fairbrother et al. (1994)
Fava et al. (1985a,b)
Feroci et al. (1997)
Finger and Bulak (1988)
Finley (1985)
Fisher and Wente (1993)
Fjeld and Rognerud (1993)
Fletcher et al. (1994)
Follett (1991)
Gerhardt (1990)
Gerhardt et al. (1991)
Gibbs and Miskiewicz (1995)
Graham et al. (1992)

Gunderson et al. (1997)
Hall (1988)
Hall et al. (1984, 1987, 1988,1992)
Hamilton et al. (1986, 2000)
Harrison et al. (1990)
Hartwell et al. (1987ab, 1988, 1997)
Hatcher et al. (1992)
Haynes et al. (1997)
Hayward et al. (1996)
Hellou et al. (1996b)
Henebry and Ross (1989)
Henny et al. (1989, 1990, 1995)
Hildebrand et al. (1976)
Hjeltnes and Julshman (1992)
Hockett and Mount (1996)
Hodson (1990)
Hoffman et al. (1988, 1991)
Homziak et al. (1993)
Hopkins et al. (2000)
Hopkins et al. (2004)
Hothem and Welsh (1994a)
Jackson (1988)
Jackson et al. (1990)
Jacquez et al. (1987)
Jay and Muncy (1979)
Jayasekera (1994)
Jayasekera and Rossbach (1996)
Jenner and Bowmer (1990) (1992)
Jin et al. (1997)
Jorgensen and Heisinger (1987)
Karlson and Frankenberger (1990)
Kemble et al. (1994)
Kennedy (1986)
Kersten et al. (1991)
King and Cromartie (1986)
King et al. (1991, 1994)
Klusek et al. (1993)
Koh and Harper (1988)
Koike et al. (1993)
Krishnaja et al. (1987)
Kruuk and Conroy (1991)
Kuehl and Haebler (1995)
Kuehl et al. (1994)
Kuss et al. (1995)
Landau et al. (1985)
Livingston et al. (1991)
Lobel et al. (1990)
Luoma and Phillips (1988)
Lundquist et al. (1994)
Lyle (1986)
MacFarlane et al. (1986)
Mann and Fyfe (1988)
Marcogliese et al. (1987)
Marvin and Howell. (1997)Maurer et al (1999)
McCloskey and Newman (1995)
McCloskey et al. (1995)
McCrea and Fischer (1986)
McLean et al. (1991)
Mehrle et al. (1987)
Metcalf-Smith (1994)
Micallef and Tyler (1989)
Mikac et al. (1985)
Miles and Tome (1997)
Miller et al. (1996)
Misitano and Schiewe (1990)
Moore (1988)
Munawar and Legner (1993)
Muskett et al. (1985)
Naddy et al. (1995)
Nielsen and Bjerregaard (1991)
Norman et al. (1992)
Nuutinen & Kukkonen (1998)
Oberbach and Hartfiel (1987, 1988)
Oberbach et al. (1989)
Ohlendorf et al. (1989, 1990, 1991)
Olson and Welsh (1993)
Peters et al.(1999)
Phillips and Gregory (1980)
Pratt and Bowers (1990)
Presser and Ohlendorf (1987)
Prevot and Sayer-Gobillard (1986)
Pritchard (1997)
Pyle et al. (2001)
Reash et al. (1988, in press)
Rhodes and Burke (1996)

Ribeyre et al. (1995)
Rice et al. (1995)
Riggs and Esch (1987)
Riggs et al. (1987)
Robertson et al. (1991)
Roper et al. (1997)
Rowe et al. (1996)
Russell et al. (1994)
Ryther et al. (1979)
Saiki and Jenings (1992)
Saiki and Ogle (1995)
Saleh et al. (1988)
Seelye et al. (1982)
Sevareid and Ichikawa (1983)
Skinner (1985)
Somerville et al. (1987)
Sorenson and Bauer (1983)
Specht et al. (1984)
Steele et al. (1992)
Stemmer et al. (1990)
Summers et al. (1995)
Thomas et al. (1980b)
Timothy et al. (2001)
Trieff et al. (1995)
Turgeon and O=Conner (1991)
Twerdok et al. (1997)
Unsal (1987)
Van Metre and Gray (1992)
Wahl et al. (1994)
Wandan and Zabik (1996)
Wang et al. (1992, 1995)
Welsh (1992)
Weres et al. (1990)
White and Geitner (1996)
Wiemeyer et al. (1986)
Wildhaber and Schmitt (1996)
Williams et al. (1989)
Wolfe et al. (1996)
Wolfenberger (1987)
Wong and Chau (1988)
Wong et al. (1982)
Wu et al. (1997)
Yamaoka et al. (1994)
Zagatto et al. (1987)
Zaidi et al. (1995)
Zhang et al. (1996)

Exposed enzymes, excised tissue or tissue extractor

Tripathi and Pandey (1985) and Heinz (1993b) used test organisms that had been previously exposed to pollutants in food or water.

Albers et al. (1996)
Al-Sabti (1994, 1995)
Arvy et al. (1995)
Augier et al. (1993a, b)
Avery et al. (1996)
Baatrup (1989)
Baatrup and Dansher (1987)
Baatrup et al. (1986)
Babich et al. (1986, 1989)
Barrington et al. (1997)
Becker et al. (1995a,b)
Bell et al. (1984, 1985, 1986a,b, 1987ab)
Berges and Harrison (1995)
Blondin et al. (1988)
Boisson et al. (1996)
Bottino et al. (1984)
Braddon (1982)
Braddon-Galloway and Balthrop (1985)
Bradford et al. (1994a,b)
Brandt et al. (1990)
Byl et al. (1994)
Chandy and Patel (1985)
Chen et al. (1997)
Cheng et al. (1993)
Christensen and Tucker (1976)
Dabbert and Powell (1993)
DeQuiroga et al. (1989)
Dierickx (1993)
Dietrich et al. (1987)
DiIlio et al. (1986)

Doyotte et al. (1997)
Drotar et al. (1987)
Dubois and Callard (1993)
Ebringer et al. (1996)
Engberg and Borsting (1994)
Engberg et al. (1993)
Eun et al. (1993)
Foltinova and Gajdosova (1993)
Foltinova et al. (1994)
Freeman and Sanglang (1977)
Grubor-Lajsic et al. (1995)
Hait and Sinha (1987)
Hanson (1997)
Heisinger and Scott (1985)
Heisinger and Wail (1989)
Henderson et al. (1987)
Henny and Bennett (1990)
Hoffman and Heinz (1988, 1998)
Hoffman et al. (1989, 1998)
Hoglund (1991)
Hontela et al. (1995)
Hsu et al. (1995)
Hsu and Goetz (1992)
Ishikawa et al. (1987)
James et al. (1993)
Jovanovic et al. (1995, 1997)
Kai et al. (1995)
Kedziroski et al. (1996)
Kelly et al. (1987)
Kralj and Stunja (1994)
Lalitha and Rani (1995)
Lan et al. (1995)
Lemaire et al. (1993)
Livingstone et al. (1992)
Low and Sin (1995, 1996)
Micallef and Tyler (1990)
Montagnese et al. (1993)
Murata et al. (1996)
Nakonieczny (1993)
Neuhierl and Boeck (1996)
Nigro (1994)
Nigro et al (1992)
Norheim and Borch-Iohnsen (1990)
Norheim et al. (1991)
O=Brien et al. (1995)
Olson and Christensen (1980)
Overbaugh and Fall (1985)
Palmisano et al. (1995)
Patel et al. (1990)
Patel and Chandy (1987)
Perez Campo et al. (1990)
Perez-Trigo et al. (1995)
Phadnis et al. (1988)
Price and Harrison (1988)
Rady et al. (1992)
Rani and Lalitha (1996)
Regoli et al. (1997)
Schmidt et al. (1985)
Schmitt et al. (1993)
Segner et al. (1994)
Sen et al. (1995)
Shigeoka et al. (1990, 1991)
Siwicki et al. (1994)
Srivastava and Srivastava (1995)
Sun et al. (1995)
Takeda et al. (1992a,b,(1993, 1997)
Treuthardt (1992)
Vazquez et al. (1994)
Veena et al. (1997)
Wise et al. (1993a,b)
Wong and Oliveira (1991b)
Yokota et al. (1988)

Test procedures test material or results were not adequately described by Botsford (1997), Botsford et al. (1997, 1998), Bovee (1978), Gissel-Nielsen and Gissel-Nielsen (1973, 1978), Greenberg and Kopec (1986), Mauk (2001), and Nassos et al. (1980) or when the test media contained an excessive amount (>200 μg/L) of EDTA (Riedel and Sanders (1996).

Some data obtained from tests conducted with just one exposure concentration to evaluate acute or chronic toxicity were not used (e.g., Bennett 1988; Heinz and Hoffman 1998; Munawar et al. 1987; Pagano et al. 1986; Wolfenberger 1986).

Kaiser (1980) calculated the toxicities of selenium(IV) and selenium(VI) to *Daphnia magna* based on physiochemical parameters. Kumar (1964) did not include a control treatment in the toxicity tests. The daphnids were probably stressed by crowding in the tests reported be Schultz et al. (1980). Siebers and Ehlers (1979) exposed too few test organisms as did Owsley (1984) in some tests.

Selenium Concentrations Reported in Wild Aquatic Organisms Were Insufficient to Calculate BAF

Abdel-Moati and Atta (1991)
Adeloju and Young (1994)
Aguirre et al. (1994)
Akesson and Srikumar (1994)
Aksnes et al. (1983)
Allen and Wilson (1990)
Ambulkar et al. (1995)
Amiard et al. (1991, 1993)
Andersen and Depledge (1997)
Andreev and Simeonov (1992)
Angulo (1996)
Arrula et al. (1996)
Arway (1988)
Ashton (1991)
Augier et al. (1991, 1993, 1995a,b)
Augspurger et al. (1998)
Avery et al. (1996)
Badsha and Goldspink (1988)
Baines and Fisher (2001)
Baldwin and Maher (1997)
Baldwin et al. (1996)
Barghigiani (1993)
Barghigiani et al. (1991)
Baron et al. (1997)
Batley (1987)
Baumann and Gillespie (1986)
Baumann and May (1984)
Beal (1974)
Beck et al. (1997)
Beland et al. (1993)
Beliaeff et al. (1997)
Bell and Cowey (1989)
Benemariya et al. (1991)
Berry et al. (1997)
Bertram et al. (1986)
Besser et al. (1994, 1993)
Birkner (1978)
Boisson and Romeo (1996)
Bowerman et al. (1994)
Braune et a. (1991)
Brezina and Arnold (1977)
Brugmann and Hennings (1994)
Brugmann and Lange (1988)
Brumbaugh and Walther (1991)
Burger (1992, 1994, 1995, 1996, 1997a,b)
Burger and Gochfeld (1992a,b, 1993, 1995 ab, 1996, 1997)
Burger et al. (1992a,b,c,1993, 1994a,b)
Byrne and DeLeon (1986)
Byrne et al. (1985)
Cantillo et al. (1997)
Capar and Yess (1996)
Capelli et al. (1987, 1991)
Cappon (1984)
Cappon and Smith (1981) (1982a,b)
Cardellicchio (1995)
Carell et al. (1987)
Carter and Porter (1997)
Caurant et al. (1994, 1996)
Chau and Riley (1965)
Chiang et al. (1994)

Chou and Uthe (1991)
Chvojka (1988)
Chvojka et al. (1990)
Clifford and Harrison (1988)
Collins (1992)
Combs et al. (1996)
Cosson et al. (1988)
Courtney et al. (1994)
Cruwys et al. (1994)
Crutchfield (2000)
Cumbie and Van Horn (1978)
Currey et al. (1992)
Custer and Hohman (1994)
Custer and Mitchell (1991, 1993)
Custer et al. (1997)
Dabeka and McKenzie (1991)
Davoren (1986)
Deaker and Maher (1997)
Demon et al. (1988)
Dietz et al. (1995, 1996)
Doherty et al. (1993)
Elliott and Scheuhammer (1997)
Eriksson et al. (1989)
Evans et al. (1993)
Felton rt al. (1990)
Felton et al. (1994)
Fitzsimons et al. (1995)
Focardi et al. (1985, 1988)
Fowler (1986)
Fowler et al. (1975, 1985)
France (1987)
Friberg (1988)
Froslie et al. (1985, 1987)
Gabrashanske and Daskalova (1985)
Gabrashanska and Nedeva (1994)
Galgan and Frank (1995)
Garcia - Hermandez et al. (2000)
Giardina et al. (1997)
Gillespie and Baumann (1986)
Gochfeld (1997)
Goede (1985, 1991, 1993a,b)
Goede et al. (1989, 1993)
Goede and DeBruin (1984, 1985)
Goede and Wolterbeek (1993, 1994a,b)
Gras et al. (1992)
Greig and Jones (1976)
Gutenmann et al. (1988)
Gutierrez-Galindo et al. (1994)
Guven et al. (1992)
Halbrook et al. (1996)
Hall and Fisher (1985)
Hamilton and Waddell (1994)
Hamilton and Wiedmeyer (1990)
Hansen et al. (1990)
Hardiman and Pearson (1995)
Hargrave et al. (1992)
Harrison and Klaverkamp (1990)
Hasunuma et al. (1993)
Haynes et al. (1995)
Hein et al. (1994)
Heiny and Tate (1997)
Heinz (1993a)
Heinz and Fitzgerald (1993a,b)
Heit (1985)
Heit and Klusek (1985)
Heit et al. (1980, 1989)
Hellou et al. (1992a,b) (1996a,b)
Henny and Herron (1989)
Hodge et al. (1996)
Hilton et al. (1982)
Honda et al. (1986)
Hothem and Ohlendorf (1989)
Hothem and Welsh (1994b)
Hothem and Zador (1995)
Hothem et al. (1995)
Houpt et al. (1988)
Hunter et al. (1995, 1997)
Ibrahim and Farrag (1992)
Ibrahim and Mat (1995)
Ishikawa et al. (1993)
Itano et al. (1984, 1985a,b)
Jarman et al. (1996)
Johns et al. (1988)
Johnson (1987)
Jop et al. (1997)

Jorhem et al. (1994)
Julshamn et al. (1987)
Kai et al. (1986a,b, 1988, 1992a,b, 1996)
Kaiser et al. (1979)
Kalas et al. (1995)
Kidwell et al. (1995)
Koeman et al. (1973)
Kovacs et al. (1984)
Krogh and Scanes (1997)
Krushevska et al. (1996)
Lakshmanan and Stephen (1994)
Lalitha et al. (1994)
LamLeung et al. (1991)
Lan et al. (1994a,b)
Langlois and Langis (1995)
Larsen and Stuerup (1994)
Larsen et al. (1997)
Lauchli (1993)
Law et al. (1996)
Lee and Fisher (1992a,b, 1993)
Leighton and Wobeser (1994)
Leland and Scudder (1990)
Lemly (1985a, 1994)
Leonzio et al. (1986, 1989, 1992)
Leskinen et al. (1986)
Li et al. (1996)
Lie et al. (1994)
Liu et al. (1987)
Lizama et al. (1989)
Lobel et al. (1989, 1991, 1992a,b)
Lonzarich et al. (1992)
Lourdes et al. (1990)
Lowe et al. (1985)
Lucas et al. (1970)
Lytle and Lytle (1982)
Mackey et al. (1996)
Maher (1987)
Maher et al. (1992, 1997)
Mann et al. (1988)
Mason et al. (2000)
Masuzawa et al. (1988)
Matsumoto (1991)
Maven et al. (1995)
May and McKinney (1981)
Mcdowell et al. (1995)
McKenzie-Parnell et al. (1988)
Meador et al. (1993)
Mehrle et al. (1982)
Meltzer et al. (1993)
Metcalfe-Smith et al. (1992, 1996)
Michot et al. (1994)
Mills et al. (1993)
Moharram et al. (1987)
Moller (1996)
Mora and Anderson (1995)
Morera et al. (1997)
Muir et al. (1988)
Mutanen et al. (1986)
Nadkarni and Primack (1993)
Nakamoto and Hassler (1992)
Narasaki and Cao (1996)
Navarrete et al. (1990)
Nettleton et al. (1990)
Nicola et al. (1987)
Nielsen and Dietz (1990)
Norheim (1987)
Norheim et al. (1992)
Norrgren et al. (1993)
Norstrom et al. (1986)
O=Conner (1996)
O=Shea et al. (1984)
Ober et al. (1987)
Oehlenschlager (1997)
Ohlendorf (1986)
Ohlendorf and Harrison (1986)
Ohlendorf and Marois (1990)
Ohlendorf et al. (1986a,b, 1987, 1988a,b)
Okazaki and Panietz (1981)
Ostapczuk et al. (1997)
Pakkala et al. (1972)
Pal et al. (1997) Palawski et al. (1991)
Palmer-Locarnini and Presley (1995)
Paludan-Miller et al. (1993)
Papadopoulou and Andreotis (1985)
Park and Presley (1997)
Park et al. (1994)

Paveglio et al. (1994)
Payer and Runkel (1978)
Payer et al. (1976)
Pennington et al. (1982)
Presley et al. (1990)
Quevauviller et al. (1993a,b)
Ramos et al. (1992)
Rao et al. (1996)
Reinfelder and Fisher (1991)
Reinfelder et al. (1993, 1998)
Renzoni et al. (1986)
Riget et al. (1996)
Risenhoover (1989)
Roditi (2000)
Roux et al. (1994)
Ruelle and Keenlyne (1993)
Sager and Cofield (1984)
Saiki (1986 ab, 1987, 1990)
Saiki and Lowe (1987)
Saiki and May (1988)
Saiki and Palawski (1990)
Saiki et al. (1992, 1993)
Sanders and Gilmour (1994)
Scanes (1997)
Scheuhammer et al. (1988)
Schantz et al. (1997)
Schmitt and Brumbaugh (1990)
Schramel and Xu (1991)
Schuler et al. (1990)
Scott and Latshaw (1993)
Secor et al. (1993)
Seelye et al. (1982)
Sharif et al. (1993)
Shen et al. (1997)
Shirasaki et al. (1996)
Shultz and Ito (1979)
Simopoulos (1997)
Skaare et al. (1990, 1994)
Smith and Flegal (1989)
Smith et al. (1992)
Sorensen (1988)
Sorensen and Bauer (1984a,b) Sorensen and Bjerregaard (1991)
Sorensen et al. (1982, 1983, 1984)
Southworth et al. (2000)
Sparling and Lowe (1996)
Speyer (1980)
Steimle et al. (1994)
Stoeppler et al. (1988)
Stone et al. (1988)
Stripp et al. (1990)
Sundarrao et al. (1991) (1992)
Svensson et al. (1992)
Tabaka et al. (1996)
Talbot and Chang (1987)
Tallandini et al. (1996)
Tan and Marshall (1997)
Tang et al. (1997)
Tao et al. (1993)
Teherani (1987)
Teigen et al. (1993)
Thomas et al. (1999)
Tilbury et al. (1997)
Topcuoglu et al. (1990)
TranVan and Teherani (1988)
Trocine and Trefry (1996)
Uthe and Bigh (1971)
Vanderstoep et al. (1990)
Varanasi et al. (1993, 1994)
Vitaliano and Zdanowicz (1992)
Vlieg (1990)
Vlieg et al. (1993)
Vos et al. (1986)
Waddell and May (1995)
Wagemann (1988)
Wagemann and Stewart (1994)
Wagemann et al. (1988) (1996) Walsh et al. (1977)
Wang (1996)
Ward and Flick (1990)
Warren et al. (1990)
Weber (1985)
Welsh and Maughan (1994)
Wen et al. (1997)
Wenzel and Gabrielsen (1995)
Whyte and Boutillier (1991)
Williams et al. (1994)
Wilson et al. (1992, 1997)
Winger and Andreasen (1985)
Winger et al. (1984, 1990)
Woock and Summers (1984)
Wren et al. (1987)
Wu and Huang (1991)
Yamaoka et al. (1996)

Yamazaki et al. (1996)
Yoshida and Yasumoto (1987)
Zatta et al. (1985)
Zeisler et al. (1988, 1993)
Zhou and Liu (1997)

APPENDIX H: CALCULATION OF EF VALUES

EPA calculated EF values by searching its database of selenium measurements and identifying all the selenium measurements from algae, detritus, or sediment. EPA then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water measurement was available for any given particulate measurement, the median was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median ratio. The geometric mean of the algae, detritus, and sediment ratios was used as the site *EF*. Because there were at most only 3 possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric mean in order to reduce the potential for one of the values to have excessive influence on the final site *EF* value. Sites with insufficient data to fulfill these criteria are left blank.

EPA evaluated differences in bioaccumulation between different categories of aquatic systems by analyzing EF values for different categories. EPA sequentially consolidated categories and examined differences in the distribution of EF values between categories. See text for a complete description of this analysis.

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Birkner 1978	East Allen Reservoir, Medicine Bow WY	20	Reservoir	Lentic	3.00		41.00	11.09	4.80	2.31
Birkner 1978	Galett Lake, Laramie WY	7	Lake	Lentic	0.18		2.80	0.70	0.80	0.88
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	30	Pond	Lentic	15.50		47.30	27.08	15.90	1.70
Birkner 1978	Meeboer Lake, Laramie WY	3	Lake	Lentic	0.10		0.30	0.17	0.30	0.58
Birkner 1978	Miller's Lake, Wellington CO	22	Lake	Lentic	4.60		44.00	14.23	6.00	2.37
Birkner 1978	Sweitzer Lake, Delta CO	27	Lake	Lentic	10.35		6.50	8.20	9.40	0.87
Birkner 1978	Twin Buttes Reservoir, Laramie WY	23	Reservoir	Lentic	7.80		10.80	9.18	7.60	1.21

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Bowie et al. 1996	Hyco Reservoir		Reservoir	Lentic	27.00			27.00	11.50	2.35
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	N2	Reservoir	Lentic	2.65		0.60	1.26	1.00	1.26
Butler et al. 1997	Large pond on Dove Creek	DCP1	Pond	Lentic	1.00		2.10	1.45	2.00	0.72
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	MNP2	Pond	Lentic	5.40		6.70	6.01	3.00	2.00
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	MNP3	Pond	Lentic	4.50		5.90	5.15	1.00	5.15
Butler et al. 1997	Pond on Cahone Canyon, west of 1 5 Road	CHP	Pond	Lentic	4.00		2.10	2.90	5.00	0.58
Butler et al. 1997	Pond on Woods Canyon at 15 Road	WCP	Pond	Lentic	2.30		3.20	2.71	3.00	0.90
Butler et al. 1997	West pond at CC Road	PVP1	Pond	Lentic	1.50		1.40	1.45	2.00	0.72
Grasso et al. 1995	Arapahoe Wetlands Pond	17	Pond	Lentic	1.87		0.40	0.86	1.00	0.86
Lemly 1985	Badin Lake		Lake	Lentic	7.70		2.07	3.99	0.32	12.48
Lemly 1985	Belews Lake		Lake	Lentic	44.10		8.27	19.10	10.91	1.75
Lemly 1985	High Rock Lake		Lake	Lentic	6.20		1.80	3.34	0.67	4.99
Muscatello and Janz 2009	Vulture Lake		Lake	Lentic	0.35		0.54	0.43	0.43	1.01
Orr et al. 2006	Barns Lake Wetland	BLW	Lake	Lentic	4.40		2.00	2.97	0.50	5.93

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Orr et al. 2006	Fording River Oxbow	FRO	Oxbow	Lentic	5.55		7.90	6.62	5.04	1.37
Orr et al. 2006	Fording Settling Pond (Clode Pond)	FSP	Pond	Lentic	5.49		2.80	3.92	42.99	0.09
Orr et al. 2006	Goddard Marsh	GM	Marsh	Lentic	3.21		26.00	9.14	90.95	0.10
Orr et al. 2012	Clode Pond 11	CL11	Pond	Lentic	25.80			25.80	36.10	0.71
Orr et al. 2012	Elk Lakes 14	EL14	Lake	Lentic	0.66			0.66	0.40	1.64
Orr et al. 2012	Flathead Wetland 17	FL17	Marsh	Lentic	1.42			1.42	0.20	7.10
Orr et al. 2012	Fording River Oxbow 10	FO10	Oxbow	Lentic	67.31			67.31	50.10	1.34
Orr et al. 2012	Goddard Marsh 13	GO13	Marsh	Lentic	18.15			18.15	16.30	1.11
Orr et al. 2012	Henretta Lake 27	HE27	Lake	Lentic	4.30			4.30	8.60	0.50
Saiki and Lowe 1987	Kesterson Pond 11		Pond	Lentic	18.15	47.95	8.56	19.53	38.60	0.51
Saiki and Lowe 1987	Kesterson Pond 2		Pond	Lentic	152.70	44.65	34.82	61.92	195.85	0.32
Saiki and Lowe 1987	Kesterson Pond 8		Pond	Lentic	136.50	92.00	6.05	42.34	70.35	0.60
Saiki and Lowe 1987	Volta Pond 26		Pond	Lentic	0.42	1.01	0.29	0.50	0.53	0.93
Saiki and Lowe 1987	Volta Pond 7		Pond	Lentic		1.39	0.39	0.74	0.63	1.17
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 7	Pond	Lentic	87.10		5.90	22.67	100.00	0.23

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 2	Pond	Lentic	52.50		9.30	22.10	90.00	0.25
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 11	Pond	Lentic	53.70		11.50	24.85	40.00	0.62
Stephens et al. 1988	Marsh 4720	*	Marsh	Lentic	2.10		4.20	2.97	31.00	0.10
Butler et al. 1991	Uncompahgre River at Colona	4	River	Lotic	0.95			0.95	1.50	0.63
Butler et al. 1993	Spring Cr. at La Boca	SP2	Creek	Lotic	1.60		0.50	0.89	5.00	0.18
Butler et al. 1995	Cahone Canyon at Highway 666	CH	Creek	Lotic	2.50		4.30	3.28	12.00	0.27
Butler et al. 1995	Hartman Draw near mouth, at Cortez	HD2	Draw	Lotic	0.45		0.20	0.30	2.00	0.15
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	ME1	Creek	Lotic	1.80			1.80	2.00	0.90
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	ME2	Creek	Lotic	1.11		1.10	1.10	3.00	0.37
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	ME4	Creek	Lotic	1.04		0.50	0.72	6.00	0.12
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	ME3	Creek	Lotic	0.82		0.40	0.57	6.00	0.10
Butler et al. 1995	Navajo Wash near Towaoc	NW	Wash	Lotic	3.45		1.60	2.35	12.00	0.20
Butler et al. 1995	San Juan River at Four Comers	SJ1	River	Lotic	0.52		0.30	0.39	1.50	0.26
Butler et al. 1995	San Juan River at Mexican Hat Utah	SJ3	River	Lotic	0.94		0.20	0.43	1.50	0.29
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	WC	Creek	Lotic	3.30		1.50	2.22	5.50	0.40

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Butler et al. 1997	Cahone Canyon at Highway 666	CH1	Creek	Lotic	2.05			2.05	10.50	0.20
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	MUD2	Creek	Lotic	1.30			1.30	18.50	0.07
Butler et al. 1997	Tributary of Cahone Canyon at 13 Road	CH2	Creek	Lotic	1.75			1.75	5.50	0.32
Butler et al. 1997	Tributary of Yellow Jacket Canyon at Highway 666	YJ1	Creek	Lotic	1.85			1.85	7.00	0.26
Butler et al. 1997	Unnamed tributary of Cow Canyon at 8 Road	COW	Creek	Lotic	1.45			1.45	3.50	0.41
Butler et al. 1997	Unnamed tributary of Cross Canyon upstream from Alkali Canyon	CCTR	Creek	Lotic	1.75			1.75	4.50	0.39
Casey 2005	Deerlick Creek		Creek	Lotic		1.00	0.20	0.45	0.20	2.24
Casey 2005	Luscar Creek		Creek	Lotic	5.50	3.20	2.40	3.48	10.70	0.33
Formation 2012	Crow Creek - 1A	CC-1A	Creek	Lotic	3.64		1.20	2.09	2.45	0.80
Formation 2012	Crow Creek - 3A	CC-3A	Creek	Lotic	3.10		0.83	1.60	2.20	0.81
Formation 2012	Crow Creek - CC150	CC-150	Creek	Lotic	1.20		0.63	0.87	0.80	1.04
Formation 2012	Crow Creek - CC350	CC-350	Creek	Lotic	1.50		0.70	1.02	0.86	1.16
Formation 2012	Crow Creek - CC75	CC-75	Creek	Lotic	1.01		0.54	0.74	0.52	1.19
Formation 2012	Deer Creek	DC-600	Creek	Lotic	4.55		1.40	2.52	1.62	1.55

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Formation 2012	Hoopes Spring - HS	HS	Spring	Lotic	12.00		2.30	5.25	20.95	0.24
Formation 2012	Hoopes Spring - HS3	HS-3	Spring	Lotic	12.00		7.00	9.17	17.05	0.54
Formation 2012	Sage Creek - LSV2C	LSV-2C	Creek	Lotic	8.09		4.60	6.10	13.80	0.45
Formation 2012	Sage Creek - LSV4	LSV-4	Creek	Lotic	9.56		3.60	5.87	8.45	0.69
Formation 2012	South Fork Tincup Cr.	SFTC-1	Creek	Lotic	0.73		0.31	0.47	0.44	1.32
Golder 2011; Teck Coal 2013	McLeod River below Cheviot Creek	MR-2	River	Lotic	1.47			1.47	2.38	0.62
Golder 2011; Teck Coal 2013	McLeod River below Luscar Dreek	MR-6	River	Lotic	0.86			0.86	4.29	0.20
Golder 2011; Teck Coal 2013	McLeod River below Whitehorse Creek	MR-4	River	Lotic	0.68			0.68	1.07	0.64
Golder 2011; Teck Coal 2013	McLeod River reference	MR-1	River	Lotic	0.75			0.75	0.30	2.50
Golder 2011; Teck Coal 2013	Prospect Creek far field	PC-3	Creek	Lotic	0.37			0.37	0.63	0.59
Golder 2011; Teck Coal 2013	Prospect Creek reference	PC-1	Creek	Lotic	0.86			0.86	0.40	2.15
Hamilton and Buhl 2004	lower East Mill Creek	LEMC	Creek	Lotic	25.70		38.90	31.62	24.00	1.32

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	ER 745	River	Lotic	0.31		1.28	0.63	0.10	6.30
McDonald and Strosher 1998	Elk R. above Fording R.	ER 750	River	Lotic	0.78		0.70	0.74	0.40	1.85
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	ER 746	River	Lotic	1.56		2.41	1.94	8.60	0.23
McDonald and Strosher 1998	Michel Cr. at Highway 3	ER 751	Creek	Lotic	1.26		2.32	1.71	7.10	0.24
Orr et al. 2006	Alexander Creek	AC	Creek	Lotic	4.49		0.90	2.01	0.90	2.23
Orr et al. 2006	Fording River	FR	River	Lotic	3.27		2.10	2.62	20.10	0.13
Orr et al. 2006	Line Creek	LC	Creek	Lotic	2.19		2.10	2.14	20.90	0.10
Orr et al. 2012	Elk River 1	EL1	River	Lotic	2.30			2.30	4.20	0.55
Orr et al. 2012	Elk River 12	EL12	River	Lotic	2.00			2.00	0.75	2.67
Orr et al. 2012	Fording River 23	FO23	River	Lotic	6.35			6.35	30.60	0.21
Orr et al. 2012	Michel Creek 2	MI2	Creek	Lotic	2.10			2.10	7.40	0.28
Presser and Luoma 2009	Upper Peters canyon (dry)	U PCW dry	Wash	Lotic	1.20		0.60	0.85	3.20	0.27
Saiki and Lowe 1987	San Luis Drain		Drain	Lotic	67.00	275.00	79.90	113.76	316.50	0.36
Saiki and Lowe 1987	Volta Wasteway		Wasteway	Lotic	0.87	2.03	0.24	0.76	0.74	1.03
Saiki et al. 1993	Mud Slough at Gun Club Road	GT5	Slough	Lotic	4.50	14.95		8.20	6.00	1.37

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	GT4	Slough	Lotic	1.39	8.40		3.42	8.00	0.43
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	SJR2	River	Lotic	1.25	5.00		2.50	7.00	0.36
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	SJR3	River	Lotic	0.45	1.25		0.75	1.00	0.75
Stephens et al. 1988	Drain J3	*	Drain	Lotic	24.00		48.00	33.94	110.00	0.31

APPENDIX I: OBSERVED VERSUS PREDICTED EGG-OVARY CONCENTRATIONS

The following table includes data for 317 individual fish tissue selenium measurements from the 64 sites where EFs could be calculated. Observed egg-ovary fish tissue measurements were compared to predicted egg-ovary fish tissue measurements calculated using equation 22 of the main text, also shown here for convenience.

$$C_{egg-ovary} = C_{water} \times TTF^{composite} \times EF \times CF \quad \text{(Equation 22)}$$

These data were used to generate the observed to predicted egg-ovary concentration Figure 6.3 of the main text. When the measured tissue type was either muscle or whole body, it was converted to egg-ovary using taxa specific conversion factors. The predicted and measured concentrations are highly correlated ($r = 0.82$, $t_{(315)} = 25.30$, $P < 0.001$).

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Birkner 1978	East Allen Reservoir, Medicine Bow WY	Iowa darter	4.80	2.31	2.87	1.45	46.14	52.68	WB
Birkner 1978	Galett Lake, Laramie WY	Iowa darter	0.80	0.88	2.87	1.45	2.91	3.05	WB
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	northern plains killifish	15.90	1.70	2.44	1.20	79.04	68.71	WB
Birkner 1978	Meeboer Lake, Laramie WY	northern plains killifish	0.30	0.58	2.44	1.20	0.51	9.22	WB
Birkner 1978	Miller's Lake, Wellington CO	fathead minnow	6.00	2.37	2.78	1.40	55.31	15.37	WB
Birkner 1978	Miller's Lake, Wellington CO	Iowa darter	6.00	2.37	2.87	1.45	59.18	33.38	WB
Birkner 1978	Sweitzer Lake, Delta CO	northern plains killifish	9.40	0.87	2.44	1.20	23.94	38.18	WB
Birkner 1978	Sweitzer Lake, Delta CO	fathead minnow	9.40	0.87	2.78	1.40	31.89	110.38	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	northern plains killifish	7.60	1.21	2.44	1.20	26.79	27.65	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	fathead minnow	7.60	1.21	2.78	1.40	35.69	48.20	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	Iowa darter	7.60	1.21	2.87	1.45	38.18	60.81	WB
Bowie et al. 1996	Hyco Reservoir	bluegill	11.50	2.35	2.00	2.13	114.97	87.47	WB
Butler et al. 1991	Uncompahgre River at Colona	flannelmouth sucker	1.50	0.63	1.52	1.41	2.03	2.40	WB
Butler et al. 1991	Uncompahgre River at Colona	white sucker	1.50	0.63	1.58	1.38	2.07	7.32	WB
Butler et al. 1991	Uncompahgre River at Colona	bluehead sucker	1.50	0.63	1.24	1.82	2.13	3.27	WB
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	3.77	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	6.39	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	4.77	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	5.06	WB
Butler et al. 1991	Uncompahgre River at Colona	rainbow trout	1.50	0.63	2.33	2.44	5.39	6.88	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	brown trout	1.00	1.26	2.78	1.45	5.08	6.20	E-O
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	channel catfish	1.00	1.26	1.35	1.45	2.47	2.32	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	2.03	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	3.05	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	5.19	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Spring Cr. at La Boca	white sucker	5.00	0.18	1.58	1.38	1.96	4.83	WB
Butler et al. 1993	Spring Cr. at La Boca	bluehead sucker	5.50	0.18	1.24	1.82	2.22	12.91	WB
Butler et al. 1993	Spring Cr. at La Boca	speckled dace	5.00	0.18	1.36	1.95	2.37	23.45	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.00	0.18	2.78	1.40	3.48	11.46	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.00	0.18	2.78	1.45	3.60	1.74	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.50	0.18	2.78	1.40	3.83	8.38	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.50	0.18	2.78	1.45	3.96	4.92	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	1.07	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	0.96	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.69	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.76	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.87	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	1.35	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.10	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.24	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	speckled dace	2.00	0.90	1.36	1.95	4.77	12.51	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	fathead minnow	2.00	0.90	2.78	1.40	7.00	7.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.25	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	1.97	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	3.10	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	1.51	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	2.36	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	speckled dace	3.00	0.37	1.36	1.95	2.92	11.92	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	fathead minnow	3.00	0.37	2.78	1.40	4.29	6.71	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.11	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	1.83	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.68	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	3.38	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	4.23	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.49	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.11	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.30	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	1.96	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	8.24	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	red shiner	6.00	0.12	2.27	1.95	3.20	9.97	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.96	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	3.38	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	5.07	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.27	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.09	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bullhead	6.00	0.10	1.62	1.45	1.34	4.35	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	5.47	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	13.68	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	10.75	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	8.45	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	9.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	green sunfish	6.00	0.10	2.29	1.45	1.91	7.26	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.01	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	7.41	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.15	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.21	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	16.91	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	13.09	WB
Butler et al. 1995	Navajo Wash near Towaoc	speckled dace	12.00	0.20	1.36	1.95	6.23	17.00	WB
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	2.98	M
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.70	M
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	5.95	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.11	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	3.10	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	0.86	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	1.55	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	5.92	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	1.71	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	8.40	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	9.97	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	5.67	WB
Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	10.18	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	6.53	WB
Butler et al. 1995	San Juan River at Four Comers	red shiner	1.50	0.26	2.27	1.95	1.75	6.84	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	channel catfish	1.50	0.29	1.35	1.45	0.85	10.88	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.68	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	1.97	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.18	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.36	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.91	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	common carp	1.50	0.29	1.58	1.92	1.31	7.49	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	25.71	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	32.00	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	36.89	WB
Butler et al. 1997	Cahone Canyon at Highway 666	green sunfish	10.50	0.20	2.29	1.45	6.83	13.79	WB
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	fathead minnow	3.00	2.00	2.78	1.40	23.39	15.37	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	4.55	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	9.45	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	10.18	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	11.03	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	10.16	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	10.76	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	16.77	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	9.08	WB
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	smallmouth bass	1.00	5.15	1.93	1.42	14.09	17.03	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	13.97	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	20.96	WB
Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	3.14	M

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	8.16	E-O
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	16.79	M
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	33.48	E-O
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	14.43	WB
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	12.10	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	15.20	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	13.49	WB
Formation 2012	Crow Creek - 1A	sculpin	2.45	0.80	2.78	1.45	7.89	11.29	WB
Formation 2012	Crow Creek - 1A	brown trout	2.45	0.80	2.96	1.45	8.37	14.39	WB
Formation 2012	Crow Creek - 1A	sculpin	2.90	0.80	2.78	1.45	9.34	25.35	WB
Formation 2012	Crow Creek - 1A	brown trout	2.90	0.80	2.96	1.45	9.91	24.36	WB
Formation 2012	Crow Creek - 1A	sculpin	4.80	0.80	2.78	1.45	15.45	18.33	WB
Formation 2012	Crow Creek - 1A	brown trout	4.80	0.80	2.96	1.45	16.40	20.29	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	20.97	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	16.91	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	15.09	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	13.30	WB
Formation 2012	Crow Creek - 3A	sculpin	2.20	0.81	2.78	1.45	7.17	16.65	WB
Formation 2012	Crow Creek - 3A	brown trout	2.20	0.81	2.97	1.45	7.60	16.27	WB
Formation 2012	Crow Creek - 3A	brown trout	2.60	0.81	2.97	1.45	8.99	22.24	WB
Formation 2012	Crow Creek - 3A	sculpin	4.20	0.81	2.78	1.45	13.68	29.32	WB
Formation 2012	Crow Creek - 3A	brown trout	4.20	0.81	2.97	1.45	14.52	28.45	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	8.72	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	7.31	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	8.43	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	12.54	WB
Formation 2012	Crow Creek - CC150	sculpin	0.80	1.04	2.74	1.45	3.31	7.46	WB
Formation 2012	Crow Creek - CC150	brown trout	0.80	1.04	2.91	1.45	3.51	7.52	WB
Formation 2012	Crow Creek - CC150	sculpin	1.40	1.04	2.74	1.45	5.79	15.57	WB
Formation 2012	Crow Creek - CC150	brown trout	1.40	1.04	2.91	1.45	6.14	14.66	WB
Formation 2012	Crow Creek - CC150	sculpin	1.50	1.04	2.74	1.45	6.20	10.67	WB
Formation 2012	Crow Creek - CC150	brown trout	1.50	1.04	2.91	1.45	6.58	11.32	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	9.39	WB
Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	10.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.86	1.16	2.79	1.45	4.02	7.66	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	9.08	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	12.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.89	1.16	2.79	1.45	4.19	14.56	WB
Formation 2012	Crow Creek - CC350	brown trout	0.86	1.16	2.97	1.45	4.27	8.36	WB
Formation 2012	Crow Creek - CC350	brown trout	0.89	1.16	2.97	1.45	4.44	16.63	WB
Formation 2012	Crow Creek - CC350	sculpin	1.10	1.16	2.79	1.45	5.15	13.83	WB
Formation 2012	Crow Creek - CC350	brown trout	1.10	1.16	2.97	1.45	5.47	11.49	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	8.10	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	7.30	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	5.86	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	7.74	WB
Formation 2012	Crow Creek - CC75	sculpin	0.52	1.19	2.69	1.45	2.39	5.47	WB
Formation 2012	Crow Creek - CC75	brown trout	0.52	1.19	2.87	1.45	2.54	4.60	WB
Formation 2012	Crow Creek - CC75	sculpin	0.85	1.19	2.69	1.45	3.94	10.43	WB
Formation 2012	Crow Creek - CC75	brown trout	0.85	1.19	2.87	1.45	4.18	14.92	WB
Formation 2012	Crow Creek - CC75	sculpin	1.00	1.19	2.69	1.45	4.64	10.28	WB
Formation 2012	Crow Creek - CC75	brown trout	1.00	1.19	2.87	1.45	4.92	9.54	WB
Formation 2012	Deer Creek	sculpin	1.45	1.55	2.81	1.45	9.17	11.07	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	12.34	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	11.42	WB
Formation 2012	Deer Creek	brown trout	1.45	1.55	3.00	1.45	9.73	8.46	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	12.35	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	8.96	WB
Formation 2012	Deer Creek	sculpin	2.00	1.55	2.81	1.45	12.65	11.55	WB
Formation 2012	Deer Creek	brown trout	2.00	1.55	3.00	1.45	13.43	18.55	WB
Formation 2012	Deer Creek	sculpin	2.40	1.55	2.81	1.45	15.18	12.51	WB
Formation 2012	Deer Creek	brown trout	2.40	1.55	3.00	1.45	16.11	15.24	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.71	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.74	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Formation 2012	Hoopes Spring - HS	sculpin	20.95	0.24	3.63	1.45	26.96	15.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	23.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	36.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.95	0.24	3.86	1.45	28.61	36.00	WB
Formation 2012	Hoopes Spring - HS	sculpin	27.30	0.24	3.63	1.45	35.13	52.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	27.30	0.24	3.86	1.45	37.28	47.18	WB
Formation 2012	Hoopes Spring - HS	sculpin	40.45	0.24	3.63	1.45	52.05	59.94	WB
Formation 2012	Hoopes Spring - HS	brown trout	40.45	0.24	3.86	1.45	55.23	32.97	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	31.71	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	26.95	WB
Formation 2012	Hoopes Spring - HS3	sculpin	17.05	0.54	2.47	1.45	32.79	38.65	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	29.78	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	27.23	WB
Formation 2012	Hoopes Spring - HS3	brown trout	17.05	0.54	2.63	1.45	34.79	25.87	WB
Formation 2012	Hoopes Spring - HS3	sculpin	26.00	0.54	2.47	1.45	49.99	34.73	WB
Formation 2012	Hoopes Spring - HS3	brown trout	26.00	0.54	2.63	1.45	53.05	34.24	WB
Formation 2012	Hoopes Spring - HS3	sculpin	31.75	0.54	2.47	1.45	61.05	34.37	WB
Formation 2012	Hoopes Spring - HS3	brown trout	31.75	0.54	2.63	1.45	64.78	41.89	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	25.35	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	16.52	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.80	0.45	2.83	1.45	25.31	27.36	WB
Formation 2012	Sage Creek - LSV2C	sculpin	14.30	0.45	2.83	1.45	26.23	37.66	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	28.12	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	18.48	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.80	0.45	3.01	1.45	26.86	32.78	WB
Formation 2012	Sage Creek - LSV2C	brown trout	14.30	0.45	3.01	1.45	27.83	28.24	WB
Formation 2012	Sage Creek - LSV2C	sculpin	18.75	0.45	2.83	1.45	34.39	29.49	WB
Formation 2012	Sage Creek - LSV2C	brown trout	18.75	0.45	3.01	1.45	36.49	30.30	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	29.04	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	26.53	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	23.42	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	21.95	WB

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Formation 2012	South Fork Tincup Cr.	sculpin	0.32	1.32	2.86	1.45	1.73	8.24	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.32	1.32	3.05	1.45	1.84	5.32	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.43	1.32	2.86	1.45	2.37	5.44	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.44	1.32	2.86	1.45	2.42	13.51	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.43	1.32	3.05	1.45	2.51	3.25	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.44	1.32	3.05	1.45	2.57	9.69	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.56	1.32	2.86	1.45	3.06	8.52	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.56	1.32	3.05	1.45	3.24	3.82	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	3.92	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.41	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.75	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.03	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.52	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.54	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.21	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.22	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	10.20	WB
Hamilton and Buhl 2004	lower East Mill Creek	cutthroat trout	24.00	1.32	2.29	1.96	142.01	102.73	WB
Lemly 1985	Badin Lake	black bullhead	0.32	12.48	1.72	1.45	9.99	4.44	M
Lemly 1985	Badin Lake	western mosquitofish	0.32	12.48	2.37	1.20	11.33	5.77	M
Lemly 1985	Badin Lake	common carp	0.32	12.48	1.58	1.92	12.10	5.81	M
Lemly 1985	Badin Lake	green sunfish	0.32	12.48	2.29	1.45	13.30	3.25	M
Lemly 1985	Badin Lake	fathead minnow	0.32	12.48	2.78	1.40	15.52	3.17	M
Lemly 1985	Badin Lake	red shiner	0.32	12.48	2.27	1.95	17.74	4.45	M
Lemly 1985	Belews Lake	black bullhead	10.91	1.75	1.72	1.45	47.79	29.84	M
Lemly 1985	Belews Lake	western mosquitofish	10.91	1.75	2.37	1.20	54.18	46.86	M
Lemly 1985	Belews Lake	common carp	10.91	1.75	1.58	1.92	57.86	38.97	M
Lemly 1985	Belews Lake	green sunfish	10.91	1.75	2.29	1.45	63.60	20.84	M
Lemly 1985	Belews Lake	fathead minnow	10.91	1.75	2.78	1.40	74.25	28.75	M
Lemly 1985	Belews Lake	red shiner	10.91	1.75	2.27	1.95	84.87	38.59	M
Lemly 1985	High Rock Lake	black bullhead	0.67	4.99	1.72	1.45	8.36	5.58	M

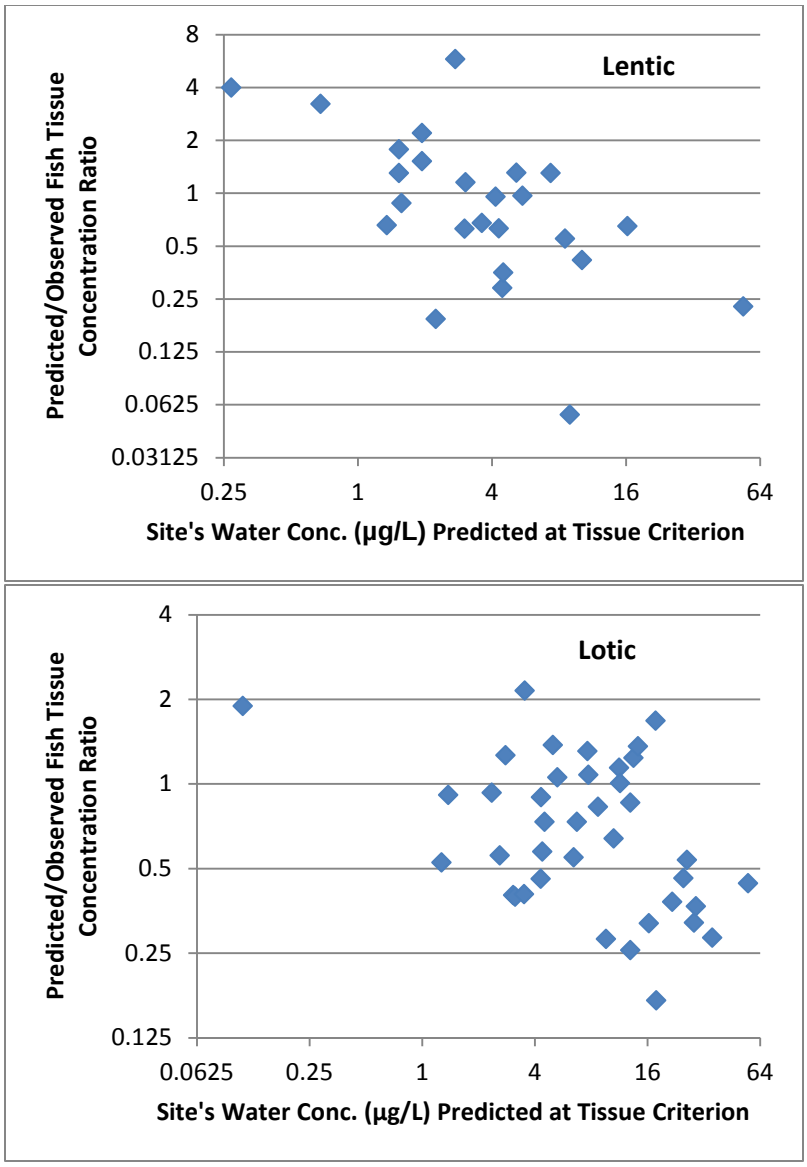
Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Lemly 1985	High Rock Lake	western mosquitofish	0.67	4.99	2.37	1.20	9.48	6.10	M
Lemly 1985	High Rock Lake	common carp	0.67	4.99	1.58	1.92	10.12	4.49	M
Lemly 1985	High Rock Lake	green sunfish	0.67	4.99	2.29	1.45	11.13	3.13	M
Lemly 1985	High Rock Lake	fathead minnow	0.67	4.99	2.78	1.40	12.99	4.00	M
Lemly 1985	High Rock Lake	red shiner	0.67	4.99	2.27	1.95	14.85	4.62	M
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	cutthroat trout	0.10	6.30	2.29	1.96	2.83	10.61	WB
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	mountain whitefish	0.10	6.30	2.97	7.39	13.83	7.11	WB
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	cutthroat trout	8.60	0.23	2.29	1.96	8.71	24.96	WB
Muscatello and Janz 2009	Vulture Lake	white sucker	0.43	1.01	1.58	1.38	0.95	4.65	WB
Muscatello and Janz 2009	Vulture Lake	burbot	0.43	1.01	2.45	1.45	1.54	15.91	WB
Muscatello and Janz 2009	Vulture Lake	ninespine stickleback	0.43	1.01	3.22	1.45	2.03	6.02	WB
Muscatello and Janz 2009	Vulture Lake	northern pike	0.43	1.01	4.02	2.39	4.17	1.83	WB
Orr et al. 2012	Clode Pond 11	cutthroat trout	36.10	0.71	2.29	1.96	115.88	81.06	E-O
Orr et al. 2012	Elk Lakes 14	cutthroat trout	0.40	1.64	2.29	1.96	2.95	14.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	11.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	15.60	E-O
Orr et al. 2012	Elk River 12	cutthroat trout	0.75	2.67	2.29	1.96	8.98	9.00	E-O
Orr et al. 2012	Fording River 23	cutthroat trout	30.60	0.21	2.29	1.96	28.52	15.56	E-O
Orr et al. 2012	Fording River Oxbow 10	cutthroat trout	50.10	1.34	2.29	1.96	302.30	47.81	E-O
Orr et al. 2012	Henretta Lake 27	cutthroat trout	8.60	0.50	2.29	1.96	19.33	13.56	E-O
Orr et al. 2012	Michel Creek 2	cutthroat trout	7.40	0.28	2.29	1.96	9.43	10.07	E-O
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	155.61	WB
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	124.49	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	268.13	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	295.66	WB

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Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	196.31	WB
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	266.93	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	178.36	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	397.41	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.53	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.48	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.62	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.63	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	10.67	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	13.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.79	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	13.17	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	19.15	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.17	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.60	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	5.68	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	6.67	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.39	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.87	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	5.76	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	7.04	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.12	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.41	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	2.63	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	5.39	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.05	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.27	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.41	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.55	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.03	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.39	WB
Stephens et al. 1988	Marsh 4720	black bullhead	31.00	0.10	1.72	1.45	7.43	10.16	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	36.49	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	40.33	WB

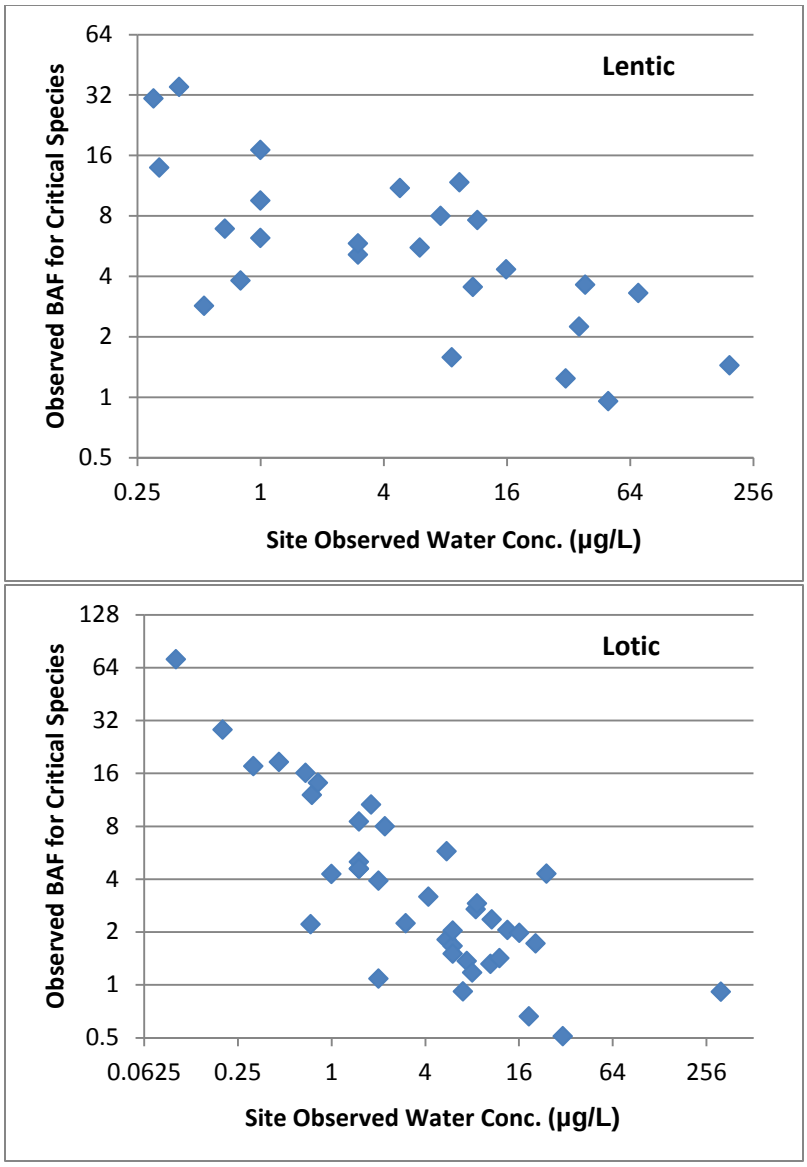
The ratio of predicted versus observed tissue concentrations in the above table can be compared against the main text Table 3.13 water concentrations that would be predicted to occur if each site's egg-ovary tissue concentration were at the criterion level. Figure I-1 shows the results. It can be seen that for those sites (in the left portion of each graph) where tissue concentrations equal to the egg-ovary criterion would be predicted to yield water concentrations not far on either side of the water criteria values, the predicted-to-observed tissue concentration ratios are not particularly biased relative to a ratio of 1.0. This indicates that the model is performing reasonably well for those sites strongly influencing the derived values of the water criteria.

The derivation of the water criteria concentrations involves an assumption of linearity in projecting the water concentration that would correspond to a tissue concentration equal to the tissue criterion. Figure I-2 suggests that high BAFs tend to be associated with low water concentrations, and low BAFs with high water concentrations. At the low concentrations associated with the 20^{th} percentile model-predicted BAF, the linearity assumption would appear to be environmentally conservative. At high concentrations, the opposite situation would occur, but overall, because the criterion is based on the 20^{th} percentile, the linearity assumption appears to be protective.



Figures I-1. For lentic (left panel) and lotic (right panel) waters, predicted-to-observed fish-tissue concentration ratio for each of the 65 sites, plotted versus each site's Table 3.13 water concentration that would be predicted to occur if its tissue levels were at the egg-ovary tissue criterion level.

Corresponding to how the water criteria concentrations were derived, for sites with multiple fish species, the plotted ratio is for the species having the highest predicted tissue-to-water ratio (i.e., highest predicted BAF). For sites having multiple samples of that species, the plotted value is the average predicted-to-observed ratio for that species.



Figures I-2. For lentic (left panel) and lotic (right panel) waters, observed BAFs (egg-ovary tissue-to-water concentration ratios) versus observed water concentration (both from the above table), for each site's fish species used in Table 3.13 (that is, for the species used in the water criteria calculations).

For sites having multiple samples of such species, tissue concentrations were averaged. Because nearly all samples were either whole body or muscle, the graphed BAFs include application of the CF, to normalize all samples to egg-ovary tissue. Since the CFs have been are assumed to be independent of concentration, the graphs do not reflect any potential CF nonlinearities, if they exist.

APPENDIX J: SUPPLEMENTARY INFORMATION ON SELENIUM BIOACCUMULATION IN AQUATIC ANIMALS

1.0 EFFECTS OF GROWTH RATE ON THE ACCUMULATION OF SELENIUM IN FISH

EPA analyzed the effect of the growth rate parameter *g* when estimating selenium bioaccumulation using the mechanistic bioaccumulation modeling described in Equation 1 of the main text. Because the addition of tissue associated with growth could have a dilution effect on the chemicals present in tissue, a parameter representing growth rate is present in the denominator of Equation 1. Indeed, growth can be an important factor in the bioaccumulation of very hydrophobic chemicals with low excretion rates such as polychlorinated biphenyls, (Connolly and Pedersen 1988). However, the effect of growth may not be as important for selenium because of its unique biogeochemical characteristics, route of exposure, and role as a micronutrient.

EPA tested the effect of the growth rate parameter *g* on estimates of selenium bioaccumulation using Equation 1 with different food web scenarios. Increasing growth rates from 0 (no growth) to 0.2/day (a relatively high rate of growth) reduced selenium concentrations in trophic level 2 and 3 organisms by as much as a factor of 10 to 20. Thus incorporating growth rate in Equation 1 could result in significant dilution of selenium and lower estimates of selenium bioaccumulation.

Although increasing the value of the growth parameter g in Equation 1 reduces estimates of selenium bioaccumulation, this simple analysis neglects an important physiological linkage between growth and food consumption. Organisms must consume enough food to support growth and meet their energy requirements for respiration, specific dynamic action, waste loss, and reproduction. These physiological requirements suggest that higher growth rates are associated with greater rates of food consumption. Because food consumption is the primary route of selenium exposure in aquatic organisms, increased selenium exposure associated with higher food consumption could counterbalance the dilution of selenium in tissue associated with higher growth rates.

EPA tested the effects of growth on estimates of selenium bioaccumulation using Equation 1 when increased food consumption was associated with higher growth rates. EPA modified Equation 1 to incorporate a simple relationship for bioenergetics (Thomann et al. 1992) and applied the model to reexamine the sensitivity of selenium bioaccumulation to growth rates in trophic level 2 and 3 organisms. The results of this analysis showed that increasing growth rates over two orders of magnitude increased selenium concentrations in trophic level 2 by a factor of 2, and decreased selenium concentrations in trophic level 3 by 10%. When growth rates were increased simultaneously in trophic levels 2 and 3, the selenium concentrations increased by less than a factor of 2. This analysis suggests that when bioenergetics is considered, selenium bioaccumulation is generally insensitive to organism growth rates. EPA believes that uncertainties in the toxicokinetic parameters of selenium far outweigh the effects on growth rate on selenium bioaccumulation. Thus, the growth rate parameter *g* was removed from Equation

1 for the purpose of deriving a translation equation that could be used to implement a tissue-based selenium water quality criterion.

2.0 Analysis of the Relative Contribution of Aqueous and Dietary Uptake on the Bioaccumulation of Selenium

EPA analyzed the relative contributions of direct aqueous uptake versus ingestion of selenium in consideration of removing the uptake rate constant k_u from Equation 1 in Section 3.2 of the main text. Because an important exposure route for some chemicals is direct contact with water, an uptake rate constant k_u is present in the numerator of Equation 1. However, fish and invertebrate organisms absorb selenium primarily through the consumption of food rather than from direct aqueous uptake (Forester 2007; Lemly 1985; Luoma et al. 1992). Thus, removing the uptake rate constant k_u could simplify Equation 1 while maintaining the key determinants of selenium bioaccumulation.

EPA tested the relative contribution of aqueous versus dietary uptake of selenium using a version of Equation 1 that incorporates both exposure pathways (Thomann et. al. 1992). For trophic level 2, selenium bioaccumulation was estimated for a range of uptake rates that varied according to the respiration rate and aqueous transfer efficiency of selenium relative to dissolved oxygen. For trophic level 3, uptake rates were varied within a range of values reported in Besser et al. (1993) and Bertram and Brooks (1986).

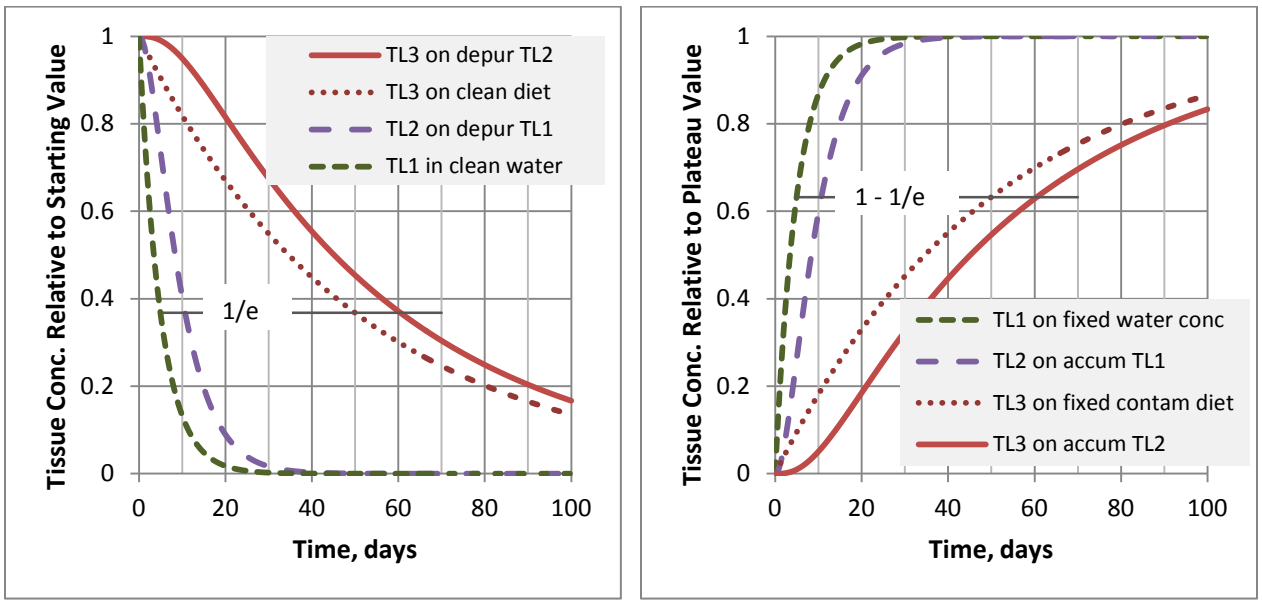
EPA's analysis showed that diet accounted for 34% - 92% of selenium bioaccumulation at trophic level 2, with a median of 74%. At trophic level 3, diet accounted for 62% - 100% of tissue selenium, with a median of 95%. Thus, disregarding aqueous uptake of selenium only resulted in a small (~5%) reduction in estimated selenium bioaccumulation in trophic level 3 organisms. These results are consistent with previous studies indicating that diet is the primary exposure route of selenium, and suggests that the uptake rate constant for selenium can be removed from Equation 1 with negligible effect for higher trophic levels organisms.

3.0 Kinetics of Accumulation and Depuration: Averaging Period

3.1 Background

For setting averaging periods for aquatic life criteria, U.S. EPA (1995b) used the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action. In the context of the water-borne direct toxicity of metals, characteristic time = 1/k, where k is the first-order kinetic coefficient in a toxico-kinetic model fitted to the relationship between LC50 and exposure duration.

In the context of selenium bioaccumulation in a single trophic level, k would the first-order depuration coefficient, and 1/k would equal the time needed to depurate to a concentration of 1/e times the initial concentration (where e=2.718). For depuration of multiple trophic levels sequentially, the characteristic time is likewise the time needed for c/c_o to reach a value of 1/e, as shown in Figure J-1a. The accumulation curve is the inverted depuration curve, as shown in Figure J-1b.



Figures J-1 a & b. Depuration and accumulation behavior for algae-detritus-sediment k=0.2/day, invertebrate k=0.2/day and fish k=0.02/day, calculated with time step = 0.1 day.
Concentration is expressed as a dimensionless ratio: concentration at time t divided by either starting concentration (J1a) or plateau concentration (J1b).

In the Figures J-1 a & b examples, the characteristic time for algae-detritus-sediment is 5 days, the characteristic time for invertebrates on an invariant diet is 5 days, the characteristic time for fish on an invariant diet is 50 days, and the characteristic time for fish on an invertebrate diet that is itself depurating or accumulating is the approximate sum of the individual characteristic times, or ~60 days.

In contrast to the model depuration rate, k, the model uptake rate (AE, assimilation efficiency, multiplied by IR, intake rate) does not affect the characteristic response time. Rather it affects the magnitude of the accumulation plateau. Uptake rate thus affects the TTF value itself but is not relevant to setting an averaging period.

Because short averaging periods are more environmentally conservative than long averaging periods, selecting parameter values for fast kinetics is more environmentally conservative. Figure J1 reflects environmentally conservative choices for k values.

3.2 Approach for Modeling Effects of Time-Variable Se Concentrations

Expression of concentrations. None of the concentrations in this analysis are expressed in ordinary units of concentration. All concentrations are modeled as values normalized to their allowable benchmark concentration – that is, concentration = 1 for a particular medium (water, algae-detritus-sediment, invertebrates, or fish) means that the medium is at its criterion concentration or corresponding benchmark. It is assumed that the benchmarks correctly align – water held at its benchmark concentration will ultimately yield Trophic Levels 1, 2, and 3 at their respective benchmark concentrations. The Trophic Level 3 benchmark is the reproductive EC10 for the 5th percentile taxon: i.e., the fish tissue criterion.

Formulation of the bioaccumulation model for kinetic analysis. For algae-detritus-sediment, for invertebrates, and for fish, accumulation at time t equals accumulation at time t-1 plus intake minus depuration, as follows:

Algae-detritus-sediment:

$$C_{TL1}[t] = C_{TL1}[t\text{-}1] + k_{uptake}\, C[t\text{-}1]water - k_{TL1}\, C_{TL1}[t\text{-}1]$$

Invertebrates:

$$C_{TL2}[t] = C_{TL2}[t\text{-}1] + AE_{TL2}\, IR_{TL2}\, C_{TL1}[t\text{-}1] - k_{TL2}\, C_{TL2}[t\text{-}1]$$

Fish:

$$C_{TL3}[t] = C_{TL3}[t\text{-}1] + AE_{TL3}\, IR_{TL3}\, C_{TL2}[t\text{-}1] - k_{TL3}\, C_{TL3}[t\text{-}1]$$

For algae-detritus-sediment, the depuration rate k is assigned a value of 0.2/day, similar to the sum of depuration and growth-dilution rate coefficients used by Brix and DeForest (2008). Because a lentic system would involve the slower kinetics of sediment exchange, the rapid rate used here implies a lotic system.

For invertebrates, a value of 0.2/day was assigned, considerably higher than those for *Lumbriculus*, Asian clam, zebra mussel, but close to those of mayfly and copepods, which are very small in size. As previously mentioned, higher k (more rapid kinetics) is an environmentally conservative assumption in this context.

For fish, the median depuration coefficient measured by Bertram and Brooks (1986) for 6-9 month-old (early adult) fathead minnows was used, providing a k_{TL3} value of 0.02/day. Because of the small size of adults of this species, this represents faster kinetics than would likely be applicable the salmonids and centrarchids of greatest concern for selenium toxicity. The striped bass k value of Baines et al. (2002) is inapplicable here because it was measured in the early juvenile life stage, a size that is too small to be relevant to reproductive impairment stemming from exposure of adult females. The concentration in fish could be equivalently viewed as either whole body or egg-ovary, relative to their

respective benchmarks. That is, partitioning within body of the fish is assumed not to involve a time delay.

The value of a TTF is given by AE x IR/k (or k_{uptake}/k for algae-detritus-sediment). Concentrations in TL1, TL2, and TL3 are normalized to their benchmarks, meaning that all benchmark concentrations have a value of 1.0. In this normalized context, the TTFs must also equal 1.0, since upon reaching steady state, TL1 at its benchmark will yield TL2 at its benchmark, which in turn will yield TL3 at its benchmark. Again, the analysis is not intended to reflect actual concentrations, merely portray temporal behavior. Since 1 = TTF = AE x IR/k, it follows that AE x IR = k within this normalized framework. Although only the product AE x IR is relevant, they are retained as distinct parameters to maintain parallelism with remainder of the criterion document. AE was assigned a value of 0.5 for fish and invertebrates, and IR = k/AE in the normalized framework.

Time step durations of 0.1-1.0 day were considered. Short time steps increase accuracy by decreasing the numerical dispersion inherent in expressing C[t] = f(C[t-1]). A time step of 0.5 day was found to yield sufficient accuracy, as measured by predicted values at the characteristic time for depuration or accumulation (per Figure J-1).

Prediction of Effects. The effect level associated with the tissue concentration at any time t is calculated via the log probit concentration-response curve, one of the commonly used sigmoid curves. It assumes that the sensitivities in the underlying population are log-normally distributed such that the concentration yielding effects on k percentage of the population is given by:

$$EC_k = EC50 \exp(\sigma z)$$

where σ is the inverse of the concentration-response curve slope and z is the normal deviate corresponding to k percent (e.g., for k=10%, z=NORMSINV(0.1)=−1.28155). Among the reproductive impairment studies presented in Appendix C, an approximate median ratio for EC50/EC10 is 1.5. This translates to σ=0.3164.

Since the fish tissue criterion concentration equals 1.0 in this normalized framework, at any time t, the fractional level of effect corresponding to any value of C_{TL3} is given by:

Fractional Effect[t] = NORMSDIST(z[t])

where z[t] is given by:

$z[t] = LN(C_{TL3}[t]/1.5)/0.3164$

Exposure Scenarios. Three exposure scenarios were evaluated under which the water criterion was just barely attained. The first two are absolute worst case scenarios, in which the 30-day average water concentration remains continuously at the criterion concentration at all times. The third is a realistic scemario.

1. Steady concentrations at the criterion: this is worst-case continuous exposure. In the real world this could not occur because water concentrations vary substantially over time. For the 30-day average concentration not to exceed more than once in three years, the realistically varying daily concentrations must remain well below the criterion concentration a large majority of the time.

2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals (i.e., separated by 29 days of zero concentration) such that the 30-day average always equals the criterion. This is the worst-case intermittent scenario, attaining the criterion through a time series that continually maximizes the 30-day average exposure at the water criterion concentration while also imposing the highest variability possible from spikes of 1-day duration. In the real world intermittent runoff sources do not occur at uniform intervals: merely averaging 30-days between discharges would yield an exceedance each time the discharge occurred with less than 30-days spacing. Further, the once-per-month peak concentrations could never be controlled at exactly 30X the chronic water criterion per the above discussion of the first scenario.

   It is because they lack real-world random variability that the above two scenarios are not realistic. They are used as absolute worst cases for purposes of comparison. The following third scenario represents a realistic and indeed typical situation for continuous exposure:

3. Log-normally distributed, smoothly variable concentrations with the 30-day average exceeding the criterion once in three years when counted using the procedure of EPA (1986). The log

standard deviation of 0.5 applied here represents typical real-world time variability for continuously flowing waters. The log serial correlation coefficient $\rho = 0.8$ represents that typical of smaller streams.

With respect to maximizing toxic effects while attaining the criterion, Scenarios #1 and #2 are absolute worst cases. In contrast, Scenario #3 represents typical time variability in ambient waters. This third scenario requires randomly generated concentrations (having specified target statistical characteristics). Multiple runs of long series are therefore needed to assure some reasonable degree of accuracy. A minimum of 20 runs of random series of 3000 days were used. The concentrations at each half-day time step were generated by the following formula:

$$C[t]water = C[t-1]water^{(\rho')} * GM^{(1-\rho')} * EXP\{\sigma * SQRT(1-\rho'^2)*NORMSINV(RAND)\}$$

where ρ' (rho prime) is the desired serial correlation coefficient between half-day time steps: $\rho'=SQRT(\rho)$ [approximation], where ρ (rho) is the desired serial correlation coefficient between daily values; GM is the desired geometric mean or median, and σ is the desired log standard deviation. The above formula allows a time series with the desired statistical characteristics to be generated.

3.2.1 Model Results

3.2.1.1 Steady concentrations at the water criterion concentration.

No graphic is needed to explain this scenario. With water steady at its criterion, algae-detritus-sediment and invertebrates are likewise steady at their benchmark concentrations, and fish tissue is at its criterion concentration. For the 5th percentile taxon, the effect would thus be 10% since the concentration is steady at the EC10.

3.2.1.2 Uniformly spaced spikes at maximum concentrations

Figure J-2. Scenario 2, uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion. Read invertebrate and fish tissue concentrations on left scale, water concentrations on right scale. Time=0 does not represent the beginning of exposure; prior to Time=0 the same exposure pattern had been going on for a long time (e.g., 10,000 days).

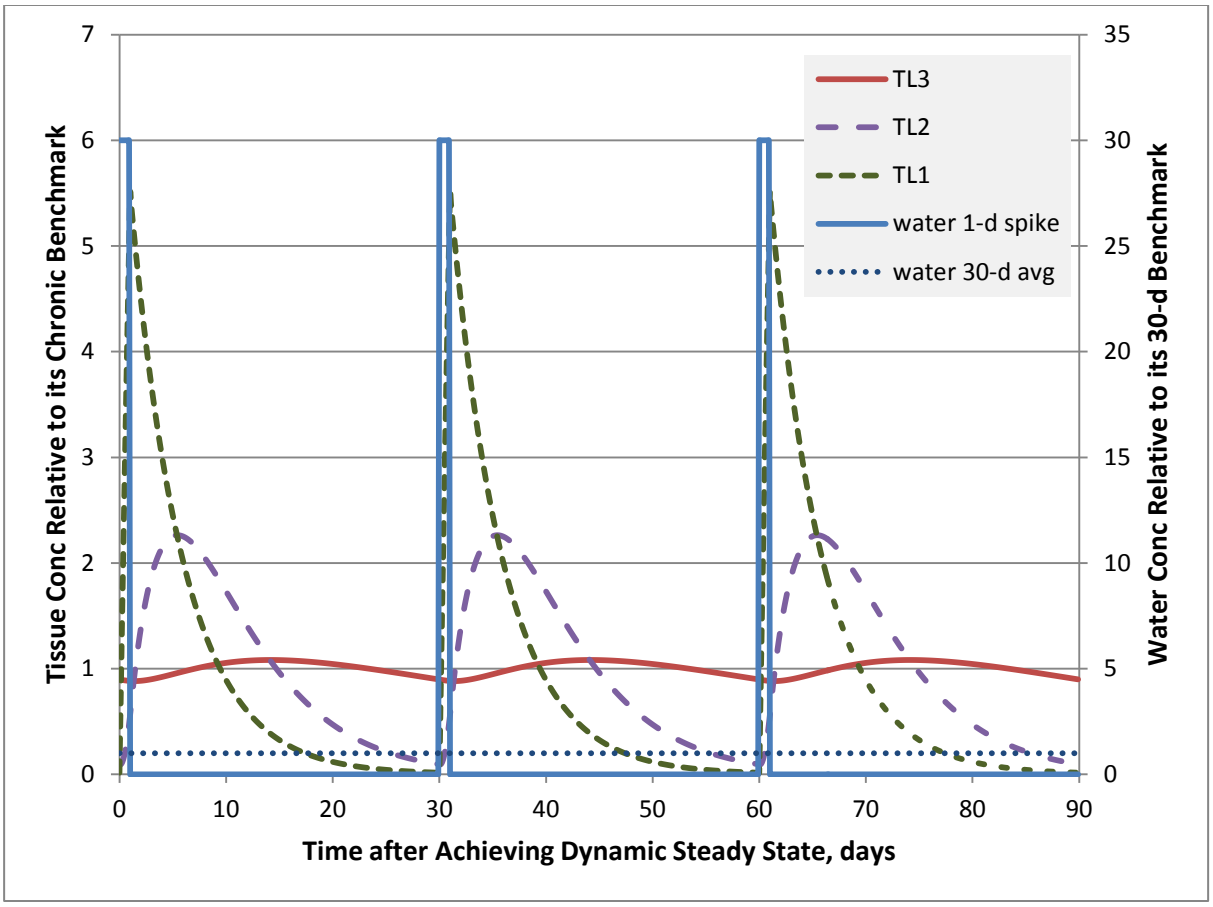


Figure J-2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion.
Tissue and water concentrations are expressed as dimensionless ratios relative to their respective criteria or benchmarks, as explained in the text.

With their more rapid kinetics, TL1 and TL2 tissue concentration swings are much more drastic than TL3 (fish) tissue concentration swings, but were the spike to continue as a steady exposure 30-fold above the water benchmark, TL1, TL2, and TL3 would all ultimately plateau at 30-fold above their respective benchmarks.

The key point here is that attaining the 30-day average via 1-day spikes spaced 30 days apart generates a small oscillation in fish tissue concentrations. Averaged over the 30-days, the fish tissue concentrations exactly attain their criterion and the predicted effect is 10%.

3.2.1.3 Log-normally distributed, smoothly varying concentrations

This is the most realistic scenarios, corresponding to typical variability observed in streams.

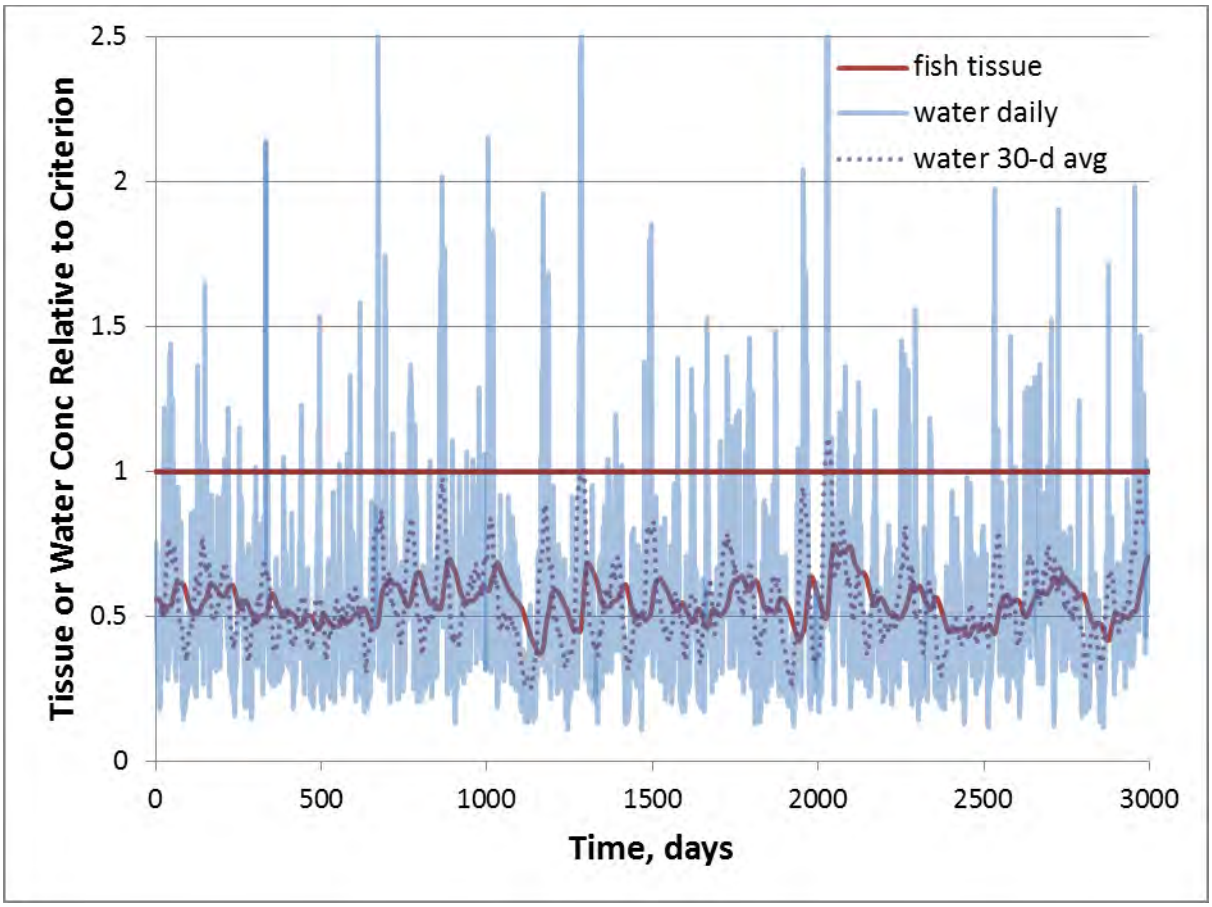


Figure J-3. A typical example of log-normally distributed, smoothly variable concentrations. The standard deviation of natural logs is 0.5 and the serial correlation coefficient of logs is 0.8 for daily values, both typical real-world situations. (The compression of 3000 days into the graph might make it difficult to recognize that the time series is smoothly varying – it has serial correlation.) At time=0, TL1, TL2, and TL3 begin at their average concentrations.

In the Figure J-3 example run, instantaneous water concentrations exceed the 30-day average criterion 7% of the time. The 30-day average concentrations exceed the criterion 1.05 times per 3 year period, counted per the EPA (1986) counting method. Tissue concentrations do not exceed their criterion at any time, and the aggregate effect is 0.12%.

In contrast to the previous scenario, the elevated concentrations here are random in their magnitude, duration, and spacing. This randomness reduces the average exposure (and aggregate effect) compatible with attainment of the 30-day average water target.

3.2.2 Summary of Scenario Results

Because Scenario 3 involves generation of random concentrations, the above graphs show just one run (3000 days) for each. Full results for the 20 runs of that scenario are shown below.

Scenario	Water: # 30-day avg. exceedances / 3-yr 1	Water: % of time exceeding	Tissue: % of time exceeding	Mean effect for 5th %ile Taxon	Comment
1. Steady	0.00	0.00	0.00	10.0	Steady at water and tissue benchmarks
2. Uniform spikes	0.00	3.33	56.7	10.0	30-d avg water conc. remains steady at benchmark (Fig. J2)
3. Smooth variable	1.01	7.8	0.00	0.18	Median=0.49 x benchmark, log stdev=0.5, rho(daily)=0.8 (e.g., Fig. 5) 2
1. Counting procedure for 30-d avg. exceedances is that of U.S. EPA (1986). 2. Results for Scenario 3 are average of 20 runs of 3000 days, each run with 0.6-1.4 exceedances / 3 yr. Runs not yielding exceedances within these bounds were not used. Among the 20 runs used, the effect CV=0.35.					

It can be concluded that the kinetics of selenium accumulation and depuration are sufficiently slow that applying a 30-day averaging period to the water criterion concentration affords protection even under unrealistic worst case conditions.

3.2.3 Example Responses to Increases in Water Concentrations

The previous Figures J-2 and J-3 illustrate situations after achievement of a dynamic steady state, where daily water concentrations change but longer-term mean water concentrations do not change. Given the same kinetic parameters as used above (i.e., yielding a 60-day characteristic time), this section addresses the rate at which tissue concentrations respond to increases in mean water concentrations, for example as would result from a new source. This is similar to the rising curve previously shown in Figure J-1b. The rapid kinetics used here for the water-TL1 step imply a small lotic system having little involvement of the bed sediments.

3.2.3.1 Step-function example

This example addresses the question: If water concentrations are increased to a level that is slightly too high, ultimately (at Time=∞) yielding fish-tissue concentrations at the EC20 instead of the EC10, how long would it take for those tissue concentrations to rise to a level that exceeds the (EC10-based) criterion?

Prior to Time=0 in this example the concentrations in TL3 had been at a moderate background concentration of 0.406 times the criterion, corresponding to the median West Virginia reference-site egg concentrations tabulated by West Virginia Department of Environmental Protection (2010). The concentrations in TL1 and TL2 are likewise assumed to have been at 0.406 normalized to their corresponding benchmarks. At Time=0 the water concentrations increase such that ultimately they will

produce an effect 10% higher than the target, thus at the EC20 of the hypothetical 5th percentile sensitive species. For typical selenium concentration-response slopes, this is 1.15-fold above the EC10. Figure J4 illustrates this scenario, which shows that 90 days are needed for TL3 concentrations to rise above the criterion.

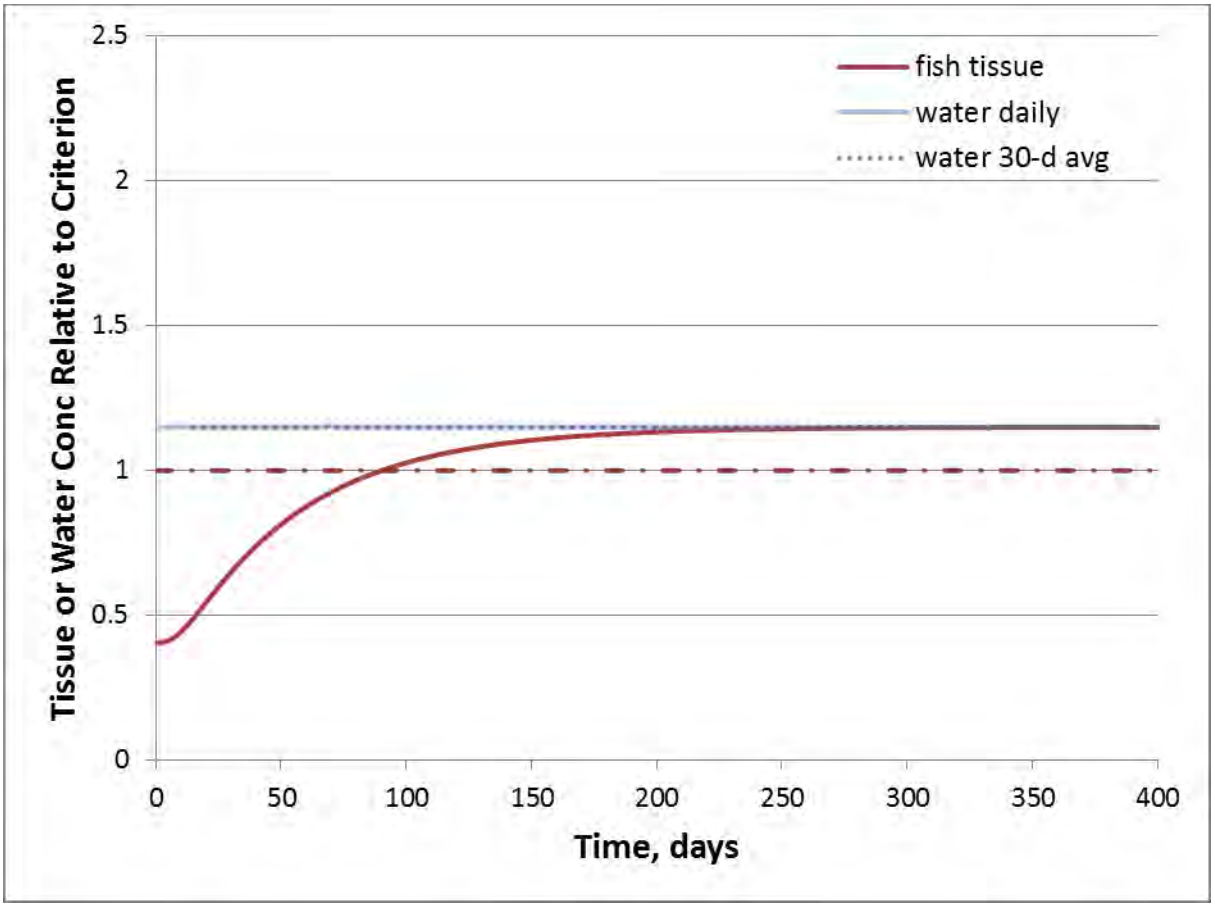


Figure J-4. TL3 concentration responding to a Time=0 step-function increase in water concentration that remains time-invariant thereafter.
Given that the water concentration is too high, ultimately yielding tissue concentrations at the hypothetical sensitive species EC20, 1.15-fold above the criterion, and given the previously presented kinetic parameters, it is calculated to take 90 days for TL3 concentrations to rise above the criterion.

3.2.3.2 Continuously time-variable example for flowing waters

To provide more realism, this example considers typical time variability, following up on Figure J3. In this example, prior to Time=0, TL1, TL2, and TL3 concentrations were at a low background concentration, 0.1 normalized to their criterion or respective benchmark. At Time=0 begin water concentrations having median = geometric mean = 0.49 normalized as a dimensionless ratio, concentration/criterion. Because the water concentrations are log-normally distributed, with log standard deviation = 0.5, the arithmetic mean is higher than the median and has the normalized value 0.56. If the simulation went on for a very long time, this time series (designed to have geometric mean 0.49 times the criterion, log standard deviation 0.5, and log serial correlation coefficient 0.8) would average one exceedance every three years, when exceedances are counted using the EPA (1986) approach. Figure J-5 shows a typical short series of 400 days.

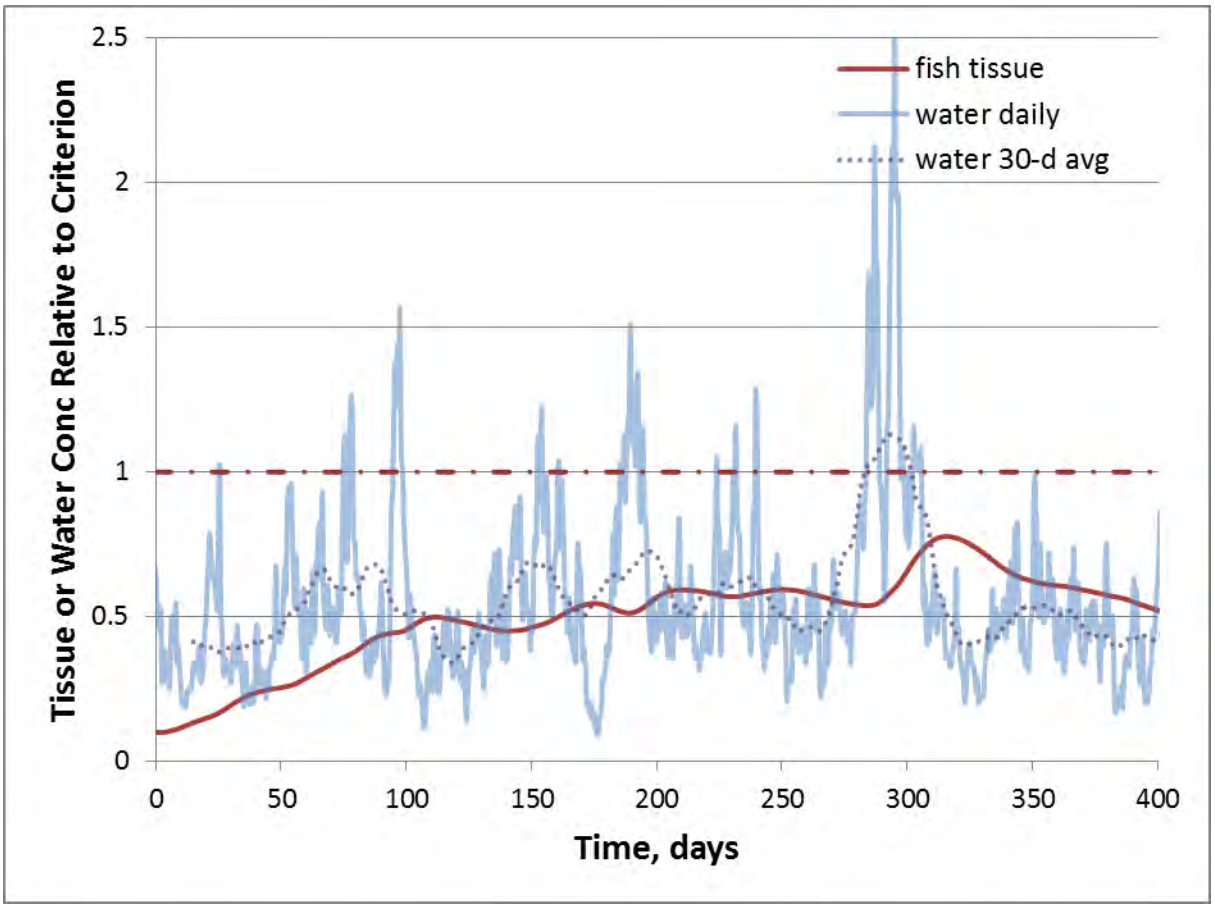


Figure J-5. Flowing water example of TL3 concentration starting at a concentration of 0.1 normalized to the criterion, and responding to randomly varying log-normally distributed water concentrations having median 0.49 (expressed as a dimensionless ratio: concentration/criterion), log standard deviation 0.5, and log serial correlation coefficient 0.8.
Again, all concentrations are as dimensionless ratios relative to the criteria concentrations.

Several points are worth noting. Because the water concentrations happen (by chance) to be below average for the first 50 days, the TL3 concentrations rise somewhat slowly during that period. Were they to be above average during that period, the TL3 concentrations would more rapidly approach their dynamically varying plateau. In such a short time series it is not graphically apparent what the long-term average TL3 concentration will be; however, because the long-term arithmetic mean water concentration would be 0.56 (normalized the its criterion), the TL3 concentration would likewise end up averaging 0.56 normalized to its criterion, if tracked for many years.

It is also worth noting that most 400-day series of the type shown in Figure J-5 would not have occurrences of 30-day average concentrations above the criterion (as suggested by Figure J-3). This particular random series does have a period of 30-day average exceedances, near Day 300, but it does not persist long enough to cause the TL3 concentration to approach its criterion.

Lastly, it should be noted that when concentrations are randomly varying as in Figure J-5, the water concentrations that one observes are highly dependent on when the samples are taken. The TL3 concentrations observed are far less dependent on when the samples are taken (after the plateau is approached), but time variations, although muted, are still present.

The example scenarios depicted here show lotic time to steady state of approximately 3 months to less than 1 year under different discharge scenarios including both continuous and intermittent discharges. The scenarios also assume that the new selenium input is from one source; multiple new sources particularly with varying discharge patterns, might have a different response time and pattern for various trophic levels.

The example is likely not appropriate for lentic systems, because they would not be expected to have the rapidly varying water concentrations of Figure J-5. In addition, the water-to-TL1 kinetics would likely be slower in lentic systems with new or time-varying sources because of the role of bottom sediments acting as a reservoir in recycling selenium. Ultimately this should yield slower rising and smoother TL3 concentrations compared to those in Figure J-5.

APPENDIX K: TRANSLATION OF A SELENIUM FISH TISSUE CRITERION ELEMENT TO A SITE-SPECIFIC WATER COLUMN VALUE

1.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING MECHANISTIC BIOACCUMULATION MODELING

Introduction:

EPA recommends fish tissue elements of the selenium criterion supersede water column elements under steady state conditions because the selenium concentration in fish tissue is a more sensitive and reliable indicator of the negative effects of selenium in aquatic life. However, implementation of a fish tissue criterion element can be challenging because many state and tribal Clean Water Act (CWA) programs prefer the expression of water quality criteria as an ambient concentration in the water-column. Therefore, EPA also recommends two monthly average water-column criterion elements, one for lotic (flowing) waters, and the other for lentic (still) waters. EPA derived all water column criterion elements from the egg/ovary criterion element representing a protective selenium concentration for fish species populations. Thus the water column criterion elements also represent protective selenium concentrations for fish species populations. If threatened or endangered fish species are present, states and tribes may need to derive alternative water column elements with a refined protection goal that account for site-specific bioaccumulation characteristics.

EPA derived water-column criterion elements by modeling selenium bioaccumulation in aquatic systems. The EPA worked with the United States Geological Survey to derive a translation equation utilizing a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al., 1992; Wang et. al., 1996; Luoma and Fisher, 1997; Wang, 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006; Presser and Luoma 2010; Presser 2013). EPA translated the selenium egg-ovary criterion element into two set(s) of site-specific water concentration values (lentic and lotic), and used the distribution(s) of those water column values to derive the respective water-column criterion elements. This appendix describes approaches that states and tribes may choose to use regarding application of this same mechanistic modeling approach (or alternatively an empirical bioaccumulation factor (BAF) approach) to translate a fish tissue criterion element (egg-ovary, whole body, or muscle) into site-specific water-column concentrations to more precisely manage selenium in specific aquatic systems.

The relationship between the concentration of selenium in the tissues of fish and the concentration of selenium in the water column can vary substantially among aquatic systems. The species of fish, the species and proportion of prey, and a variety of site-specific biogeochemical factors affect selenium bioaccumulation and thus determine the allowable concentration of selenium in ambient water protective of aquatic life. States and tribes may choose to adopt the results of site-specific water column translations as site-specific criteria (SSC) or adopt a translation procedure into state or tribal water quality

standards. Under both options, the water quality standards revisions must be approved by EPA under Section 303(c) of the Clean Water Act. If a state or tribe adopts a translation procedure that will be implemented by other CWA programs, it must be scientifically defensible, produce repeatable, predictable outcomes, and result in criteria that protect the applicable designated use. Examples of such approaches include the mechanistic modeling approach and the empirical BAF approach described within this Appendix.

EPA considered both mechanistic and empirical modeling approaches to translate the selenium egg-ovary criterion element into water column concentration elements. A mechanistic modeling approach uses scientific knowledge of the physical and chemical processes underlying bioaccumulation to establish a relationship between the concentrations of selenium in the water column and the concentration of selenium in the tissue of aquatic organisms. The mechanistic modeling approach enables formulation of site-specific models of trophic transfer of selenium through aquatic food webs and translation of the egg-ovary criterion element into an equivalent site-specific water concentration. The empirical modeling approach establishes a relationship between concentrations of selenium in fish tissue and ambient water directly by measuring selenium concentrations in both media and calculating the ratio of the two concentrations. The ratio (BAF) can then be used to estimate the target concentration of selenium in the water column as related to the adopted fish tissue element.

Both the mechanistic and empirical modeling approaches have advantages and disadvantages that should be considered before deciding which approach to use. On the one hand, the mechanistic modeling approach has the advantage of not requiring extensive fish tissue sampling and analysis by using knowledge of aquatic system food webs. However, uncertainty in the selection of model parameters increases uncertainty in the outcome leading to a reduction in defensibility. Of particular concern with respect to the mechanistic model EPA developed is the selection of the value for the enrichment factor parameter *EF* (discussed in more detail below). On the other hand, the empirical BAF approach is conceptually and computationally simpler because it relies only on field measurements and does not require extensive knowledge of the physical, chemical, or biological characteristics of the aquatic system. However, obtaining a sufficient number of measurements in fish tissue and water may be logistically difficult and/or more expensive.

The appropriate modeling approach to use when translating the selenium egg-ovary criterion element to a site-specific water-column concentration depends on individual circumstances and site-specific characteristics. The mechanistic modeling approach may be a useful method in situations where there is little or no data on the amount of selenium in an aquatic system, the empirical BAF approach may be desirable in circumstances where in fish tissue and water data are available. Below is a description of

methodology than can be used to translate the egg-ovary criterion element to a site-specific water-column concentration for site-specific management of selenium.

1.1 Relating the Concentration of Selenium in Fish Tissue and Water using the Mechanistic Modeling Approach

The relationship between the concentration of selenium in the eggs or ovaries of fish and the concentration of selenium in the water column is given in Equation K-1 (Equation 18 from the main text):

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \textit{(Equation K-1)}$$

Where:

C_{water} = the concentration of selenium in water (µg/L),

$C_{egg\text{-}ovary}$ = the concentration of selenium in the eggs or ovaries of fish (µg/g),

$TTF^{composite}$ = the product of the trophic transfer factor (*TTF*) values of the fish species that is the target of the egg-ovary criterion element and the *TTF* values of all lower trophic levels in its food web (no units of measurement, see explanation below).

EF = the steady state proportional bioconcentration of dissolved selenium at the base of the aquatic food web (L/g),

CF = the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues (no units of measurement).

The basic principles expressed in Equation K-1 are illustrated in the conceptual model shown in Figure K-1.

Selenium dissolved in surface water enters aquatic food webs by becoming associated with trophic level 1 primary producer organisms (e.g., algae) and other biotic (e.g., detritus) and abiotic (e.g., sediment) particulate material. An enrichment function (*EF*) quantifies the bioconcentration of selenium in particulate material and thus its bioavailability in the aquatic system. The parameter *EF* is a single value that represents the steady state proportional concentration of selenium in particulate material relative to the concentration of selenium dissolved in water.

Organic particulate material is consumed by trophic level 2 organisms (usually aquatic invertebrates, but also some fish species that are herbivores/detritivores) resulting in the accumulation of selenium in the tissues of those organisms. Trophic level 2 invertebrates are consumed by trophic level 3 fishes resulting in further accumulation of selenium in the tissues of those fish. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer factor (*TTF)*. A *TTF* is a single value that represents the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. Different species of organisms metabolize selenium in different ways. Thus each species is associated with a specific *TTF* value. Because the trophic transfer of selenium through all trophic levels is mathematically equal to the product of the individual *TTF* values, all consumer-resource interactions in a particular aquatic ecosystem are simplified in Equation K-1 by representing the product of all the individual *TTF* values as the single parameter $TTF^{composite}$.

Fish accumulate selenium in different tissues of the body in differing amounts. Species physiology, age, diet, sex, and spawning status are some of the factors that affect selenium partitioning in body tissues. Because the primary selenium criterion element is expressed as a concentration in the eggs and/or ovaries, a conversion factor (*CF*) quantifies the relationship between the concentration of selenium in the eggs and/or ovaries and the average concentration of selenium in the whole body or muscle tissues. The parameter *CF* in Equation K-1 is a single value that represents the steady state proportional concentration of selenium in the eggs and/or ovaries relative to the average concentration of selenium in all body tissues. Different species of fish accumulate selenium in their eggs and ovaries to different degrees. Thus each species of fish is associated with a specific *CF* value.

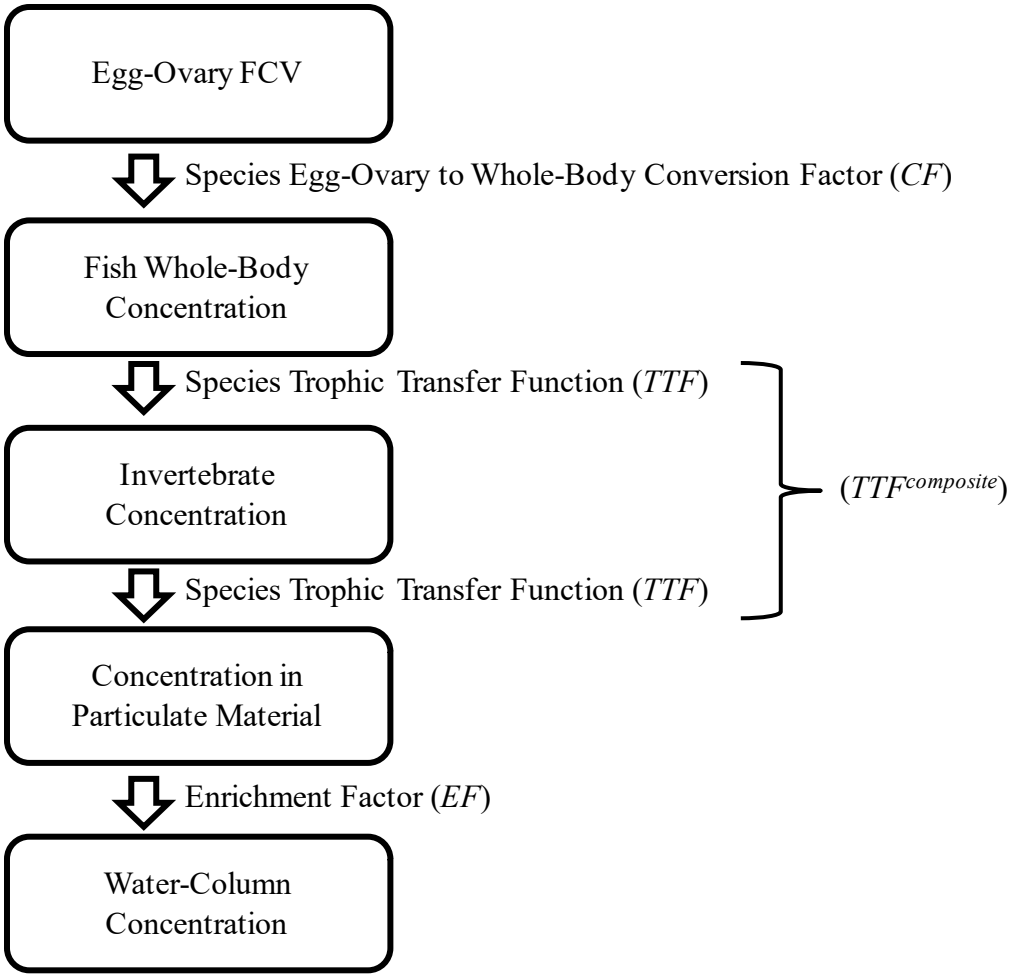


Figure K-1. Conceptual model for translating the egg-ovary FCV to a water-column concentration.
Note: States may want to use the whole body or muscle criterion elements as the starting point for site specific translation to a water column concentration.

Once the parameters that quantify the transfer of selenium through each step in this pathway are identified, they can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific concentration of selenium in the water column (i.e., target water column concentration).

Because each *TTF* value is species-specific, it is possible to differentiate bioaccumulation in different aquatic systems by modeling the food web of the target fish species. For example, where the food web contains more than 3 trophic levels, $TTF^{composite}$ can be represented as the product of all *TTF* values for each trophic level given in Equation K-2, which is a generalization of Equation 10 from the main text:

$$TTF^{composite} = TTF^{TL2} \times TTF^{TL3} \times \ldots \times TTF^{TLn} \qquad \textit{(Equation K-2)}$$

Where:

$TTF^{composite}$ = the product of all *TTF* values at all trophic levels.

TTF^{TLn} = the *TTF* value of the highest trophic level.

The consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* value at a particular trophic level as the average *TTF* values of all species at that trophic level weighted by the proportion of species consumed given as Equation K-3 (Equation 11 in the main text):

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \textit{(Equation K-3)}$$

Where:

TTF_i^{TLx} = the trophic transfer factor of the i^{th} species at a particular trophic level

w_i = the proportion of the i^{th} species consumed.

These concepts can be used to formulate a mathematical expression of $TTF^{composite}$ that models selenium bioaccumulation in a variety of aquatic ecosystems. Figure K-2 illustrates five hypothetical food web scenarios and the formulation of $TTF^{composite}$ for each of them. For each scenario, the value of $TTF^{composite}$, the *CF* value associated with the targeted fish species, and the site-specific *EF* value can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific water concentration value. The hypothetical food web models in Figure K-2 are a few possible examples of food web models for illustrative purposes. It is desirable to derive and use of a food web model that best represents the aquatic system for which the water column translation will apply. The general steps for deriving a site-specific translation of the egg-ovary criterion element to a water concentration value are described below.

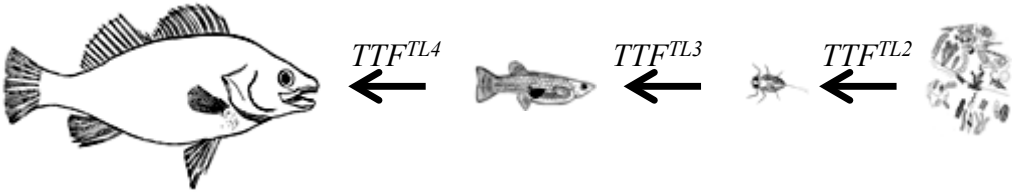
A) Three trophic levels (simple):

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$



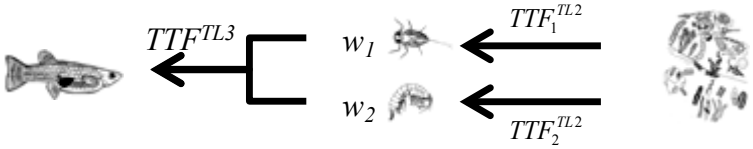
B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$



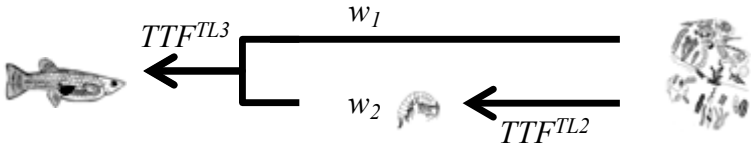
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

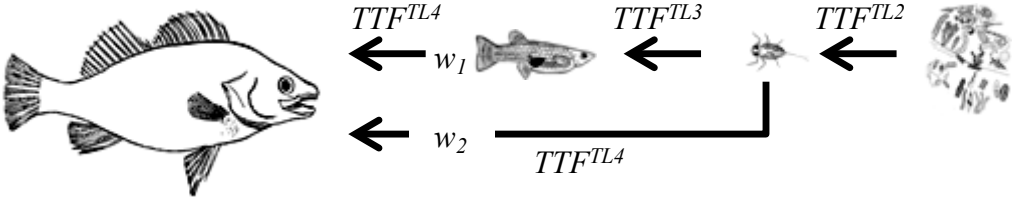


Figure K-2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios.

$TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food web model is used to calculate a value for $TTF^{composite}$ using appropriate species-specific *TTF* values and the proportions of each species consumed at each trophic level. See text for further explanation.

1.2 Steps for Deriving a Site-Specific Water Concentration Value from the Egg-Ovary Criterion Element

Below are the steps for deriving a site-specific water concentration value from the selenium egg-ovary criterion element using EPA's mechanistic model approach:

1) Identify the appropriate target fish species.
2) Model the food web of the targeted fish species.
3) Identify appropriate *TTF* values by either:
    a. selecting the appropriate *TTF* values from a list of EPA-derived values, or
    b. deriving *TTF* values from existing data, or
    c. deriving *TTF* values by conducting additional studies, or
    d. extrapolating *TTF* values from existing values.
4) Determine the appropriate value of *EF* by either
    a. deriving a site-specific *EF* value from field measurements, or
    b. deriving an appropriate *EF* value from existing data, or
    c. extrapolating from *EF* values of similar waters.
5) Determine the appropriate *CF* value by either,
    a. selecting the appropriate *CF* value from a list of EPA-derived values, or
    b. deriving a *CF* value from existing data, or
    c. deriving a *CF* value by conducting additional studies, or
    d. extrapolating a *CF* value from existing values.
6) Translate the selenium egg-ovary criterion element into a site-specific water concentration value using Equation K-1.

Below are detailed descriptions of each step followed by example calculations using a variety of hypothetical scenarios. EPA is providing this information to support help states and tribes that choose to develop selenium water column values from the egg-ovary criterion element or develop translation procedures. Successful application of the mechanistic approach described here requires use of particular food web models and parameter values that are appropriate for particular aquatic systems.

1.2.1 Identify the Appropriate Target Fish Species

1.2.1.1 When fish are present

In developing a site-specific translation of the egg-ovary criterion element, the user wshould select whether to use a mechanistic model or empricial (BAF) approach. This decision will in large part

determine the data and information requirements. A mechanistic model approach will likely require information on the spatial and temporal distribution of aquatic organisms, and may require measurements of selenium in ambient water and particulate material. An empirical model approach will use measurements of selenium is fish tissue and ambient water.

Developing a site-specific translation of the egg-ovary criterion element will also entail selection of which species of fish to target. The concentration of selenium in eggs and ovaries is the most sensitive and consistent indicator of toxicity. However, toxicity and bioaccumulation potential can vary among species. Species in the families Acipenseridae, Centrarchidae, and Salmonidae are particularly sensitive to selenium (Table 3.3 in the main document), whereas species such as stoneroller species, creek chub, blackside dace, and white sucker have documented tolerance to selenium and can be found in selenium contaminated systems (NAMC 2008, Presser 2012). Green sunfish accumulate less selenium than other species with comparable exposures in the same aquatic system (Hitt and Smith 2015). Selection of the fish species in the aquatic system with the greatest selenium sensitivity and bioaccumulation potential is recommended.

Several additional factors should also be considered in deciding which species to target when developing a site-specific translation of the egg-ovary criterion element. Anadromous species (species that migrate from salt water to spawn in fresh water) should generally avoided because selenium exposure and bioaccumulation occurs over a relatively long period through the consumption of locally contaminated aquatic organisms. Additionally considerations include whether the fish species selected typically consume organisms known or suspected to readily bioaccumulate selenium (e.g., mollusks). For example, high concentrations of selenium in San Francisco Bay white sturgeon are associated with their consumption of *Potamocorbula amurensis*, a bivalve in close proximity to selenium-contaminated sediments that rapidly and efficiently accumulates selenium (Stewart et al. 2004). In contrast, striped bass from the same aquatic system have substantially lower concentration of selenium in their tissues because their zooplankton-based food web has substantially lower selenium bioaccumulation characteristics (Schlekat et al. 2004; Stewart et al. 2004). The 2016 selenium criterion was developed for freshwater, but if considering other ecosystems, it may be worth noting that salinity may also affect bioaccumulation of selenium. Freshwater mollusks tend to have relatively higher *TTF* values when compared to other freshwater invertebrate taxa (e.g., aquatic insects), but they are lower than mollusks in marine or brackish systems (and particularly *P. amurensis*, an invasive clam in the San Francisco Bay). In aquatic systems with resident fish species of unknown selenium sensitivity and bioaccumulation potential, other factors such as ecological significance could be considered when choosing a target species.

Data from fisheries or biological surveys or other biological assessments could be considered to determine the fish species that reside in specific surface waters. State and tribal resource agency personnel

familiar with fish sampling activities could also be a source of information on resident fish species. General information on the fish species present in state and tribal surface waters may also be found at:

- State Fish and Game agencies
- U.S. Fish and Wildlife Service (http://www.fws.gov)
- U.S. Geological Survey (http://www.usgs.gov)
- NatureServe.org (http://www.natureserve.org)
- Fishbase (http://www.fishbase.org)
- State or local sources of biological information (e.g. Biota Information System of New Mexico at http://www.bison-m.org)

Measurements of selenium in fish tissue would most reflect the ecosystem if adult (reproductively mature) fish are sampled. Selenium measurements in fish tissue will likely be more stable in adult fish because they are more likely to have a stable prey base. Reproductively mature (ripe or gravid) females would be needed for measures selenium in eggs and/or ovary tissue for comparison to the the egg-ovary tissue criterion element. It would be prudent to avoid sampling ovary tissue "post-spawn" due to a potential decrease in selenium concentration presumably due to the loss of selenium through spawning and release of eggs with relatively high concentrations of selenium. Consideration of closely related taxonomic surrogates (same genus or family) for threatened or endangered species may be useful.

Figure K-3 shows an example decision tree that may help in selection of the appropriate fish species for deriving a site-specific water concentration value from the selenium egg-ovary, whole-body, or muscle FCV. The use of taxonomic hierarchies for anlysis utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993). Additional information on fish tissue sampling (e.g., species selection, temporal and spatial considerations) is under development and will be published in the form of a technical support document (TSD) by the EPA in the near future.

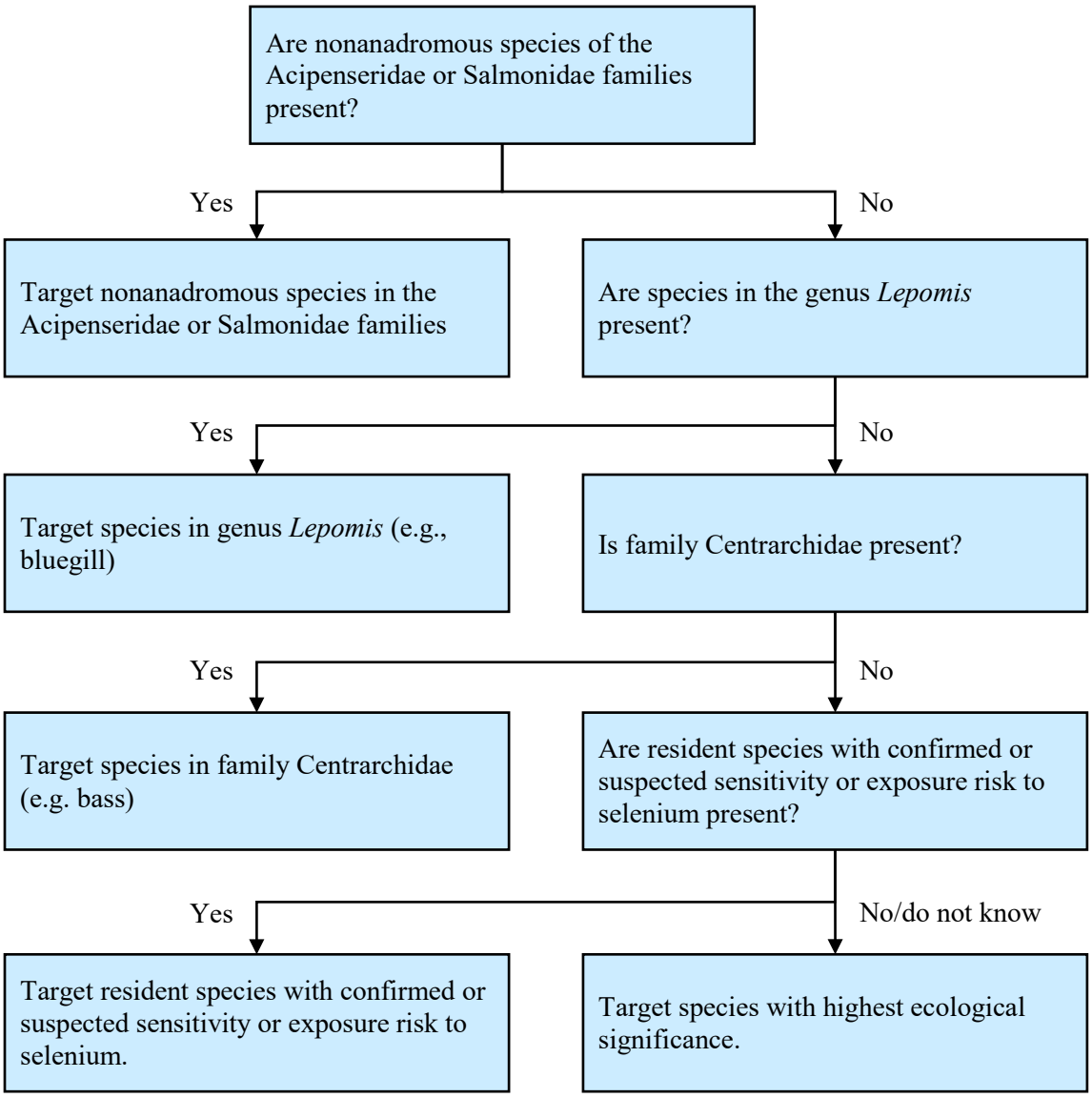


Figure K-3. Recommendeed decision process for selection of the fish species to use when deriving a water concentration from the selenium egg-ovary FCV.
This decision tree is also generally applicable when using the whole body or muscle tissue as the starting point for development of SSC, particularly when using the BAF approach.

1.2.1.2 When fish are absent from a site

Some aquatic systems do not contain resident fish. Fish may be absent from a waterbody because of intermittent or persistent low flows, physical impediments such as waterfalls or impoundments, lack of adequate habitat for feeding and/or spawning, or intolerable aquatic conditions related to pH, turbidity, temperature, salinity, total dissolved solids, chemical contaminants, or pathogens. These conditions could be due to natural or anthropogenic causes. Some streams may be naturally intermittent or ephemeral, or

they might exhibit low or intermittent flows because of impoundments or water draw-down for agricultural irrigation, industrial uses, drinking water supply, or other uses.

When fish are absent from a waterbody, consideration of sampling the most sensitive fish species inhabiting nearby, most proximate downstream waters may be useful in order to understand selenium bioaccumulation potential in such systems. Although the upper reaches of some aquatic systems may not support fish communities, the invertebrate organisms that reside there may tolerate high concentrations of selenium and pose a selenium risk to predator fish if transported downstream. Users may choose to evaluate upstream waters without fish by measuring the selenium concentration in water, biotic and/or abiotic particulate material, and/or the tissues of invertebrate aquatic organisms that reside there. Because selenium associated with particulate material and invertebrate organisms can be transported downstream during intermittent high flows, elevated concentrations of selenium in the tissues of downstream fish could indicate upstream sources of selenium that require a more detailed evaluation of upstream conditions.

1.2.2 Model the Food-Web of the Targeted Fish Species

After selecting the target fish species, model users should formulate a mathematical expression of the target species food-web that will be used to calculate the value of $TTF^{composite}$. As discussed previously, $TTF^{composite}$ is the product of the *TTF* values across trophic levels of the target fish species food-web. The complexity of the food-web model will depend on the species of fish that is targeted, the diversity of prey species in the aquatic system, and the amount of information that is available. Many of the same information sources used to identify the targeted fish species in a waterbody could also be used to obtain information about its food web. The types and proportions of food organisms the targeted fish species consumes can be directly assessed through studies that examine stomach contents or from information gathered through biological assessments. If site-specific information is not available, model users could estimate the target fish species food-web using publicly available databases such as NatureServe (http://www.natureserve.org). For example, the NatureServe database record for fathead minnow in the HUC watershed #5040004 in Ohio indicates under the heading: "Ecology and Life History - Food Comments," the fathead minnow "feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990)."

Additional sources of information include:

- FishBase (http://www.fishbase.org). FishBase is a relational database developed at the World Fish Center in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and many other partners.

- Carlander, K.D. Handbook of Freshwater Fishery Biology, volumes 1, 2 and 3. Iowa state University Press, Ames, Iowa. 1969-1997.

1.2.3 Identify Appropriate *TTF* Values

The food-web model uses appropriately selected species-specific *TTF* values (and, if appropriate, proportions within the same trophic level). Model users identify the appropriate *TTF* values by using one of the following four procedures, or by using other scientifically defensible methods.

1.2.3.1 Select the appropriate *TTF* values from the provided list of EPA-derived values

Species-specific TTF values represent the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. EPA-derived *TTF* values for aquatic invertebrates and fish are provided in Tables K-1 and K-2 (Tables 3.10 and 3.11 in main text; see also main text for a complete explanation of the procedure EPA used to derive these values).

Table K-1. EPA-derived Trophic Transfer Factor (TTF) values for freshwater aquatic invertebrates.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Crustaceans					
amphipod	*Hyalella azteca*	-	-	-	1.22
copepod	copepods	0.520	0.420	0.155	1.41
crayfish	*Astacidae*	-	-	-	1.46
water flea	*Daphnia magna*	0.406	0.210	0.116	0.74
Insects					
dragonfly	*Anisoptera*	-	-	-	1.97
damselfly	*Coenagrionidae*	-	-	-	2.88
mayfly	*Centroptilum triangulifer*	-	-	-	2.38
midge	*Chironimidae*	-	-	-	1.90
water boatman	*Corixidae*	-	-	-	1.48
Mollusks					
asian clam[a]	*Corbicula fluminea*	0.550	0.050	0.006	4.58
zebra mussel	*Dreissena polymorpha*	0.260	0.400	0.026	4.00
Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	zooplankton	-	-	-	1.89

[a] Not to be confused with *Potamocorbula amurensis*

Table K-2. EPA-derived Trophic Transfer Factor (*TTF*) values for freshwater fish.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79

Common name	Scientific name	AE	IR	k_e	TTF
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

The *TTF* values from these lists could be used exclusively, or in conjunction with *TTF* values obtained from other sources (see below). Note that these tables do not represent an exhaustive list of all *TTF* values that may be required to calculate a site-specific water concentration value. If this list does not include a required *TTF* value, another approach could be considered to obtain an appropriate value.

1.2.3.2 Deriving *TTF* values from existing data

If model users cannot obtain one or more required *TTF* values from Tables K-1 and/or K-2, species-specific *TTF* values could be derived using existing data. One approach for deriving species-specific *TTF* values is to use the physiological coefficients representing food ingestion rate (*IR)*, selenium efflux rate (k_e), and selenium assimilation efficiency (*AE*) to calculate a *TTF* value using Equation K-4 (Equation 3 from the main text, Reinfelder et al. 1998**)** given as:

$$TTF = \frac{AE \times IR}{k_e} \qquad \textit{(Equation K-4)}$$

Where:

TTF = species-specific trophic transfer factor

AE = species-specific assimilation efficiency (%)

IR = species-specific ingestion rate (g/g-d)

k_e = species-specific efflux rate constant (/d)

The physiological coefficients *IR*, *AE* and are species-specific values. Values for AE and k_e can only be derived from laboratory studies. Values for IR may be derived from laboratory studies or obtained from published literature. After the three physiological coefficients are obtained, a *TTF* value can be calculated using Equation K-4.

Another way to derive species-specific *TTF* values is to empirically assess the relationship between the selenium concentration in the tissue of organisms and the selenium concentration in the food they consume using paired measurements from field studies. Species-specific *TTF* values can be derived from such measurements by calculating ratios, using regression techniques, or other scientifically defensible methods.

Model users could choose to use the same approach EPA used to calculate species-specific *TTF* values. EPA derived *TTF* values using a combination median and regression approach. EPA defined the *TTF* value for any trophic level as:

$$TTF^{TLn} = \frac{C_{tissue}^{TLn}}{C_{food}^{TLn}} \qquad \textit{(Equation K-5)}$$

Where:

TTF^{TLn} = The trophic transfer factor of a given trophic level,

C_{tissue}^{TLn} = The selenium concentration (mg/kg dw) in the tissues of the consumer organism,

C_{food}^{TLn} = The selenium concentration (mg/kg dw) in the consumer organism's food.

EPA used the median of the ratios given in Equation K-5 as the species-specific *TTF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear

regression analysis. EPA considered the relationship acceptable if a linear regression of tissue selenium concentration on food selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope (i.e., selenium concentrations in the consumer increases with increasing selenium in food).

1.2.3.3 Deriving *TTF* values by conducting additional studies

Additional studies could be conducted to obtain the data needed to derive *TTF* values for specific needs, or to revise existing *TTF* values, if the existing *TTF* values do not appear to be appropriate for a particular aquatic system.

1.2.3.4 Extrapolating *TTF* values from existing values

If one or more necessary *TTF* values are not available, and the information needed to derive a species-specific *TTF* value is not available or impractical to obtain, model users could consider extrapolating a new *TTF* value from other known *TTF* values. One possible method to extrapolate a *TTF* value is to sequentially consider higher taxonomic classifications until one or more of the organisms with a known *TTF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *TTF* values, the average *TTF* from the matching table entries could be used. The use of taxonomic hierarchies in this way utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993).

EPA used such an extrapolation approach to derive some of the *TTF* values necessary to develop the water column criterion elements. For example, the *TTF* value for *Chrosomus eos* (northern redbelly dace) was not available. *TTF* values were also not available for other species in the genus *Chrosomus*, but *TTF* values were available for species in the family Cyprinidae, including *Rhinichthys atratulus* (blacknose dace), *Cyprinus carpio* (common carp), *Semotilus atromaculatus* (creek chub), *Pimephales promelas* (fathead minnow), *Cyprinella lutrensis* (red shiner), *Richardsonius balteatus* (redside shiner), and *Notropis stramineus* (sand shiner). Because Cyprinidae is the lowest taxonomic classification where *Chrosomus eos* matches one or more species with an available *TTF* value, EPA used the median *TTF* value of blacknose dace, common carp, creek chub, fathead minnow, red shiner, redside shiner, and sand shiner as the *TTF* value for northern redbelly dace.

1.2.4 Determine the Appropriate *EF* Value

The selenium enrichment function (*EF*) value represents the bioavailability of selenium at the base of the aquatic food web. The base of the aquatic food web includes phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma, 2010). EPA refers to this mixture of living and non-living entities as particulate material. The parameter *EF* varies more widely across aquatic systems than any other parameter, and is influenced by

the source and form of selenium, water residence time, the biogeochemical characteristics of the waterbody, and the type of particulate matter collected. Because *EF* can vary greatly across waterbodies, this parameter has the greatest potential to introduce uncertainty in the translation from an egg-ovary selenium concentration to a water column concentration. For this reason, use *EF* values derived from site-specific data is recommended whenever possible in applying the model. One of the following four procedures could be used to derive *EF* values, or other scientifically defensible methods could be used.

1.2.4.1 Deriving a site-specific *EF* value from field measurements

Equation 12 from the main text defines the parameter *EF* as the ratio of the concentration of selenium in particulate material to the concentration of selenium dissolved in water given as:

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \textit{(Equation K-6)}$$

Where:

$C_{particulate}$ = Concentration of selenium in particulate material (µg/g)

C_{water} = Concentration of selenium dissolved in water (µg/L)

EF = Enrichment Function (L/g)

To calculate a site-specific *EF* value, EPA first calculates the ratio of each individual particulate measurement and its associated water measurement (if more than one water measurement is available for any given particulate measurement, the median water measurement is used). If more than one ratio for any given category of particulate material is available (e.g., more than one ratio of algae to water), EPA takes the median of the ratios. EPA then calculates the geometric mean of the median ratios for each category of particular material as the site *EF* value. EPA only uses sediment measurements if there are at least one measurement from either algae or detritus.

Deriving a site-specific *EF* value in this manner is a relatively straightforward procedure. However, consideration of data that appropriately accounts for the spatial and temporal variability of an aquatic system would be useful in the development of any sampling plan. Aquatic system characteristics such as dimension, volume, shape, residence time, velocity, and growing season are a few important factors that should be considered in designing a sampling plan that will adequately account for variability. State and Federal agencies (USGS, ACOE) as well as watershed groups may be useful sources of information that can help characterize the temporal and spatial variability at a particular aquatic system. When developing the selenium criterion, EPA observed a relatively lower correlation between the selenium concentration in water and abiotic (benthic sediments) particulate samples compared to the same analysis between water and biotic (algae and detritus) particulate samples, resulting in EPA's decision

that calculation of any site-specific *EF* values include information from at least one type of biotic particulate indeveloping its criteiron. Prioritization of sampling of biotic particulate material over abiotic samples should be considered. Regaridng selenium measurements from abiotic particulate material, consideration of utilizing at least one type of biotic particulate material when deriving the *EF* value of an aquatic system is recommended.

Site-specific *EF* values using particulate and water samples that are as spatially and temporally coincident as possible would be considered the most robust. Although EPA's analysis of particulate and water samples from a sample population of aquatic systems found that samples taken within one year of each other, based on data availability, were appropriate in deriving the national criterion (Figure 3.5 in the main document), a site-specific EF value would ideally involve collecting particulate and water samples at the same location and time to ensure their representativeness of sirte-specific conditions. One simple and effective sampling and analysis scenario would be to collect water samples or a combination of particulate and water samples, separate the particulate material from the water in each sample by filtering, measure the concentration of selenium in the separated water and particulate material, compute the ratio of the two measurements from each sample, and then calculate the mean or median of all the ratios.

Selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as lakes, reservoirs, oxbows, and wetlands) and with fine particulate sediments high in organic carbon. A well-planned sampling protocol was developed in association with the development of a site-specific water-column criterion for selenium in the San Francisco Bay Delta[2]. States and tribes may also want to consult Doblin et al. (2006) for specific particulate sampling methods. EPA's National Rivers and Streams Assessment[3] also provides methods for quantitative periphyton sampling that commonly represents the base of many aquatic food webs. Analytical methods to measure selenium in particulate material and in water are discussed in Appendix L.

1.2.4.2 Deriving an appropriate *EF* value from existing data

If suitable and sufficient site-specific measurements of selenium in particulate material and water are already available, the model user may be able to use that data to derive an appropriate *EF* value. However, it would be important to ensure that the data represents current conditions, were collected and analyzed using scientifically sound sampling and analytical techniques, and proper quality assurance and quality control protocols were implemented.

1.2.4.3 Extrapolating from *EF* values of similar waters

[2] https://www3.epa.gov/region9/water/ctr/selenium-modeling_admin-report.pdf

[3] https://www.nemi.gov/methods/method_summary/12558/ (EPA-841-B-07-009) and https://www.nemi.gov/methods/method_summary/12565/ (EPA-841-B-12-009)

In circumstances where a site-specific, field-derived *EF* value is not available or practical to develop, an *EF* value from one or more aquatic systems with similar hydrological, geochemical, and biological characteristics could be used to estimate *EF*. However, there is a possibility of introducing significant uncertainty when using *EF* values extrapolated from other aquatic systems. More information on this topic is contained in Appendix H of this document.

1.2.5 Determine the Appropriate *CF* Value

1.2.5.1 Selecting the appropriate *CF* value from the list of values that were used to derive EPA's recommended water criteria concentration values

The parameter *CF* represents the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues. EPA derived species-specific *CF* values for 20 species of fish from studies that measured selenium concentrations in both eggs and/or ovaries and in whole body and/or muscle. These *CF* values can be found in Appendix B and are reproduced below (Table K-3).

Table K-3. Selenium Whole Body to Egg-Ovary Conversion Factors (*CF*).

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Species				
Bluegill	2.13			2.13
Bluehead sucker	1.82			1.82
Brook trout		1.09	1.27	1.38
Brown trout	1.45			1.45
Creek chub	1.99			1.99
Common carp	1.92			1.92
Cutthroat trout	1.96			1.96
Desert pupfish	1.20			1.20
Dolly Varden		1.26	1.27	1.61
Fathead minnow	1.40			1.40
Flannelmouth sucker	1.41			1.41
Green sunfish	1.45			1.45
Mountain whitefish		5.80	1.27	7.39
Northern pike		1.88	1.27	2.39
Rainbow trout		1.92	1.27	2.44

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Razorback sucker		2.31	1.34	3.11
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sturgeon		1.33	1.27	1.69
White sucker	1.38			1.38
Genus				
Catostomus				1.41
Gila				2.07
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				1.96
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				1.95
Salmonidae				1.71
Order				
Cyprinodontiformes				1.20
Perciformes				1.45
Class				
Actinopterygii				1.45

The data and methods used to derive the *CF* in this table are described in Appendix B.

1.2.5.2 Deriving a *CF* value from existing data

The parameter *CF* is mathematically expressed as Equation K-7 (Equation 16 in the main text):

$$CF = \frac{C_{egg-o\,\mathrm{var}\,y}}{C_{whole-body}} \qquad \textit{(Equation K-7)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (mg/kg).

If suitable and sufficient data are available, a model user could derive a species-specific *CF* value using the same numerical methods described above to calculate the parameter *EF*. The median of the ratios given in Equation K-7 could be used as the species-specific *CF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear regression analysis. IN deriving the national criterion, EPA considered it to be acceptable if a linear regression of egg-ovary selenium concentration on whole body selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope. Other scientifically defensible methods could be used. Regardless of the method used, the user should ensure that the data used to derive *CF* values were collected using adequate quality assurance and quality control protocols.

1.2.5.3 Deriving a *CF* value by conducting additional studies

Additional studies could be performed to obtain data needed to derive *CF* values for specific needs or to revise existing *CF* values if there is reason to believe doing so may increase the accuracy of the resulting water concentration value. Analytical methods to measure selenium in tissue are discussed in Appendix L. Where appropriate, additional data could be obtained as part of a NPDES permit application by invoking authority under CWA section 308 (or comparable state or tribal authority) to require NPDES-regulated facilities to collect information necessary to develop permit limits**.**

1.2.5.4 Extrapolating the CF value from the list of values that were used to derive EPA's recommended water criteria concentration values

If one or more necessary *CF* values are not available, and the information needed to derive a species-specific *CF* value is not available or impractical to obtain, a model user could could consider extrapolating a new *CF* value from other known *CF* values. One possible method to extrapolate a *CF* value is to use the same taxonomic approach EPA uses for *TTF* values that are not available for specific

species (Section 1.2.3.4). Sequentially consider higher taxonomic classifications could be considered until one or more of the fish species with a known *CF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *CF* values, the average *CF* value from the matching table entries could be used.

1.2.6 Translate the Selenium Egg-Ovary Criterion Element into a Site-Specific Water Concentration Value using Equation K-1

Model users could derive a site-specific water concentration value from the egg-ovary criterion element value using Equation K-1 with appropriate values of *CF*, $TTF^{composite}$ (derived from the product of the individual *TTF* values from each trophic level) and *EF*. Note that NPDES permitting regulations at 40 CFR § 122.45(c) requires that a Water Quality-Based Effluent Limit (WQBEL) for metals be expressed as total recoverable metal, unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Equation K-1 assumes selenium concentrations dissolved in water. While states and tribes may express ambient water quality criteria in water quality standards as dissolved selenium, an additional step would be necessary to convert the dissolved selenium concentration to a total recoverable selenium concentration for the purpose of NPDES permitting. Guidance for converting expression of metal concentrations in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996).

1.3 Managing Uncertainty using the Mechanistic Modeling Approach

Uncertainty in the translation of the egg-ovary criterion element to a water column value using the mechanistic bioaccumulation modeling approach (Equation K-1) can arise from several sources. These include:

- Measurement error when deriving input parameters,
- Inaccurate food web models due to misidentification and/or incorrect proportions of prey organisms,
- Inaccurate or inappropriate *EF*, *TTF*, and/or *CF* values,
- Biological variability,
- Unaccounted factors affecting bioaccumulation (e.g. selenium speciation), and
- Other unknown factors.

The most influential step in selenium bioaccumulation occurs at the base of aquatic food webs (Chapman et al. 2010). The parameter *EF* characterizes this step by quantifying the partitioning of

selenium between the dissolved and particulate state. *EF* can vary by at least two orders of magnitude across aquatic systems (Presser and Luoma 2010). The greatest reduction in uncertainty could be achieved when translating a fish tissue concentration of selenium to a water column concentration using Equation K-1 by using temporally and spatially coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity to accurately characterize *EF*.

Presser (2013) provides several recommendation to reduce uncertainty in an ecosystem scale approach to deriving a site-specific selenium water column criterion in a coal mining impacted area of West Virginia. Suggested actions to reduce uncertainty include:

- Obtaining temporally matched pairs of selenium measurements in dissolved and particulate material across a broad range of sites to ensure the samples accurately characterize the aquatic system and to assess sample variability;
- Characterizing particulate material across seasons to better define the base of the food web;
- Evaluating aquatic systems variables such as residence time, watershed dilution, and physical habitat attributes on as fine a scale as possible;
- Refining model assumptions to accurately characterize dietary preferences and composition of fish, and develop additional *TTF* values if necessary;
- Identify and target fish species particularly sensitive to selenium;
- Consider temporal changes in the bioaccumulation potential of the aquatic system and changes in selenium sensitivity over the life cycle of fish; and
- Consider variability in hydrology and selenium discharges.

The suitability of selected equation parameters could be determined by obtaining fish tissue and water column measurements of selenium from small-scale field studies, use of equation K-1 to estimate one measurements using the other, and comparison of the estimated concentration with the actual concentration (see Section 6.2.1 of the main document for a description of EPA's validation approach).

1.4 Example Calculations

Below are six hypothetical examples that demonstrate how to translate the egg-ovary FCV to a site-specific water concentration criterion using Equation K-1. These examples encompass a variety of hypothetical aquatic systems with various fish species and food webs. For these hypothetical examples, species-specific *TTF* values were taken from Tables K-1 and K-2, and *CF* values were taken from Table K-3. To calculate EF in these examples, the EPA used a hypothetical water concentration of 5 µg/L and the hypothetical particulate concentrations of 4.25 µg/g and 8.75 µg/g in lotic and lentic aquatic systems, respectively.

1.4.1 Example 1

Bluegill (*Lepomis macrochirus*) in a river that consume mostly amphipods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for amphipods (TTF^{TL2})	1.22
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$\mathrm{EF} = \frac{\mathrm{C_{particulate}}}{\mathrm{C_{water}}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$
$$= 1.03 \times 1.22$$
$$= 1.26$$

$$C_{water} = \frac{15.1}{1.26 \times 0.85 \times 2.13}$$

= 6.62 µg/L

1.4.2 Example 2

Fathead minnow (*Pimephales promelas*) in a river that consume mostly copepods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods (TTF^{TL2})	1.41
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$
$$= 1.57 \times 1.41$$
$$= 2.21$$

$$C_{water} = \frac{15.1}{2.21 \times 0.85 \times 1.40}$$

= 5.74 µg/L

1.4.3 Example 3

Bluegill (*Lepomis macrochirus*) in a lake that consume mostly aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	8.75
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for aquatic insects (median of Odonates, Water boatman, Midges, and Mayflies) (TTF^{TL2})	2.14
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{8.75}{5.00}$$

= 1.75 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$TTF^{composite}$ $= TTF^{TL3} \times TTF^{TL2}$

= 1.03 x 2.14

= 2.20

$$C_{water} = \frac{15.1}{2.20 \times 1.75 \times 2.13}$$

= 1.84 µg/L

1.4.4 Example 4

Fathead minnow (*Pimephales promelas*) in a river that consume approximately ⅔ copepods and ⅓ aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods and aquatic insects (TTF^{TL2}) Copepods = 1.41 Average of all aquatic insects = 2.14 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i \times w_i\right)$ $= (1.41 \times ⅔) + (2.14 \times ⅓)$ $= 1.65$	1.65
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$
= 1.57 × 1.65
= 2.59

$$C_{water} = \frac{15.1}{2.59 \times 0.85 \times 1.40}$$

= 4.90 µg/L

1.5.5 Example 5

Flathead chub (*Platygobio gracilis*) in a river with a diet of approximately 80% aquatic insects and 20% algae:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of flathead chub: Lowest matching taxon is the family Cyprinidae. Therefore, the TTF value of Cyprinidae is used (TTF^{TL3})	1.20
Trophic transfer factor for insects (TTF^{TL2}) Average of all aquatic insects = 2.14	2.14
Egg-ovary to whole-body conversion factor for flathead chub (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	1.95
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times w_2]$$

Where:
w_1 = Proportion of fathead chub diet from insects; and
w_2 = Proportion of fathead chub diet from algae

$$TTF^{comb} = [1.20 \times 2.14 \times 0.8] + [1.20 \times 0.2]$$
= 2.29

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{2.29 \times 0.85 \times 1.95}$$

= 3.98 µg/L

1.5.6 Example 6

Largemouth bass (*Micropterus salmoides*) in a large river that consume mostly Western mosquitofish (*Gambusia affinis*) that consume approximately ¾ insects and ¼ crustaceans:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of largemouth bass (TTF^{TL4})	1.39
Trophic transfer factor of Western mosquitofish (TTF^{TL3})	1.21
Trophic transfer factor for insects and crustaceans (TTF^{TL2}) Median all Insects – 2.14 Median all Crustaceans – 1.41 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i^{TL2} w_i\right)$ = (2.14 x 0.75) + (1.41 x 0.25) = 1.96	1.96
Egg-ovary to whole-body conversion factor for largemouth bass (species-specific value not available, so median CF for genus Micropterus is used) (CF)	1.42
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$
$= 1.39 \times 1.21 \times 1.96$
$= 3.30$

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{3.30 \times 0.85 \times 1.42}$$

= 3.79 µg/L

2.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING BIOACCUMULATION FACTORS (BAF)

2.1 Summary of the BAF Approach

A bioaccumulation factor (BAF) is the ratio (in milligrams/kilogram per milligrams/liter, or liters per kilogram) of the concentration of a chemical in the tissue of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). BAFs are used to relate chemical concentrations in aquatic organisms to concentrations in the ambient media of aquatic ecosystems where both the organism and its food are exposed and the ratio does not change substantially over time. The BAF is expressed mathematically as:

$$BAF = \frac{C_{tissue}}{C_{water}} \qquad \textit{(Equation K-8)}$$

Where:

BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)
C_{tissue}	=	concentration of chemical in fish tissue (mg/kg)
C_{water}	=	ambient concentration of chemical in water (mg/L)

The site-specific BAF can then be applied to the tissue criterion to solve for a target site-specific water column criterion (C_{target}):

$$C_{target} \times \frac{C_{egg-ovary\ criterion}}{BAF} \qquad \textit{(Equation K-9)}$$

Where:

C_{target}	=	site-specific water criterion concentration (mg/L)
$C_{egg\text{-}ovary\ criterion}$	=	national egg-ovary tissue criterion (15.1 mg Se/kg dw)
BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)

To translate a fish tissue criterion to a water concentration value, a site-specific, field-measured BAF for the waterbody could be developed, and then a water concentration criterion could be calculated using Equation K-9. Detailed information about how to derive a site-specific, field-measured BAF is provided in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume 3: Development of Site-specific Bioaccumulation*

Factors (U.S. EPA 2009). Although this guidance was developed for deriving human health criteria, the methodological approach is also applicable to the derivation of aquatic life criteria. The following example illustrates the calculation of a site specific water column criterion using the BAF approach.

2.1.1 Example: Derivation of a site specific water column criterion for a waterbody impacted by selenium

Available data for a hypothetical site indicate that the average egg/ovary tissue concentration of selenium for the bluegill (*Lepomis macrochirus*) is 22 mg/kg (dw). This concentration exceeds the USEPA proposed egg/ovary criterion of 15.1 mg/kg (dw). The ambient selenium water column concentration at that hypothetical site is 4.0 µg/L. The following calculation shows how to derive a target water column that would achieve a site-specific criterion using the bioaccumulation factor (BAF) approach.

Site specific selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.1
Ambient selenium water column concentration (µg/L)	4.0
Target water column concentration (µg/L)	X

Set up proportional equation to solve for allowable water column concentration:

$$\frac{Site\ specific\ egg/ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Site\ specific\ water\ concentration\ (\frac{\mu g\ Se}{L})} = \frac{Criterion\ egg\ ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Solve for the target water concentration that will achieve a site-specific criterion:

$$\frac{22.0\ (\frac{mg\ Se}{kg\ dw})}{4.0\ (\frac{\mu g\ Se}{L})} = \frac{15.1\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Target water concentration = 2.75 µg/L.

2.2 Managing Uncertainty using the BAF Approach

Uncertainty can be introduced when using the BAF approach to derive a water concentration value from a fish tissue criterion concentration. Inaccurate water concentration values can result when BAFs are derived from water and fish tissue concentration measurements that are obtained from sources that do not closely represent site characteristics, or from field data collected from large-scale sites that encompass multiple water bodies or ecosystems. Most of this uncertainty results from differences in the

bioavailability of selenium between the study sites where measurements are made to derive the BAF, and the site(s) to which the BAF is used to derive needed water concentration values.

Because of uncertainties associated with the BAF approach, EPA does not recommend developing BAFs from data extrapolated from different sites or across large spatial scales. EPA's Framework for Metals Risk Assessment (U.S. EPA 2007) outlines key principles about metals and describes how they should be considered in conducting human health and ecological risk assessments due the the effects of water chemistry on bioavilability of such chemicals. The current science does not support the use of a single, generic threshold BAF value as an indicator of metal bioaccumulation. The use of BAFs are appropriate only for site-specific applications where sufficient measurements have been taken from the site of interest and there is little or no extrapolation of BAF values across differing exposure conditions and species.

The preferred approach for using a BAF to implement the selenium fish tissue criterion is to calculate a site-specific, field-measured BAF from data gathered at the site of interest, and to apply that BAF to that site. A site-specific, field-measured BAF is a direct measure of bioaccumulation in an aquatic system because the data are collected from the aquatic ecosystem itself and thus reflects real-world exposure through all relevant exposure routes. A site-specific, field-measured BAF also reflects biotic and abiotic factors that influence the bioavailability, biomagnification, metabolism, and biogeochemical cycling of selenium that might affect bioaccumulation in the aquatic organism or its food web. Appropriately developed site-specific, field-measured BAFs are appropriate for all bioaccumulative chemicals, regardless of the extent of chemical metabolism in biota from a site (U.S. EPA 2000).

Although a site-specific, field-measured BAF is a direct measure of bioaccumulation, its predictive power depends on a number of important factors being properly addressed in the design of the field sampling effort. For example, sampling in areas with relatively long water residence times should be a priority because selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as wetlands, oxbows, and estuaries) and with fine particulate sediments high in organic carbon. In addition, migratory species should generally not be used because their exposure to selenium could reflect selenium concentrations in areas other than where the fish were caught. Fish may also need to be sampled and BAF values recalculated if selenium levels significantly change over time because BAFs are known to be affected by the ambient concentration of the metals in the aquatic environment (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). States and tribes should refer to *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume* (U.S. EPA 2009) for guidance on appropriate methods for developing a site-specific, field-derive BAF.

The advantage of using the BAF approach is its relative simplicity, especially when the data necessary to derive the BAF is already available. Furthermore, the BAF approach is completely empirical and does not require any specific knowledge about the physical, chemical, or biological characteristics of the waterbody. The relationship between the concentration of selenium in fish tissue and water is directly determined by direct measurements in these media. This may be advantageous when there are uncertainties with how to collect a particulate sample that is representative of the base of the food web, or dilution concerns (e.g., sandy streams with little surface area for algae sampling and high potential for sand contamination of a benthic sediment sample).

Limitations of the BAF approach should be considered before deciding if this method is appropriate for translating the selenium FCV to a water concentration value. One disadvantage of the BAF approach is the considerable effort and resources necessary to collect sufficient data to establish the relationship between tissue and water concentrations. Resource use increases as the spatial scale and complexity of the aquatic system increases. Furthermore, the BAF approach does not allow extrapolation across species, space, and large time scales because the site-specific factors that might influence bioaccumulation are integrated within the tissue concentration measurements and thus cannot be individually adjusted to extrapolate to other conditions. Thus, site-specific, field-measured BAFs only provide an accounting of the uptake and accumulation of selenium for an organism at a specific site and point in time. This is more important in lotic habitats, since the kinetics of selenium bioaccumulation may be very different at a site upstream or downstream from the site of interest.

As noted previously, NPDES permitting regulations at 40 CFR § 122.45(c) require WQBELs for metals be expressed as total recoverable metal unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Guidance for converting expression of metals in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996). Whether or not a water concentration value derived from a site-specific, field-derived BAF requires conversion from dissolved to total recoverable selenium depends on how the BAF is developed. Generally, conversion would not be necessary if the BAF is derived from water concentration values that measure total selenium; however, conversion would be necessary if the BAF was derived from water concentration values that measured dissolved selenium. Table K-4 compares some of the principle characteristics of the mechanistic bioaccumulation modeling approach or the BAF approach for translating the selenium FCV to a water concentration.

3.0 COMPARISON OF MECHANISTIC BIOACCUMULATION MODELING AND BAF APPROACHES

Data from Saiki et al. (1993) are used here to illustrate an example comparison of the two translation approaches, the mechanistic bioaccumulation modeling approach and the bioaccumulation factor (BAF) model approach. Definitive selenium measurements for all ecosystem compartments (e.g., water, algae, etc.) are available for two species, bluegill and largemouth bass, at four sites. Food web pathways were calculated using results of gut content analysis. Although Saiki et al. (1993) satisfies the minimum requirements for a site specific translation, it represents a sparse dataset, with only two measurements per ecosystem compartment, one for the spring and fall of 1987, respectively. For purposes of this exercise, samples from the same site collected at different time periods will be treated as replicate data; however, EPA recommends using larger sample sizes collected during the same time period when calculating a site specific criterion.

Selenium data used to calculate site specific water criteria are included in Table K-4. Median concentrations and coefficients of variation for each ecosystem compartment at each site are included in Table K-5. Because at most only two concentrations were available for each ecosystem, site median are equal to site averages. Site specific translations for both approaches will be calculated using median selenium concentrations.

Table K-4. Selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw.

Site	Date	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	Fall 1987	3	7.40	22	4.6	8.9	5.2	2.4	6.4	6.8
Mud Slough at Gun Club Road	Spring 1987	9	1.60	7.9	3.3	7.2	4.4	5.4	5	6.9
Salt Slough at the San Luis National Wildlife Refuge	Fall 1987	3	0.38	8.9	3.4	5.4	3.1	4.5	4.5	4.7
Salt Slough at the San Luis National Wildlife Refuge	Spring 1987	13	2.40	7.9	3.7	6.9	3.2	4.4	4.3	4
San Joaquin R. above Hills Ferry Road	Fall 1987	3	1.20	6.6	3.8	6	1.7	2.6	3.3	2.2
San Joaquin R. above Hills Ferry Road	Spring 1987	11	1.30	3.4	2.8	4.1	1.9	4.3	2.7	2.4
San Joaquin R. at Durham Ferry State Recreation Area	Fall 1987	1	0.39	1.2	1.5	1.5	0.77	1.6	2	1.8
San Joaquin R. at Durham Ferry State Recreation Area	Spring 1987		0.50	1.3	1.1	1.6	1.3	1.8	1.9	1.7

Table K-5. Median selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
For purposes of this exercise, spring and fall samples measured at the same site are treated as replicates. Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw. Coefficients of determination included in parentheses.

Site	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	6.0 (0.71)	4.50 (0.91)	14.95 (0.67)	3.95 (0.23)	8.05 (0.15)	4.80 (0.12)	3.90 (0.54)	5.70 (0.17)	6.85 (0.01)
Salt Slough at the San Luis National Wildlife Refuge	8.0 (0.88)	1.39 (1.03)	8.40 (0.08)	3.55 (0.06)	6.15 (0.17)	3.15 (0.02)	4.45 (0.02)	4.40 (0.03)	4.35 (0.11)
San Joaquin R. above Hills Ferry Road	7.0 (0.81)	1.25 (0.06)	5.00 (0.45)	3.30 (0.21)	5.05 (0.27)	1.80 (0.08)	3.45 (0.35)	3.00 (0.14)	2.30 (0.06)
San Joaquin R. at Durham Ferry State Recreation Area	1.0 (na)	0.45 (0.17)	1.25 (0.06)	1.30 (0.22)	1.55 (0.05)	1.04 (0.36)	1.70 (0.08)	1.95 (0.04)	1.75 (0.04)

3.1 Translation using the BAF Approach

Site specific BAFs were calculated for bluegill and largemouth bass at each of the four sites (Table K-6). A site-specific water criterion was calculated for each species at the four sites using equation K-8, which is equivalent to the BAF example shown in the previous section. The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$BAF = \frac{C_{tissue}}{C_{water}} = \frac{4.4\ \mu g/g}{8\ \mu g/L} = 0.55\ L/g$$

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{BAF} = \frac{8.5\ mg/kg}{0.55\ L/g} = 15.5\ \mu g/L$$

The whole body tissue criterion of 8.5 mg/kg is used here because whole body fish tissue selenium measurements were made. If site specific egg ovary fish tissue had been measured, then the egg ovary tissue criterion of 15.1 mg/kg would have been used.

Table K-6. Site and species specific translated water concentrations using the BAF translation approach.

		Bluegill:			Largemouth Bass:		
Site	Water (µg/L)	WB Se (mg/kg)	BAF (L/g)	Water SSC[a] (µg/L)	WB Se (mg/kg)	BAF (L/g)	Water SSC[a] (µg/L)
Mud Slough at Gun Club Road	6.0	5.70	0.95	8.95	6.85	1.14	7.45
Salt Slough at the San Luis National Wildlife Refuge	8.0	4.40	0.55	15.45	4.35	0.54	15.63
San Joaquin R. above Hills Ferry Road	7.0	3.00	0.43	19.83	2.30	0.33	25.87
San Joaquin R. at Durham Ferry State Recreation Area	1.0	1.95	1.95	4.36	1.75	1.75	4.86

a – Site specific criterion based on BAF for respective species.

At each site, the lowest translated water criterion for all species is used as the site specific criterion. At site "Mud Slough at Gun Club Road," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the BAF approach range from 4.4 to 19.8 µg/L Table K-6).

3.2 Translation using the Mechanistic Bioaccumulation Modeling Approach

The first step in the bioaccumulation modeling approach is the calculation of site specific enrichment factors (*EF*s). Because both algae and detritus selenium concentrations were available, the first step was the calculation of separate *EF*s for algae and detritus at each site, following the procedures described in section 1.2.4.1. Algal and detrital *EF*s, respectively, were calculated using the median of all Se concentrations in algae (or detritus) at a site by the median of all Se concentrations in water at the same site. After calculating separate algal and detrital *EF*s, the final *EF* at each site was calculated as the geometric mean of the algal and detrital *EF* at a given site. Algal, detrital, and site *EF*s are shown in Table K-7.

Table K-7. Se concentrations in water, algae, detritus, and site specific *EF*s.

Site	Water (µg/L)	Algae (mg/kg)	Detritus (mg/kg)	EF (L/g)
Mud Slough at Gun Club Road	6.0	4.50	14.95	1.37
Salt Slough at the San Luis National Wildlife Refuge	8.0	1.39	8.40	0.43
San Joaquin R. above Hills Ferry Road	7.0	1.25	5.00	0.36
San Joaquin R. at Durham Ferry State Recreation Area	1.0	0.45	1.25	0.75

As an example, the EF calculation for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$EF_{algae} = \frac{C_{algae}}{water}\ ;\ EF_{detritus} = \frac{C_{detritus}}{water}$$

$$EF_{site} = \sqrt{\left(EF_{algae} \times EF_{detritus}\right)}$$

$$EF_{algae} = \frac{1.39\ mg/kg}{8.0\ \mu g/L};\ EF_{detritus} = \frac{8.4\ mg/kg}{8.0\ \mu g/L}$$

$$EF_{site} = \sqrt{(0.17 \times 1.05)}$$

$$EF_{site} = 0.43\ L/g$$

The second step in the bioaccumulation modeling approach is the calculation of site specific composite trophic transfer factors ($TTF^{composite}$). Based on gut content analysis, bluegill diets consisted of

47% amphipods, 23% chironomids, and 30% zooplankton, while largemouth bass diets consisted of 73% bluegill and 27% crayfish.

The composite TTF for bluegill was calculated using the following equation:

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times TTF^{TL2} \times w_2] + [TTF^{TL3} \times TTF^{TL2} \times w_3]$$

Where:

W_1 = proportion of diet from amphipods,

W_2 = proportion of diet from chironomids, and

W_3 = proportion of diet from zooplankton.

For each of the 3 species in the bluegill diet, site specific TTF^{TL3} and TTF^{TL2} were calculated separately. Using median concentrations from Table K-5, $TTF^{composite}$ were calculated for each of the sites and are included in Table K-8.

Table K-8. Trophic transfer factors (TTFs) for bluegill and bluegill prey.

	TL2 TTFs:			**TL3 TTFs:**			**$TTF^{composite}$:**
Site	**Amphipod**	**Chironomid**	**Zooplankton**	**BG-Amph**	**BG-Chiro**	**BG-Zoo**	**Bluegill**
Mud Slough at Gun Club Road	0.41	0.83	0.40	1.44	0.71	1.46	0.59
Salt Slough at the San Luis National Wildlife Refuge	0.73	1.26	0.91	1.24	0.72	0.99	0.90
San Joaquin R. above Hills Ferry Road	1.06	1.62	1.10	0.91	0.59	0.87	0.96
San Joaquin R. at Durham Ferry State Recreation Area	1.53	1.83	2.01	1.50	1.26	1.15	2.30

As an example, the bluegill $TTF^{composite}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$TTF^{composite} = [1.24 \times 0.73 \times 0.47] + [0.72 \times 1.26 \times 0.23] + [0.99 \times 0.91 \times 0.30]$$

$$TTF^{composite} = 0.90$$

The composite TTF for largemouth bass was calculated using the following equation:

$$TTF^{composite} = [TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times TTF^{TL2} \times w_2]$$

Where:

W_1 = proportion of diet from bluegill, and

W_2 = proportion of diet from crayfish

For the proportion of the largemouth bass diet consisting of bluegill, TTF^{TL3} x TTF^{TL2} was equal to the $TTF^{composite}$ for bluegill. As was the case for bluegill, site specific TTFs were calculated for each species, and are included in Table K-9.

Table K-9. Trophic transfer factors (TTFs) for largemouth bass and largemouth bass prey.

	Crayfish dietary fraction:		Bluegill dietary fraction:		$TTF^{composite}$:
Site	Crayfish	LMB-Cray	Bluegill[a]	LMB-BG	LMB
Mud Slough at Gun Club Road	0.49	1.43	0.59	0.70	0.49
Salt Slough at the San Luis National Wildlife Refuge	0.64	1.38	0.90	0.89	0.82
San Joaquin R. above Hills Ferry Road	0.58	1.28	0.96	0.74	0.71
San Joaquin R. at Durham Ferry State Recreation Area	1.22	1.69	2.30	2.06	4.03

a – $TTF^{composite}$ for bluegill.

As an example, the largemouth bass $TTF^{combined}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$TTF^{composite} = \left[TTF^{TL4} \times TTF_{bluegill}^{composite} \times w_1\right] + [TTF^{TL3} \times TTF^{TL2} \times w_2]$$

$$TTF^{composite} = [0.89 \times 0.90 \times 0.73] + [1.38 \times 0.64 \times 0.27]$$

$$TTF^{composite} = 0.82$$

After calculating site and species specific EF and $TTF^{combined}$, site specific water criterion concentrations were calculated using a modified version of equation K-1, shown below.

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{EF\ x\ TTF^{composite}}$$

The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$C_{water\ criterion} = \frac{8.5\ mg/kg}{0.43\ L/g\ x\ 0.90} = 22.1\ \mu g/L$$

Because the selenium in fish tissue at these sites were measured as whole body concentrations, the whole body criterion of 8.5 µg/L is used, and an egg-ovary to whole body conversion factor is not required. As with the BAF approach, the lowest translated water criterion for all species is used as the site specific criterion. At site "San Joaquin R. at Durham Ferry State Recreation Area," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the mechanistic bioaccumulation modeling approach are more variable than concentrations calculated using the BAF approach, and range from 2.8 to 33.3 µg/L Table K-10). At all sites using both methods, the translated site specific water concentration criteria were higher than the measured water concentrations.

Table K-10. Site and species specific translated water concentrations using the mechanistic bioaccumulation modeling approach.

		Bluegill:			Largemouth Bass:		
Site	EF (L/g)	WB Se (mg/kg)	TTF	Water SSC (µg/L)	WB Se (mg/kg)	TTF	Water SSC (µg/L)
Mud Slough at Gun Club Road	1.37	5.70	0.59	10.61	6.85	0.49	12.65
Salt Slough at the San Luis National Wildlife Refuge	0.43	4.40	0.90	22.14	4.35	0.82	24.18
San Joaquin R. above Hills Ferry Road	0.36	3.00	0.96	24.79	2.30	0.71	33.31
San Joaquin R. at Durham Ferry State Recreation Area	0.75	1.95	2.30	4.95	1.75	4.03	2.83

3.3 Summary Comparison of the Mechanistic Bioaccumulation and BAF Approaches

A comparison of the mechanistic bioaccumulation and BAF approaches is included in Table K-11.

Table K-11. Comparison of mechanistic bioaccumulation and BAF approaches.

Mechanistic bioaccumulation modeling	Bioaccumulation Factor (BAF)
Knowledge of the aquatic system needed	No information on aquatic system needed
Choice of input parameters at discretion of state or tribe	No input parameters to choose
Species-specific	Species-specific
Can be applied at different sites if site *EF* can be estimated.	Site-specific
Fish tissue sampling not required for translation	Fish tissue and water sampling required

APPENDIX L: ANALYTICAL METHODS FOR MEASURING SELENIUM

The Clean Water Act (CWA) establishes an EPA approval process for certain analytical methods used in the National Pollutant Discharge Elimination System (NPDES) program and for section 401 certifications. EPA has several approved methods for measuring selenium in water under 40 CFR § 136. EPA generally requires the use of EPA-approved methods for the NPDES program and for CWA section 401 certifications issued by states and tribes (40 CFR § 136.1). However, since there are no EPA approved methods for the analysis of selenium in fish tissue, states and tribes may use analytical methods not approved by EPA to evaluate the attainment of water quality standards or to develop or implement Total Maximum Daily Loads provided that these methods are scientifically sound (40 CFR 122.21(g)(7)).

Implementation of a water quality standard for selenium may require the ability to detect and measure the concentration of selenium in effluent, ambient water, tissue, and other media that is below the detection limit or limit of quantitation that some analytical methods can provide. States and tribes should choose an analytical method that is sufficiently sensitive to implement its water quality standard for selenium. Below are descriptions of some of the methods available for measuring selenium concentrations with sufficient sensitivity to implement EPA's recommended selenium criterion. Complete descriptions of analytical methods appropriate for analyzing selenium in different media can be found in the National Environmental Methods Index at http://www.nemi.gov.

1.0 General Considerations when Measuring Concentrations of Selenium

The oxidation states of selenium dissolved in surface water are usually selenate (+6), selenite (+4), and organo-selenium (-2). The presence of selenium in different oxidation states complicates some analytical methods (Presser and Ohlendorf 1987). EPA recommends using standard reference samples to check for the percentage recovery of each species of selenium (selenate, selenite and organo-selenium) during initial testing of selenium methodologies for quality control and assurance.

If water samples are not filtered, particulate species such as elemental selenium and particulate organo-selenium will also be measured. In addition, federal regulations at 40 CFR §122.45(c) generally requires considering total recoverable metals when establishing effluent limits and reporting requirements.

2.0 Analytical Methods Recommended for Measuring Selenium in Water

EPA has several approved analytical methods under 40 CFR § 136 specifically for measuring total selenium in water. These regulations state that measurements for NPDES permit applications and permittee reporting should be made using analytical methods approved by EPA. Because EPA has

approved methods for analyzing selenium in water, these methods must be used for NPDES permits (40 CFR § 122.21(g)(7), 122.41(j), 136.1, 136.3, and 136.6).

A complete list of EPA-approved analytical methods for selenium can be found at: http://www.epa.gov/waterscience/methods/method/. Three EPA-approved methods that may be sufficiently sensitive[4] for the purposes of implementing a selenium water quality criterion are listed below (Table L-1).

Table L-1. Suggested EPA-Approved Methods for Selenium in Water

Method	Technique	Method detection limit
American Public Health Standard Method 3114 B (2009) or 3114 C (2009)	Hydride generation atomic absorption spectrometry (HG-AAS)	2 µg/L
EPA Method 200.8, Rev 5.4 (1998)	Inductively coupled plasma mass spectrometry (ICP-MS)	7.9 µg/L
EPA Method 200.9, Rev.2.2 (1994)	Stabilized temperature graphite furnace atomic absorption (STGF-AA)	0.6 µg/L

2.1 American Public Health Standard Method 3114 B

For measuring selenium in water, American Public Health Standard Method 3114 B uses the HG-AAS technique. Method 3114 B has a method detection limit (MDL) of 2 µg/L. Samples for dissolved analytes should be filtered on-site through 0.45-micron capsule filters certified free of trace-element contamination or other appropriate filtering equipment (Wilde et al. 1999). Dissolved samples should be preserved with high purity hydrochloric acid or nitric acid to a pH less than 2.

For measuring total selenium, samples should not be filtered. In addition, all selenium in the sample should be in the form of selenite (+4). Thus, the following pre-treatment steps to convert all selenium in the sample to selenite are critical when using the HG-AAS method:

1. Boiling with persulfate to oxidize and digest organic material.
2. Boiling with hydrochloric acid to reduce selenate species to selenite.
3. Reduction by sodium borohydride to hydrogen selenide in the quartz tube of the AAS.

[4]For more information on choosing a sufficiently sensitive method, see the memorandum *Analytical Methods for Mercury in National Pollutant Discharge Elimination System (NPDES) Permits* from James A. Hanlon, Director of the Office of Wastewater Management, dated August 23, 2007, available at http://www.epa.gov/npdes/pubs/mercurymemo_analyticalmethods.pdf.

Optimal conversion conditions are essential for accurate results because too mild a reduction could lead to incomplete reduction of selenate and too rigorous a reduction could lead to plating out of elemental selenium (Cutter 1987, 1983; Presser and Barnes 1984, 1985).

Method 3114 B has the advantage that it is a fully validated method, is commonly used by many laboratories, is relatively inexpensive, is less susceptible to background interference (Cutter 1987, 1983; Presser and Barnes 1984, 1985), and has sufficient sensitivity to accurately measure what can be expected in many lotic aquatic systems. However, this method may not be sufficiently sensitive for some lentic aquatic systems where relatively lower selenium concentrations may need to be measured. If no selenium is detected in a lentic system using this method, EPA recommends using a more sensitive analytical method.

2.2 EPA Method 200.8

EPA method 200.8 has a MDL of 7.9 µg/L using the ICP-MS analytical technique. This method has the advantage that no pre-treatment steps are necessary. However, this method may not be sufficiently sensitive in many applications of the selenium criterion (Lamothe et al. 1999). If no selenium is detected using this method, EPA recommends monitoring with a more sensitive method.

2.3 EPA Method 200.9

Method 200.9 has a MDL of 0.6 µg/L using the STGF-AA analytical technique. This method has the advantage that it can detect selenium at very low concentrations. However, graphite furnace techniques require careful matrix matching.

Of these three EPA approved methods, Method 3114B using the HG-AAS technique is the most cost-effective, with sufficient sensitivity and relatively low risk of interference in most cases. EPA Method 200.8 may be used where appropriate, such as when selenium concentrations in effluent are known to be higher than 7.9 µg/L. EPA Method 200.9 may be used if a very low MDL is needed. Some additional methods not approved by EPA that states and tribes might consider are:

- Collision/Reaction Cell Inductively Coupled Plasma Mass Spectroscopy (cICP-MS) (Garbarino et al. 2005) - A relatively new technique that is a sensitive and selective detector for metal analysis. However, isobaric interference can cause problems for quantitative determination as well as identification based on the analyte pattern. Collision cells, reaction cells or other interfaces reducing sample matrix effects that might otherwise interfere in the mass selective determinative step are allowed in CWA analyses provided the method performance specifications relevant to ICP-MS measurements are met

- Fluorometric Analysis_- a wet chemistry technique using diaminonapthalene. This method also achieves acceptable precision and accuracy on standard reference samples (Olson 1969; Olson et al. 1975; American Public Health Association Standard Method 3500, on-line version).

Methods for measuring different species of selenium dissolved in water are also available. These methods determine the species of dissolved selenium present in a sample through differential digestion and hydride generation atomic adsorption spectrophotometry (Cutter 1978, 1983; Presser and Barnes, 1984; 1985; May et al. 2007). Selenite can be measured in samples with no pre-treatment. Selenate plus selenite can be measured in samples subjected to boiling with hydrochloric acid. Subtraction of the measured selenite fraction from the measured combined fraction would yield a measure of the selenate fraction. If a sample is analyzed to measure total dissolved selenium as described above, then measurements of the combined fraction can be subtracted to yield measurements of the dissolved organo-selenium fraction.

3.0 ANALYTICAL METHODS AVAILABLE FOR MEASURING SELENIUM IN FISH TISSUE

EPA does not have approved methods under 40 CFR § 136 for measuring selenium in fish tissue. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in fish tissue if the samples are made soluble. Tissue samples are homogenized and digested prior to analysis using strong acid or dry-ashing digestion as described below. A review of sample digestion techniques has been published (Ihnat 1992). Standard reference materials, analytical duplicates, and matrix spike samples are recommended to determine the applicability of a selected digestion procedure.

3.1 Strong acid digestion

Solid samples can be subjected to strong acid digestion to break down mineral and organic matrices. Samples are typically dried and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Note that some strong acid digestion methods may not be suitable for fish tissue. Strong acid digestion methods are categorized by the type of material or amount of organic material present (e.g., solid waste; biological tissue, plants, soil, sediment, rock, coal) and degrees of tissue solubilization needed (extraction, leachate, or complete destruction). Methods differ in acid mixture and degree and type of heating (EPA Method 3050B, Revision 2, 1996; EPA Method 200.2,

Revision 2.8, 1994; Briggs and Crock, 1986; Taggart, 2002, chapters I, J, and K). High boiling acids (perchloric and sulfuric) may lead to a loss of selenium if solutions are heated to dryness.

3.2 Dry-ashing digestion

Dry-ashing digestion is applicable to biological samples (Brumbaugh and Walther, 1989; May et al., 2007). Biological samples are normally lyophilized (freeze-dried) and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Dried solid samples are:

1. Boiled in nitric acid for solubilization and oxidation
2. Ashed at 500º C with magnesium nitrate to complete oxidation and decompose remaining organic material
3. Heated with hydrochloric acid to dissolve the ash and reduce selenium to the selenite (+4) state required for detection by HG-AAS.

3.3 Analytical methods available for measuring selenium in particulate material

There are no 40 CFR § 136 methods for analyzing selenium in particulate material. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in particulate material after the sample has been separated from the water and pre-treated using the same methods used for fish tissue. In order to obtain a particulate material sample, a water column sample should be filtered to separate the particulate material and bed sediment. Various techniques for collection of suspended particulate material using filtration are available from the EPA (e.g. Method 1669) and the U.S. Geological Survey (Moulton et al. 2002; USGS, Britton and Greeson 1987). These techniques include:

- EPA Method 1669 (1996) includes filtration through a 0.45 µm capsule filter at the field site.
- USGS protocols for collection of phytoplankton and seston in rivers and streams as part of their National Water Quality Assessment Program for watershed and habitat assessment (http://water.usgs.gov/nawqa/protocols.html).
- Textbooks such as *Limnological Analyses* address sampling of lakes using traditional techniques including phytoplankton nets. (Wetzel and Likens 1991).
- Sampling of suspended material from estuaries where particulates are a substantial part of the ecosystem is described in Doblin et al. (2005) as part of their work on the San Francisco Bay-Delta Estuary.
- Separating suspended sediment using high-speed centrifugation and decantation when the concentration of particulate material is relatively low (Horowitz et al. 1989).

APPENDIX M: ABBREVIATIONS

REFERENCE AND SITE ABBREVIATIONS

Reference	Site		Species	
Bi:	22	Miller's Lake, Wellington CO	FM	Fathead minnow
Birkner 1978	27	Sweitzer Lake, Delta CO	FM	Fathead minnow
	23	Twin Buttes Reservoir, Laramie WY	FM	Fathead minnow
	20	East Allen Reservoir, Medicine Bow WY	ID	Iowa darter
	7	Galett Lake, Laramie WY	ID	Iowa darter
	22	Miller's Lake, Wellington CO	ID	Iowa darter
	23	Twin Buttes Reservoir, Laramie WY	ID	Iowa darter
	30	Larimer Highway 9 Pond, Fort Collins CO	NPK	Northern plains killfish
	3	Meeboer Lake, Laramie WY	NPK	Northern plains killfish
	27	Sweitzer Lake, Delta CO	NPK	Northern plains killfish
	23	Twin Buttes Reservoir, Laramie WY	NPK	Northern plains killfish
Bu91:	4	Uncompahgre River at Colona	BhS	Bluehead sucker
Butler et al. 1991	4	Uncompahgre River at Colona	BnT	Brown trout
	4	Uncompahgre River at Colona	FS	Flannelmouth sucker
	4	Uncompahgre River at Colona	MS	Mottled sculpin
	4	Uncompahgre River at Colona	RT	Rainbow trout
	4	Uncompahgre River at Colona	WS	White sucker
Bu93:	SP2	Spring Creek at La Boca	BhS	Bluehead sucker
Butler et al. 1993	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BT	Brown trout
	SP2	Spring Creek at La Boca	BT	Brown trout
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BB	Black bullhead
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	ChC	Channel catfish
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	CC	Common carp

Reference	Site		Species	
	SP2	Spring Creek at La Boca	FM	Fathead minnow
	SP2	Spring Creek at La Boca	SD	Speckled dace
	SP2	Spring Creek at La Boca	WS	White sucker
Bu95:	ME2	McElmo Cr., downstream from Alkali Canyon	BhS	Bluehead sucker
Butler et al. 1995	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BhS	Bluehead sucker
	NW	Navajo Wash near Towaoc	BhS	Bluehead sucker
	SJ1	San Juan R. at Four Corners	BhS	Bluehead sucker
	SJ3	San Juan R. at Mexican Hat Utah	BhS	Bluehead sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BB	Black bullhead
	SJ1	San Juan R. at Four Corners	ChC	Channel catfish
	SJ3	San Juan R. at Mexican Hat Utah	ChC	Channel catfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	CC	Common carp
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	CC	Common carp
	SJ1	San Juan R. at Four Corners	CC	Common carp
	SJ3	San Juan R. at Mexican Hat Utah	CC	Common carp
	HD2	Hartman Draw near mouth, at Cortez	FM	Fathead minnow
	ME1	McElmo Cr. at Hwy. 160, near Cortez	FM	Fathead minnow
	ME2	McElmo Cr., downstream from Alkali Canyon	FM	Fathead minnow
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FM	Fathead minnow
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FM	Fathead minnow
	WC	Woods Canyon near Yellow Jacket	FM	Fathead minnow
	SJ1	San Juan R. at Four Corners	FS	Flannelmouth sucker
	HD2	Hartman Draw near mouth, at Cortez	FS	Flannelmouth sucker
	ME2	McElmo Cr., downstream from Alkali Canyon	FS	Flannelmouth sucker
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	SJ3	San Juan R. at Mexican Hat Utah	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	GnS	Green sunfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	RSh	Red shiner
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	RSh	Red shiner

Reference	Site		Species	
	SJ1	San Juan R. at Four Corners	RSh	Red shiner
	ME1	McElmo Cr. at Hwy. 160, near Cortez	SD	Speckled dace
	ME2	McElmo Cr., downstream from Alkali Canyon	SD	Speckled dace
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	SD	Speckled dace
	NW	Navajo Wash near Towaoc	SD	Speckled dace
	SJ1	San Juan R. at Four Corners	SD	Speckled dace
	HD2	Hartman Draw near mouth, at Cortez	Su	Sucker
Bu97:	MUD2	Mud Cr. at Hwy. 32, near Cortez	BhS	Bluehead sucker
Butler et al. 1997	MNP2	Large pond south of G Road, southern Mancos Valley	FM	Fathead minnow
	MUD2	Mud Cr. at Hwy. 32, near Cortez	FM	Fathead minnow
	WCP	Pond on Woods Canyon at 15 Road	FM	Fathead minnow
	CH1	Cahone Canyon at Hwy. 666	GnS	Green sunfish
	MUD2	Mud Cr. at Hwy. 32, near Cortez	GnS	Green sunfish
	MNP3	Pond downstream from site MNP2, southern Mancos Valley	SB	Smallmouth bass
Ca:	DC	Deerlick Creek	RT	Rainbow trout
Casey **2005**	LC	Luscar Creek	RT	Rainbow trout
Fo:	CC-1A	Crow Creek – 1A	BnT	Brown trout
Formation 2012	CC-3A	Crow Creek – 3A	BnT	Brown trout
	CC-150	Crow Creek – 150	BnT	Brown trout
	CC-350	Crow Creek – 350	BnT	Brown trout
	CC-75	Crow Creek – 75	BnT	Brown trout
	DC	Deer Creek	BnT	Brown trout
	HS	Hoopes Spring	BnT	Brown trout
	HS-3	Hoopes Spring – 3	BnT	Brown trout
	LSV-2C	Sage Creek – 2C	BnT	Brown trout
	LSV-4	Sage Creek – 4	BnT	Brown trout
	SFTC	South Fork Tincup Creek	BnT	Brown trout

Reference	Site		Species	
	CC-1A	Crow Creek – 1A	Sc	Sculpin
	CC-3A	Crow Creek – 3A	Sc	Sculpin
	CC-150	Crow Creek – 150	Sc	Sculpin
	CC-350	Crow Creek – 350	Sc	Sculpin
	CC-75	Crow Creek – 75	Sc	Sculpin
	DC	Deer Creek	Sc	Sculpin
	HS	Hoopes Spring	Sc	Sculpin
	HS-3	Hoopes Spring – 3	Sc	Sculpin
	LSV-2C	Sage Creek – 2C	Sc	Sculpin
	LSV-4	Sage Creek – 4	Sc	Sculpin
	SFTC	South Fork Tincup Creek	Sc	Sculpin
Gr:	17	Arapahoe Wetlands Pond	FM	Fathead minnow
Grasso et al. 1995	17	Arapahoe Wetlands Pond	WS	White sucker
HB:	LEMC	Lower East Mill Creek	CT	Cutthroat trout
Hamilton and				
Buhl 2004				
Le:	BA	Badin Lake	BB	Black bullhead
Lemly 1985	BE	Belews Lake	BB	Black bullhead
	HR	High Rock Lake	BB	Black bullhead
	BA	Badin Lake	CC	Common carp
	BE	Belews Lake	CC	Common carp
	HR	High Rock Lake	CC	Common carp
	BA	Badin Lake	FM	Fathead minnow
	BE	Belews Lake	FM	Fathead minnow
	HR	High Rock Lake	FM	Fathead minnow
	BA	Badin Lake	GnS	Green sunfish
	BE	Belews Lake	GnS	Green sunfish
	HR	High Rock Lake	GnS	Green sunfish

Reference	Site		Species	
	BA	Badin Lake	WM	Western mosquitofish
	BE	Belews Lake	WM	Western mosquitofish
	HR	High Rock Lake	WM	Western mosquitofish
	BA	Badin Lake	RSh	Red shiner
	BE	Belews Lake	RSh	Red shiner
	HR	High Rock Lake	RSh	Red shiner
Sa87:	KP11	Kesterson Pond 11	WM	Western mosquitofish
Saiki and	KP2	Kesterson Pond 2	WM	Western mosquitofish
Lowe 1987	KP8	Kesterson Pond 8	WM	Western mosquitofish
	SLD	San Luis Drain	WM	Western mosquitofish
	VP26	Volta Pond 26	WM	Western mosquitofish
	VW	Volta Wasteway	WM	Western mosquitofish
Sa93:	GT4	Salt Slough at San Luis Wildlife Refuge	Bg	Bluegill
Saiki et al. 1993	GT5	Mud Slough at San Luis Wildlife Refuge	Bg	Bluegill
	SJR2	San Joaquin R. above Hills Ferry Rd.	Bg	Bluegill
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	Bg	Bluegill
	GT4	Salt Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	GT5	Mud Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	SJR2	San Joaquin R. above Hills Ferry Rd.	LMB	Largemouth bass
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	LMB	Largemouth bass
	GT4	Salt Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	GT5	Mud Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	SJR2	San Joaquin R. above Hills Ferry Rd.	WM	Western mosquitofish
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	WM	Western mosquitofish
St:	M4720	Marsh 4720	BB	Black bullhead
Stephens et al. 1988	M4720	Marsh 4720	CC	Common carp

APPENDIX N: COMPARISON OF APPROACHES FOR CALCULATING SELENIUM TISSUE CONVERSION FACTORS

1.0 COMPARISON OF THE MEDIAN RATIO AND REGRESSION APPROACHES

Regression analysis and the application of median ratios are two approaches that can be used to quantify the relationship between two variables, such as the concentration of selenium within two tissue types. When concentrations in the two tissues are plotted, each point represents the ratio of one tissue type to another. A regression analysis calculates the line that best fits those tissue concentrations, which is characterized by both a slope and a y-intercept. In contrast, the median ratio is a single value representing the 50^{th} centile of all ratios. Conversion factors (CFs) are presently calculated as the median ratio of two tissue types. The use of median ratios grew out of the goal of patterning the translation procedure after the Luoma and Presser selenium bioaccumulation model, where field derived factors representing the transfer of selenium from one ecosystem compartment to another were represented as single values calculated using constrained (y-intercept passes through the origin) regression. Median ratios were implemented to produce a single value that was operationally similar to a constrained regression slope, but that was free from the issues associated with constrained regression, particularly cases where the y-intercept was notably different from zero, which would result in slopes that were highly divergent from slopes derived using conventional regression. Both median ratios and conventional regression (with or without log transformation) are far superior to constrained (no y-intercept) regression. The following discussion will compare median ratios and conventional linear regression.

A median is a measure of central tendency that is free from all parametric assumptions associated with linear regression. As the 50^{th} centile of all y/x ratios, it is independent of the effects of outliers or the overall distribution of ratios. As implemented in the criterion document, median ratios were assumed to be representative of the linear relationship between the concentration in tissue y to the concentration in tissue x. This assumption was tested with a pre-screening procedure using conventional linear regression. If the regression model had a positive slope and was statistically significant at $P<0.05$, then the relationship was assumed to be positive and linear, and a median ratio was used as representative of that linear relationship.

A log-log regression includes both a slope and a y-intercept. Because they apply to log space, these parameters mean something different than similar parameters in arithmetic space. Linear relationships in log space translate back to power functions in arithmetic space. That is, the log space straight line function:

$$\log(EO) = m \cdot \log(WB) + b \qquad (1)$$

translates to:

$$EO = a \cdot WB^{m} \qquad (2)$$

where the coefficient $a=10^{b}$. The log-log plot intercept b represents the value of log EO when WB=1 mg/kg (that is when log(WB)=0).

The key point when comparing log-log regression to the median ratio approach is that when log-log slope m=1, then Equation 2 reduces to a simple direct proportion EO = a·WB in arithmetic space**.** Figure N-1 illustrates the behavior of CF (that is, the ratio EO/WB), depending on whether the log-log slope m of the plot of log(EO) versus log(WB) has a value 1, >1, or <1.

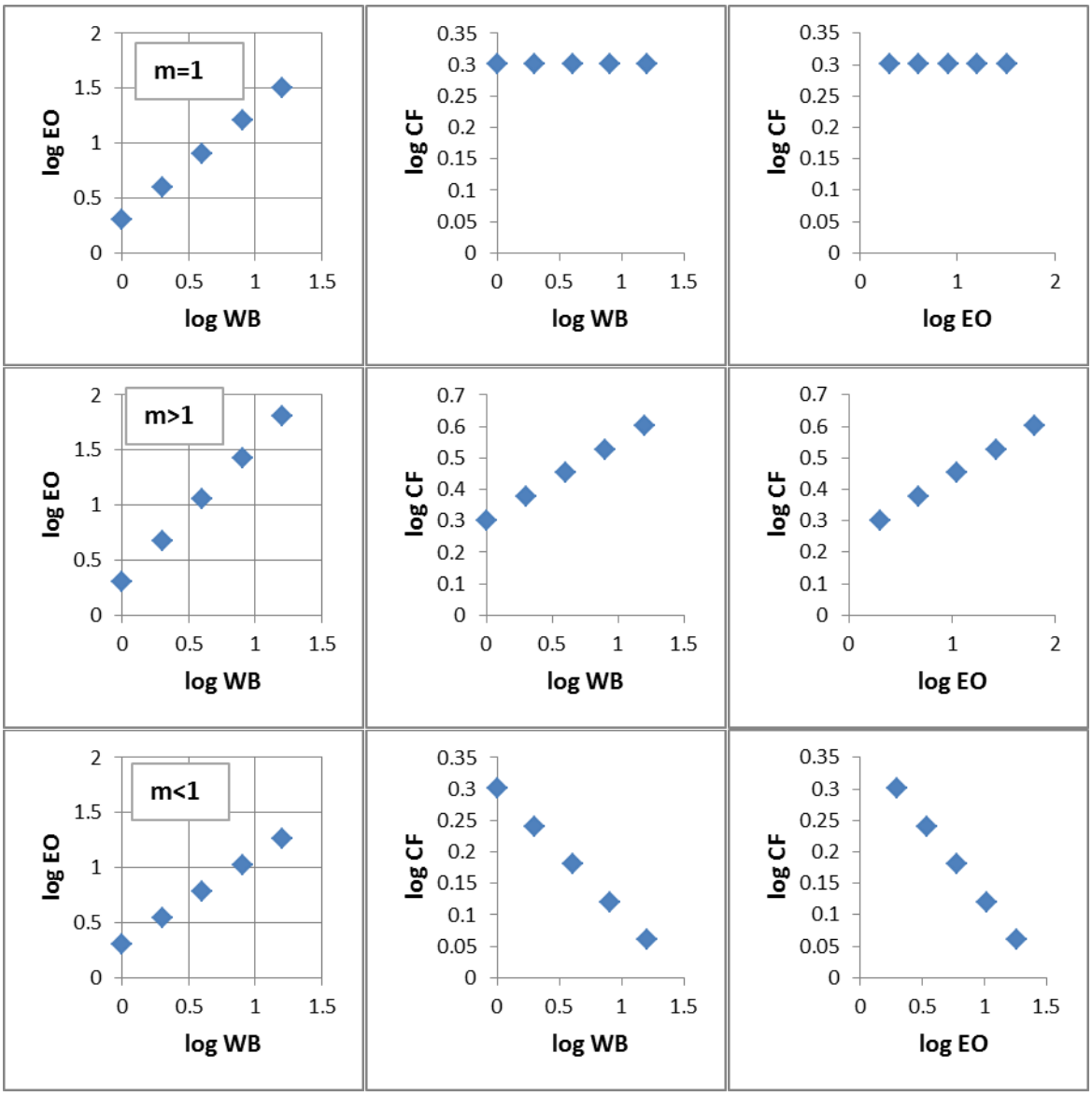


Figure N-1. Idealized illustration (sans scatter) of the effect of log-log slope, m, on whether CF is stable, increases, or decreases with concentration (whether measured as WB in column 2 or EO in column 3).

Top row: m=1. Second row: m=1.25. Bottom row: m=0.8. In all cases, CF=EO/WB, but the three rows were not designed to yield the same median CF. Were the five idealized data points replaced by a large number of well-behaved real-world data points, the straight lines would tend to be replaced by oval clouds of points having the illustrated slopes.

When the log-log slope m≈1, CF does not change with concentration. In that case, CF is the simple proportionality constant as assumed in all previous versions of the criterion document. When m is noticeably different from 1, CF changes with concentration, and we would solve for its value at the EO criterion concentration. If the EO criterion concentration is not near the median EO value in the graphed data, then the regression-calculated CF value may differ somewhat from the median CF.

While both a median ratio and a linear regression account for all of the plotted values within a particular relationship, the regression model is derived from the specific locations of every data point, whereas the median is derived independent of the specific distribution of the data points. In this way, a regression contains more information about the entire data distribution, and as such, is more affected by deviations from linearity. This second point can be an advantage or a disadvantage, depending on the data distribution. For some CF relationships in the database, there is evidence of slight non-proportionality, in which the y/x ratios at higher concentrations are different than the y/x ratios at lower concentrations. In these instances, a log transformation of the tissue concentrations will serve to better linearize the overall relationship, so that the resulting regression model will better capture the y/x relationship across the full concentration range. The median ratio of these models will be the same regardless of whether or not the data are transformed. However, because the use of median ratios is based on the assumption of proportionality, CFs calculated using regression of log transformed values will provide slightly more accurate representations of the relationship across the full concentration range than a median ratio, for those datasets that show some evidence of non-proportionality. An exception would be a case where the midpoint of the data distribution, where the median ratio is more likely to be located, is similar to the criterion concentration. In these instances the median ratio would be expected to be similar the regression based CF regardless of slope. Finally, for those datasets that do not show this effect, selection of either the median ratio or the regression based CF approach are both equally valid approaches.

Another source of uncertainty can occur for species with a CF derived from a narrow concentration range that does not encompass the criterion concentration. In these instances, the slope of the regression model may not be representative of the slope had there been concentrations bracketing the criterion. Similarly, the median of the concentration ratios within this small range may not be representative of the median ratio if there had been concentrations bracketing the criterion. However, it may be preferable, or "safer", to use a non-parametric median rather than the result of an extrapolated regression equation, particularly when the regression is based on few data points (no matter how good r^2 is).

To conclude, CFs calculated from median ratios have the advantages of simplicity, being easier to explain and implement, and they are "safer" in the sense that they are not affected by outliers or the distribution of variance across the data range. CFs calculated from log regression include more

information about the entire data distribution, but can be sensitive to outliers. CFs calculated from the two approaches can diverge in cases where the data range does not encompass the criterion concentration, particularly in cases those where the log transformed slope is also much greater or less than one. Overall, the median ratio and log regression approaches generate similar CFs for this dataset, and have little effect on the translated water criterion elements.

In general, as indicated by the idealized data in Exhibit A, the median and the TLS regression-predicted CF will be similar under either of the following conditions: (a) log-log EO/WB slope near 1.0, or (b) criterion near the middle of the observed data range of tissue concentrations for the species. They are likely to differ from each other when both of the following conditions simultaneously occur: (c) log-log slope distant from 1.0, and (d) criterion distant from the center of the data range.

2.0 COMPARISON OF THE ORDINARY LEAST SQUARES AND TOTAL LEAST SQUARES REGRESSION APPROACHES

The calculation of conversion factors using linear regression following log transformation addresses the issues associated with non-proportional relationships between Se in different tissues, and is the approach recommended by several public commenters. Conventional ordinary least squares (OLS) regression results can vary depending on which tissue type is assigned to the x and y axis, respectively. This is because OLS regression assumes that the variable on the y axis is dependent on the variable on the x axis, and the resulting regression is the line that minimizes the sum of the squared distances between observed y-values and predicted y-values. OLS regression assumes that the values on the x-axis have no uncertainty. For datasets such as the paired tissue concentrations used to calculate CF, there is no dependency between the selenium concentrations in one tissue type to another tissue type, and concentrations in both tissue types are equally uncertain. Because of this, we could assign either tissue type to either axis, and the resulting CF would be slightly different. By convention, we assign egg-ovary to the y-axis when comparing it to whole-body or muscle, and we assign muscle to the y-axis when comparing it to whole-body, because these are the ratios used in the translation equations. While CFs using median ratios are not affected by axis assignment, CFs using OLS are, for reasons described above.

An alternative regression approach that corrects this issue is total least squares (TLS) regression. TLS regression is preferable to OLS regression in cases where there is error associated with each of the variables, and there is no dependency of one variable on the other. With TLS, the regression is the line that minimizes the sum of the squared distances between observed predicted x- and y-values, and produced the same result regardless of which variable is assigned to which axis. Curves drawn by eye tend to mimic TLS, not OLS. Without thinking about it, the person drawing the line naturally attempts to

minimize both vertical and horizontal errors. However, a significant disadvantage of TLS regression is that Excel has no built-in function to perform it, and many readers will be unfamiliar with it.

Table N-1 shows the effect of the different calculation procedures (median ratio, log OLS regression - xyOLS, log OLS regression with reversed axes - yxOLS, and log TLS regression) on all directly measured CFs. Median ratio CFs tend to diverge from regression based CFs for datasets where log-log slopes are markedly different than 1 and the criterion is not near the center of the observed concentrations. CFs calculated from TLS regression nearly always fall between CFs calculated from OLS regression with and without the axes reversed, and are not affected by axis order.

	Directly calculated conversion factors for each tissue ratio, by method											
	EO/WB				M/WB				EO/M			
Species	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS
bluegill	2.13	1.90	2.04	1.98	1.32	1.36	1.37	1.36	1.38	1.11	1.24	1.18
bluehead sucker	1.82	1.41	1.50	1.45	1.23	1.70	1.67	1.59	1.48	0.82	0.91	0.85
brook trout									1.09	0.96		0.99
brown trout	1.45	1.53	1.77	1.74								
common carp	1.92	1.62	1.63	1.62	1.61	1.36	1.41	1.45	1.14	1.06	1.18	1.14
creek chub	1.99	2.05	2.01	2.03								
cutthroat trout	1.96	1.37	1.67	1.48					1.81	1.97	1.83	1.89
desert pupfish	1.20	1.14	1.14	1.14								
dolly varden									1.26	1.64	1.52	1.59
fathead minnow	1.40	1.71	1.56	1.64								
flannelmouth sucker	1.41	1.14	1.84	1.49	1.46	1.94	1.89	1.85	1.08	0.51	1.06	0.69
green sunfish	1.45	1.35	1.45	1.40	1.23	1.28	1.32	1.24	1.21	1.08	1.17	1.12
mountain whitefish									5.80	10.47	4.98	7.35
northern pike									1.88	1.65	1.78	1.70
rainbow trout									1.92	1.82	1.88	1.82
razorback sucker									2.31	1.93	1.89	1.90
roundtail chub	2.07	2.22	2.26	2.24	1.05	1.08	1.10	1.05	2.04	1.99	2.10	2.06
smallmouth bass	1.42	1.31	1.68	1.52	1.23	1.88	1.97	1.68	1.19	0.67	0.88	0.72
white sturgeon									1.33	0.97	1.07	1.01
white sucker	1.38	1.02	1.25	1.12	1.34	1.43	1.54	1.45	1.00	0.59	0.84	0.67

Table N-1. Comparison of all directly calculated conversion factors by method.
Methods include median ratio (Ratio), log ordinary least squares (xyOLS), log ordinary least squares with axes reversed (yxOLS), and log total least squares (logTLS). Regression based CFs were calculated at the egg ovary criterion concentration of 15.1 mg/kg. Muscle to whole body (M/WB) CFs were calculated at the muscle concentration at the egg-ovary criterion.

The following examples illustrate the differences between OLS and TLS regressions, and the effect of axis assignment on CF.

2.1 Example 1 – Flannelmouth Sucker (Egg-ovary/Whole-body)

CF by approach: (1.41 –median ratio, 1.13 – log OLS, 1.86 – log OLS with reversed axes, 1.48 – log TLS)

Model comparison 1a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log (Egg-ovary) = (0.7966 x (log Whole-body)) + 0.2857

log (Whole-Body) = (log (16 mg/kg) – 0.2857)/0.7966 = 1.153

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.13

TLS: log (Egg-ovary) = (0.9877 x (log Whole-body)) + 0.1843

log (Whole-Body) = (log (16 mg/kg) – 0.1843)/0.9877 = 1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

In Figure N-2, the fitted regression lines do not appear particularly divergent; however, these points cover a relatively narrow (and low), concentration range. At the criterion concentration (log E/O = 1.204), the predictions lines are more divergent, resulting in the differences between the CFs. Also, note that the TLS slope is close to 1. The resulting TLS-derived CF is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in a CF for the OLS model that is notably different than the median ratio CF (1.13 vs 1.41).

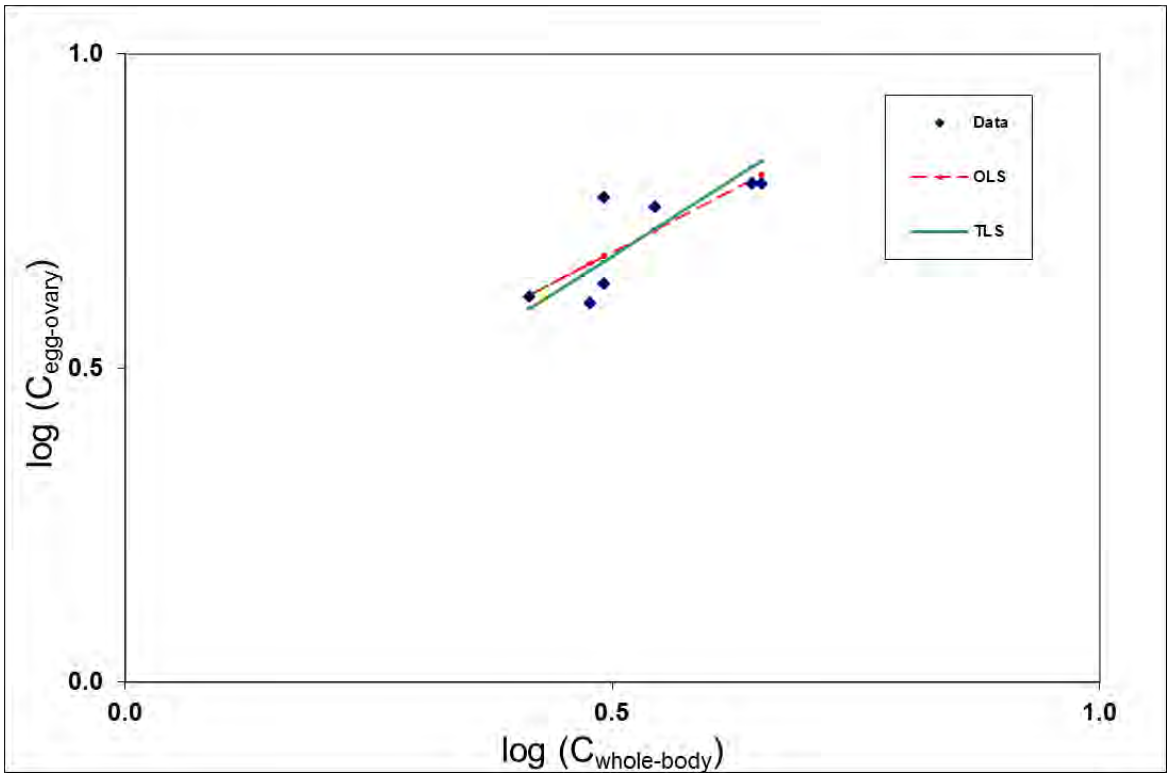


Figure N-2. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 1b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.8126 x (log Egg-ovary)) - 0.0450 = 0.9335

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.86

TLS: log(Whole-body) = (1.012 x (log Egg-ovary)) - 0.1866 =1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

At first glance, Figure N-3 appears very similar to Figure N-2. However, note that the axes are reversed, and because we are now solving for y (whole body concentration at egg-ovary criterion concentration), the shallower slope of the reverse OLS figure results a whole body concentration at the egg-ovary criterion lower than in the upper figures, which in turn results in a larger CF. Also, note that the TLS model is a mirror image of the model in Figure N-3, and as such has the same calculated CF. As above, the TLS slope is close to 1, with a TLS-derived CF that is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in an OLS-derived CF that is notably different than the median ratio CF (1.86 vs 1.41).

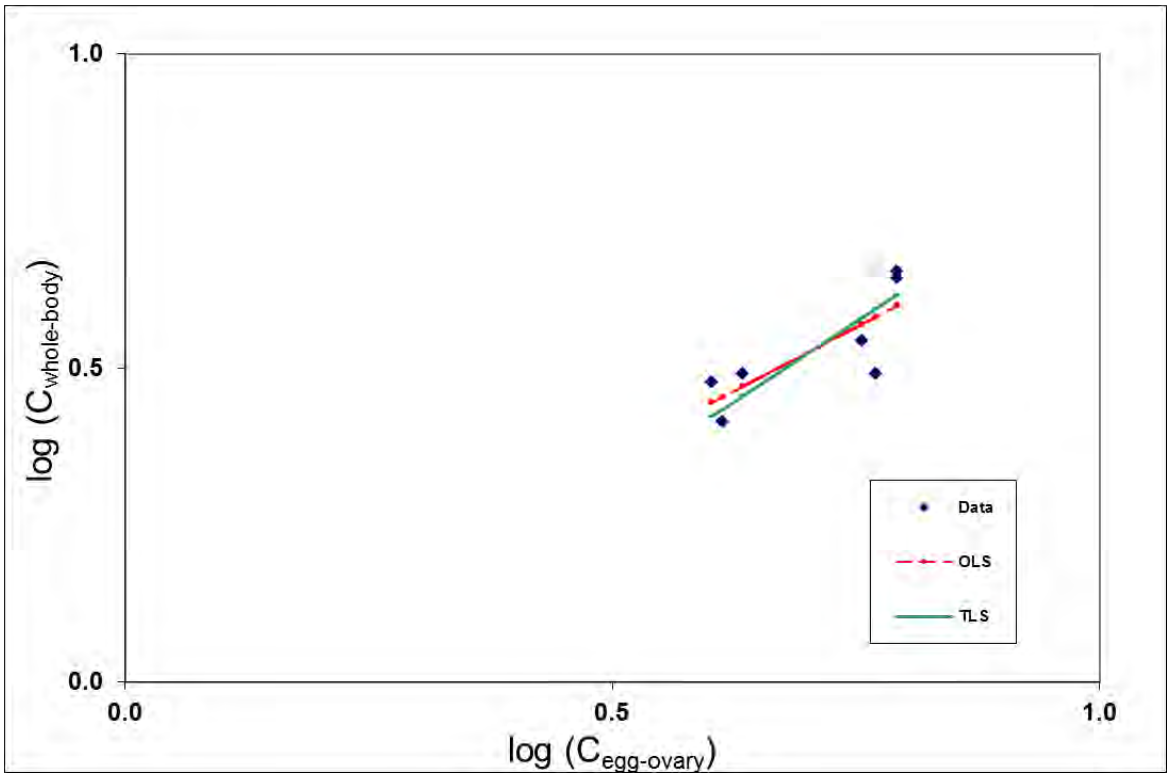


Figure N-3. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

2.2 Example 2 – Bluegill (Egg-ovary/Whole-body)

CF by approach: (2.13 –median ratio, 1.90 – log OLS, 2.07 – log OLS with reversed axes, 2.01 – log TLS)

Model comparison 2a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log(Egg-ovary) = (1.061 x (log Whole-body)) + 0.2227

log (Whole-Body) = (log (16 mg/kg) – 0.2227)/1.061 = 0.9250

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.90

TLS: log(Egg-ovary) = (1.240 x (log Whole-body)) + 0.0.0861

log (Whole-Body) = (log (16 mg/kg) – 0.0861)/1.240 = 0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly larger calculated CF (Figure N-4). Even though the slopes are larger than 1, the data range encompasses the criterion concentration, which is close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

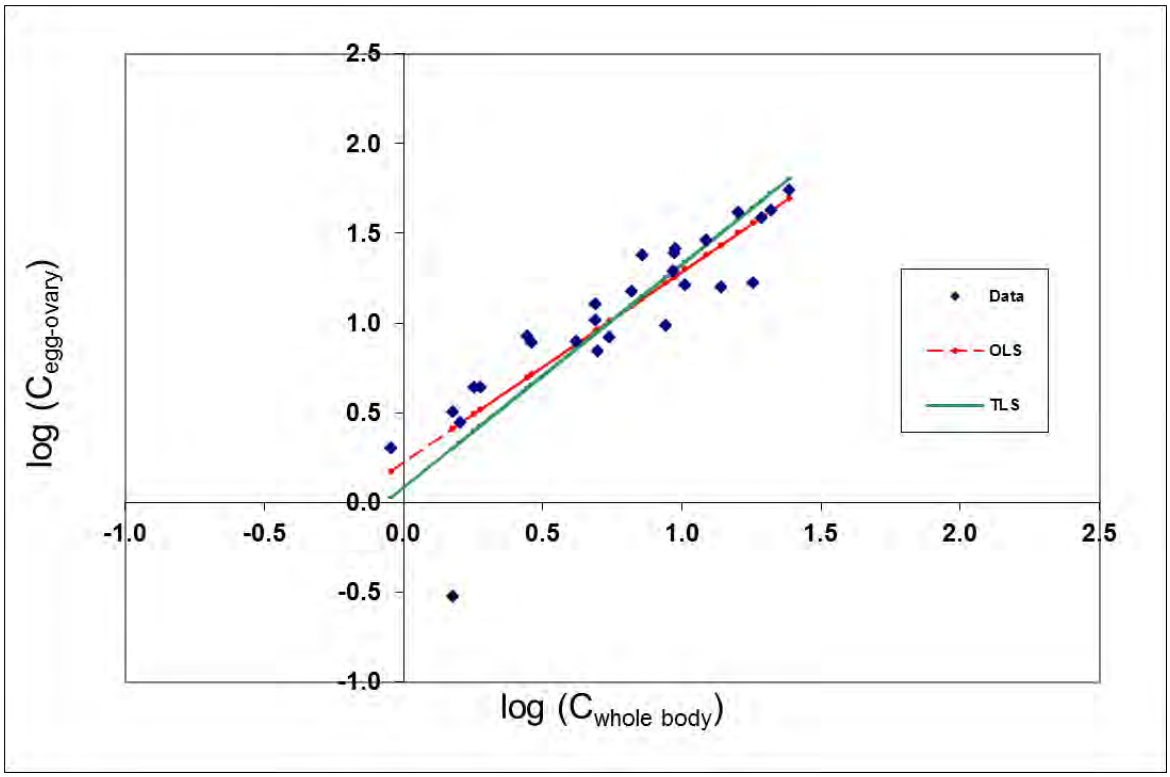


Figure N-4. OLS vs. TLS regression model fits for bluegill. Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 2b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.7269 x (log Egg-ovary)) + 0.0129 = 0.8883

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.07

TLS: log(Whole-body) = (0.8066 x (log Egg-ovary)) - 0.0695 =0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly smaller calculated CF (Figure N-5). Even though the slopes are less than 1, the data range encompasses the criterion concentration, which is also close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

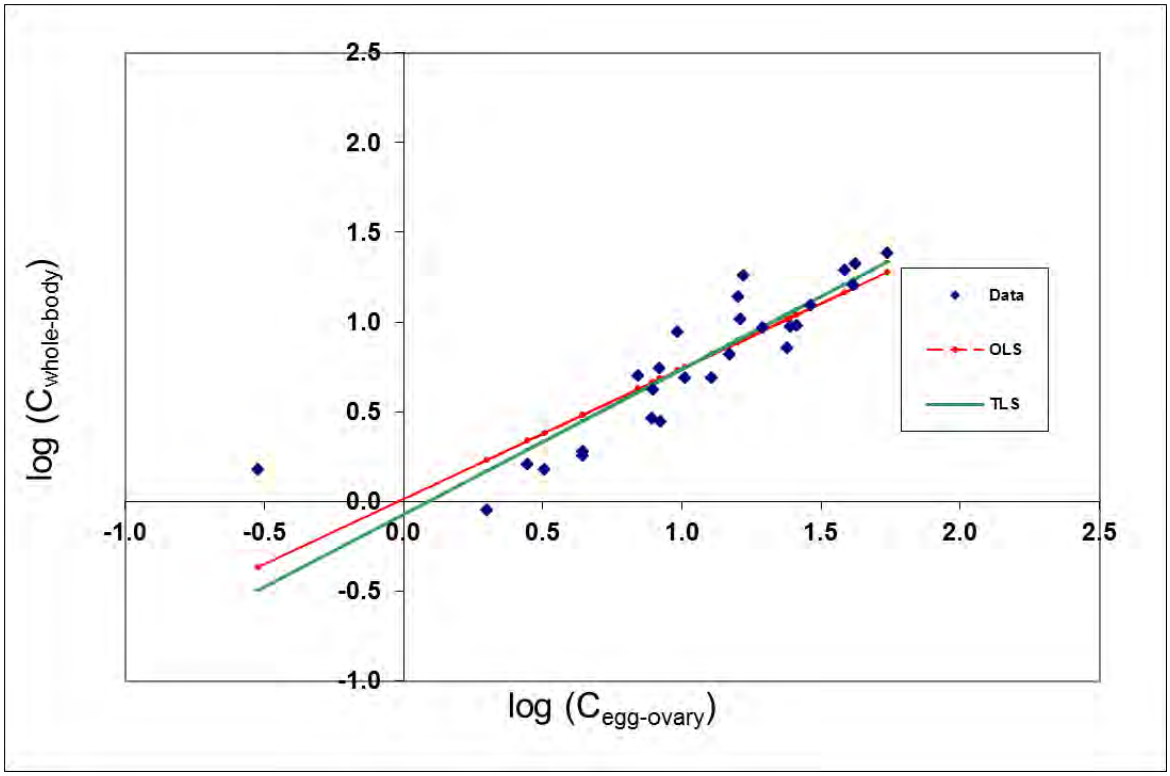


Figure N-5. OLS vs. TLS regression model fits for bluegill.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

The effect of the CFs calculated by the different approaches has a minor effect on the final translated water criterion elements. Compared to the median ratio method, translated water criterion element concentrations are slightly higher using CFs calculated from the log OLS regression methods, CFs calculated from the reverse axis log OLS are slightly lower. Lentic translated water criterion element

concentrations are the same using CFs from median ratios and log TLS regression methods, while lotic concentrations calculated from log TLS CFs are slightly lower compared to those calculated using median ratio CFs (**Table N-2**).

Table N-2. Translated water concentration criterion element criterion concentrations by CF calculation method.

Method	Lentic (µg/L)	Lotic (µg/L)
Median Ratio	1.5	3.1
log OLS regression	1.6	3.3
inverse log OLS regression	1.4	2.9
log TLS regression	1.5	2.9

3.0 COMPARISON OF MEDIAN- AND REGRESSION-BASED CONVERSION FACTORS TO CALCULATE CHRONIC VALUES FOR MUSCLE AND WHOLE BODY TISSUES

Besides being used in the translation of the egg-ovary (EO) tissue criterion to water, conversion factors (CF) were also used to convert egg-ovary (EO) chronic values (CV) to muscle or whole body tissue concentrations. These conversions were done when the data from a reproductive toxicity study did not have muscle or whole body selenium concentrations or if the latter tissue data was not usable to determine a chronic value. Directly calculated CVs using either muscle or whole body selenium measurements from a study was preferred over converted CVs in the determination of the final chronic value (= criterion).

Table N-3 provides a comparison of median-based and regression-based CFs when they are used to convert an EO selenium concentration to muscle or whole body. Regression-based CFs used total least squares (TLS) regression for the reasons stated above. The table lists each taxon in the reproductive toxicity data set and presents CVs that are either directly calculated or converted from the EO CV using either the median or TLS CF. Generally, the median-based and TLS-based CFs were similar for both tissue types and this similarity resulted in similar criteria (bottom row). The muscle criterion for the data set that contained directly calculated CVs and converted CVs was similar whether median or TLS CFs were used, 11.3 and 10.6, respectively. The whole body criterion was also similar using these two approaches, 8.5 and 9.6, respectively. The median-based CFs were selected based on reasons stated in the previous section.

Table N-3. Comparison of muscle and whole body chronic values when calculated directly and converted from egg-ovary concentrations using median- and TLS regression-based conversion factors.

Taxon	EO CV	Muscle chronic values (CV) and conversion factors (CF)						Whole body chronic values (CV) and conversion factors (CF)					
		Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV	Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV
Salvelinus	56.22	44.48	35.36	1.26	44.48	1.59	35.36	34.92	24.34	1.61	34.92	2.31	24.34
Esox	34	21.70[d]	21.70[d]	1.88	18.13	1.70	20.00	14.23	13.77	2.39	14.23	2.47	13.77
Cyprinodon	27	28.72	34.18	0.94	28.72	0.79	34.18	22.50	23.68	1.20	22.50	1.14	23.68
O. mykiss	24.5	12.79	13.46	1.92	12.79	1.82	13.46	10.04	9.28	2.44	10.04	2.64	9.28
O. clarkii, Rudolph	24.7	16.6[d]	16.6[d]	1.81	13.65	1.89	13.07	12.60	16.69	1.96	12.60	1.48	16.69
O. clarkii, Nautilus	27.7	15.30	14.66	1.81	15.30	1.89	14.66	14.13	18.72	1.96	14.13	1.48	18.72
Oncorhynchus	25.31	14.28	14.49	NA	13.59	NA	13.65	11.58	12.81	NA	11.58	NA	12.81
Micropterus	26.3	22.16	36.53	1.19	22.16	0.72	36.53	18.52	17.30	1.42	18.52	1.52	17.30
L. macrochirus, Coyle	26.3	19.13	22.29	1.38	NA	1.18	NA	8.6[d]	8.6[d]	NA	NA	NA	NA
L. macrochirus, Doroshov	22.6	15.7[d]	15.7[d]	NA	NA	NA	NA	10.61	11.41	2.13	NA	1.98	11.41
L. macrochirus, Hermanutz	14.7	13.4[d]	13.4[d]	NA	NA	NA	NA	10.6[d]	10.6[d]	NA	NA	NA	NA
Lepomis	20.60	15.91	16.74	1.38	14.98	1.18	17.45	9.890	10.13	2.13	9.656	1.98	10.40
Salmo	21	18.50	17.50	1.14	18.50	1.20	17.50	13.2[d]	13.2[d]	1.45	14.48	1.74	12.07
Acipenser	15.6	11.9[d]	11.9[d]	1.33	11.73	1.01	15.45	9.209	10.68	1.69	9.209	1.46	10.68
Criterion	**15.10**	**11.34**	**11.57**	NA	**10.99**	NA	**13.35**	**8.538**	**9.567**	NA	**8.189**	NA	**9.879**

[d] directly calculated from muscle or whole body selenium concentrations

Appendix B

Fish Tissue Sampling Standard Operating Procedure

February 25, 2015

WQPBWQS-29

Approvals:

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ACRONYMS

Acronym	Definition
CFR	Code of Federal Regulations
COC	Chain of Custody
CVM	Center for Veterinary Medicine (FDA)
DEQ	Department of Environmental Quality (Montana)
DELTs	Deformities, eroded fins, lesions, and tumors
EDP	EQuIS Data Processor
EPA	Environmental Protection Agency (U.S.)
FDA	US Food and Drug Administration
FWP	Fish, Wildlife & Parks (Montana)
FWS	Fish & Wildlife Service (US)
HASP	Health and Safety Plan
HAZWOPER	Hazardous Waste Operations and Emergency Response
HUC	Hydrologic Unit Code
ID	Identification
INAD	Investigational New Animal Drug
JHA	Job Hazard Analysis
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
SAP	Sampling and Analysis Plan
SDS	Safety Data Sheet (formerly Material Safety Data Sheet)
SOP	Standard Operating Procedure
T/E	Threatened or Endangered
TMS	Tricaine methanesulfonate
U.S.	United States
USGS	United States Geological Survey
WQPB	Water Quality Planning Bureau (DEQ)
WQS	Water Quality Standards

DEFINITIONS

Word	Definition
Cervical Dislocation	Separation of the spinal column from the skull and/or brain.
Chain-of-Custody	Chain of custody (COC) is the protocol that provides a record of the persons having control of, and access to, a sample.
Composite Sample	A homogeneous mixture of samples from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample (U.S. Environmental Protection Agency, 2000).
Contaminant Reduction Zone	The location at a hazardous waste site where decontamination occurs.
Data Quality Objective	A statement of an acceptable quantitative criterion relative to the quality and quantity of the data to be collected and the ultimate use of the data.
Exclusion Zone	Also called the "hot zone," the area with the known suspected contamination and therefore the highest potential for exposure to hazardous substances.
Exophthalmia	An abnormal physical condition characterized by marked protrusion (bulging) of the eyeballs.
Formaldehyde	A colorless flammable gas with a pungent odor that is highly reactive with many substances and used as the basis of Formalin preservative solution.
Formalin	A solution of formaldehyde gas in water containing a certain percentage of methanol as a stabilizer; used as a disinfectant and as a preservative of biological samples
Health and Safety Plan	A document or collection of documents the objective of which is to assure that all work conducted as part of the project is done as safely as possible and with full consideration and awareness of potential risks.
Ichthyological Taxonomy	A branch of science dealing with the naming and identification of species of fish.
Introgression	The transfer of genetic information from one species to another as a result of cross-breeding.
Medical Monitoring Program	A schedule of periodic medical examinations performed for field personnel with the objective of preventing and identifying any degradation in health due to occupational exposure.
Neoplasm	An abnormal mass of tissue or a tumor.
Quality Assurance Project Plan	A document containing the elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for a project.
Safety Data Sheets	A document required by 29 Code of Federal Regulations (CFR) 1910.1200(g) to be provided by a chemical manufacturer, distributor, or importer the objective of which is to communicate the hazards associated with a chemical to the user.
Spawning	Manual stripping of eggs from live female fish.
Righting Response	The ability of a fish to correctly orient itself (dorsal side up) in water; an indicator of stress level or other conditions.
Taxon	A group of organisms determined by taxonomists to form a distinct unit.
Voucher	A preserved specimen representing selected species and maintained for validating taxonomic identifications and documenting spatial and temporal distributions (U.S. Geological Survey, 2002). May also be in photographic form.

1.0 SCOPE AND APPLICABILITY

Since 1998, the United States Environmental Protection Agency (EPA) has conducted or collaborated in ongoing freshwater fish contamination studies (U.S. Environmental Protection Agency, 2014b). At the drafting of this standard operating procedure (SOP), EPA is updating the national recommended aquatic life criteria for selenium. The updated criteria contain two water-column-based and two fish tissue-based elements, and EPA recommends that delegated authorities adopt all four elements (U.S. Environmental Protection Agency, 2014a). In anticipation of the continuing need for accurate data representing the levels of chemicals found in fish species in Montana, the Montana Department of Environmental Quality (DEQ) has drafted this SOP.

This SOP is intended to serve as a guide for and to assure integrity and consistency in the collection of tissue samples, including whole fish, fillet, and biopsy plugs, from fish populations in Montana waters by or for DEQ, and it should be referenced in development of project planning and design documents. Sample design elements, fish capture and collection methods (electrofishing, netting, etc.), and laboratory methodologies for analysis of chemicals in fish tissue samples are beyond the scope of this SOP.

2.0 FIELD SAMPLE COLLECTION METHODS

Sample collection procedures must be followed diligently to ensure the integrity of the information used in reporting and upon which any actionable decisions are based. Methods outside those described herein may be appropriate or necessary on a case-by-case basis, but any such deviations from the following methods should be included in event planning documents if anticipated and otherwise noted in sampling event documentation (e.g. logbooks) and final reporting.

2.1 PERSONNEL QUALIFICATIONS AND TRAINING

Fish tissue sampling teams must be staffed with personnel experienced in ichthyological taxonomy and fish handling, tissue sampling, and sample preparation and preservation. At a minimum, the sampling team should be composed of a fisheries biologist knowledgeable in local fish species, a field technician experienced with these sampling protocols, and a quality control person if the biologist and technician do not have the expertise to serve this role (Tetra Tech, 2000). Field personnel should be in adequate physical condition for the performance of sampling work in potentially adverse field conditions including extremes of heat, cold, elevation, precipitation; near dangerous wildlife; etc. **Note:** if the personnel performing fish specimen collection do not include a Montana Fish, Wildlife and Parks (FWP) representative, a fish collection permit must be obtained from FWP for the project.

All team members should be provided with a copy of any project documents pertinent to their role including but not limited to the sampling and analysis plan (SAP), the quality assurance project plan (QAPP), this SOP, field documentation and chain of custody (COC) forms, and sample labels. Team members should ensure they read and are familiar with the SAP, QAPP, and SOP. Personnel must be familiar with any health hazards associated with fixing or preserving agents to be used. Safety Data Sheets (SDS) for all chemicals used in field operations must be available to sampling team members. If

sampling will be performed in an exclusion or contaminant reduction zone of a hazardous waste site, sampling personnel are required to have Hazardous Waste Operations and Emergency Response (HAZWOPER) training.

Due to time or resource constraints, it may only be feasible to have one sampling team member fully trained or certified in all areas; typically this would be the team leader. However, pre-field orientation or training sessions for sampling team members should be conducted whenever possible. These sessions should emphasize strict adherence to sampling protocols, health and safety measures, and nuance specific to the project and should include hands-on activity in addition to classroom instruction. During field sampling activity, inexperienced sampling team members should be accompanied by technically proficient personnel until such time as they are able to perform tasks adequately on an individual basis.

2.2 HEALTH AND SAFETY

Personnel health and safety must be the highest priorities for any activity performed for DEQ, and these priorities are everyone's responsibility. Fish tissue sampling tasks are likely to occur in the vicinity of surface waterbodies and possibly in harsh outdoor environments. In such instances, refer to **Section 3.3** of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring," (Montana Department of Environmental Quality, 2012). If a health and safety plan (HASP) is available for the project or site, it should be reviewed and adhered to by sampling team personnel. The sampling team leader or project planner should complete the Job Hazard Analysis (JHA) form (WQPB-JHA-01), including project-specific information. All sampling team members must read and sign the form, and the form must be signed by a supervisor.

Sampling team members with potentially incapacitating medical conditions (e.g. MedicAlert medical issues) should provide enough information regarding such condition(s) to their team leader prior to entering the field to allow them to be prepared in the event of an emergency. Participation in a medical monitoring program may be appropriate if known or suspected hazardous materials will be encountered; however, it is not typically necessary for fish tissue sampling work. Additional safety precautions that should be taken specific to fish tissue sampling include the following:

- Proper handling of chemical fixatives (e.g. formaldehyde and formalin) to prevent inhalation or dermal contact issues;
- Safe use, storage, and transport of sampling sharps such as fillet knives, biopsy punches, and fish scalers;
- Use of gloves or pliers, as appropriate, to prevent cuts, punctures, or lacerations when handling fish species having sharp fins, spines, and teeth;
- Awareness of dangerous local wildlife that may be attracted to fish sampling activities; and
- Following correct lifting practices for moving heavy objects such as full sample coolers.

2.3 EQUIPMENT AND FIELD SUPPLIES

Attachment A of this SOP is a list of equipment and supplies specific to fish tissue sampling events that is intended to supplement field work checklist items such as those found in the general sections of **Attachment A** - Field Supply List of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring" (Montana Department of Environmental Quality, 2012).

Additions to or subtractions from this list may be appropriate dependent upon specific project objectives and conditions. Equipment and supplies should be inventoried and checked for condition and functionality prior to departure for the field and upon return. Restock, repair, and replacement of equipment as appropriate should occur immediately following field work to aid planning and scheduling for the next sampling team and/or event. Sample containers and labels should be prepared prior to sampling, to the extent possible, to increase efficiency in the field and reduce holding times.

2.4 FISH HANDLING

Maintaining sample integrity from the point of collection through sample processing and analysis is critical to meeting data quality objectives. All potential sources of extraneous contamination should be identified before sample collection and handling, if possible, and steps should be taken to ensure their elimination or minimization (Tetra Tech, 2000). Fish should be handled using clean nitrile gloves at all phases of sample processing.

Whether whole fish, fillet, biopsy punch sampling, and/or compositing will be employed, fish-to-sample processing should occur as quickly as possible following collection of fish from the waterbody. Despite efforts to limit handling and stress to fish, some mortality is likely. Mortality can be minimized by recognizing which species are sensitive to handling and prolonged confinement and processing them before others (U.S. Geological Survey, 2002). Shorter processing duration will typically result in increased sample integrity and survival rates for no-kill sampling specimens. **Threatened/Endangered (T/E) species should be released immediately upon capture unless included in the project, in which case they should be processed first and returned to the water as quickly as possible.**

2.4.1 Lethal vs. No-Kill

Lethal fish tissue sampling techniques may be appropriate in circumstances where determination of effects on aquatic life or human health warrant whole fish, fillet, or target tissue sampling. In such cases, fish should be euthanized as humanely as possible by anesthetizing or delivering a sharp, forceful blow to the head using a foil wrapped or inert, decontaminated instrument, followed by cervical dislocation while taking care not to break the skin. The force of the blow should be similar (slightly less) to that needed to drive a nail into wood (Erway et al., 2004). If this blunt trauma method is not feasible, asphyxiation by wrapping in foil and placing in a plastic bag on ice is an alternative (Parametrix, Inc., 2009) or an overdose of anesthetic may be administered.

When lethal sampling methods are not necessary or not possible (e.g. T/E species), the no-kill, biopsy punch method may provide data that meets quality objectives for certain analytes. This method involves inserting a biopsy punch into a de-scaled muscular section of a live fish. Following sample extraction, an antibiotic salve is placed over the excised area and the fish is released. In addition to eliminating the need to euthanize fish, biopsy punch sampling uses clean equipment and supplies each time a sample is collected, thus providing some reduction in risk of sample contamination (Syracuse Research Corporation, 2003). Mercury analysis concentration results have been demonstrated to be similar to fillet sample results (within 6%) under certain analysis techniques without causing fish mortality (Baker et al., 2004). See **Section 3.2.2** for details on performing biopsy punch sampling.

2.4.2 Anesthetizing

Anesthesia can facilitate sampling operations such as taking fish measurements and biopsy punch samples and may also lessen injury and/or mortality to fish. The following substances have been found to be effective anesthetizing agents for fish:

- Tricaine methanesulfonate (commonly as Tricaine methanesulfonate (TMS), MS-222, or Tricaine-S®) – TMS has been approved by the U.S. Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) for aquaculture purposes. Though an effective anesthetic, TMS has a very long withdrawal time (21 days) potentially making its use for field work impractical (Bowker et al., 2011). TMS should be used in accordance with the manufacturer's instructions.
- Eugenole (the active ingredient in clove oil and AQUI-S® E/20E) – AQUI-S® E and AQUI-S® 20E have been approved by FDA for use through the U.S. Fish and Wildlife Service's (FWS) Aquatic Animal Drug Approval Partnership (AADAP) as an Investigational New Animal Drug (INAD) on fish that will be released to public waters. AQUI-S® E contains 50% eugenole and AQUI-S® 20E contains 10% eugenole (U.S. Food and Drug Administration, 2015). The withdrawal period for AQUI-S® 20E is 72 hours (Bowker et al., 2011). AQUI-S® E/20E should be used in accordance with the manufacturer's guidelines and its FDA approval. Clove oil is typically 85-95% eugenole (U.S. Food and Drug Administration for Veterinary Medicine, 2007). Though many governmental and non-governmental organizations, including the U.S. Geological Survey (USGS) and FWS, provide information regarding methods of use of clove oil as a fish anesthetic, it has not been approved for use by the FDA.
- Carbon dioxide (CO_2) – CO_2 sedation can be accomplished using sodium bicarbonate at 142 to 642 ppm for 5 minutes, carbonated water, or gaseous carbon dioxide (Walsh and Meador, 1998). CO_2 may not be as effective in inducing deeper stages of anesthesia, making it less effective for sampling involving surgical procedures; however, recovery times are likely to be shorter (Peak, 1998). The following method of anesthesia using CO_2 is adapted from "Revised Protocols for Sampling Algal, invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program" (U.S. Geological Survey, 2002):

    1. Place subject fish in a 19 L (5 gal) bucket containing about 12 L of ambient water. (Only a few fish should be anesthetized at a time to minimize potential mortality resulting from prolonged sedation.)
    2. Add 350 mL of carbonated water or two CO_2-producing tablets to the bucket. Determining the CO_2 dosage in the field can be difficult because by the time the fish have responded to the sedation, the concentration of CO_2 may be too high (overdose can cause mortality). If the concentration is too high (onset of sedation is rapid), the fish should be moved to ambient water or processed immediately.
    3. Leave the fish in the bucket until the desired level of sedation is achieved (about 2 to 5 minutes).
    4. Recovery time for CO_2 anesthesia may be approximately 5 minutes (Peak, 1998).

Following sedation and sampling procedures, recovery time in a holding vessel of ambient water or an instream cage must be provided until full recovery is observed and the necessary withdrawal period has expired. Recovery time may vary depending on species, size, type of anesthetic, and time under

anesthesia. Release should occur downstream of the sampling reach to minimize the likelihood of re-sampling the same specimens.

2.4.3 Fish Welfare

Main stressors on fish during sampling include water quality, water temperature, duration of holding, and stress due to handling (U.S. Fish and Wildlife Service, 2012). Signs of captive fish stress include loss of righting response, gaping, gulping air, and exuding excessive mucus (Joy, 2013). The following handling techniques should be practiced and are important in the assurance of survival when handling T/E species:

- Proper body support of fish during handling (not holding by jaw or covering eyes or gills);
- Using wetted, gloved hands to reduce loss of protective mucus;
- Minimizing time out of the water;
- Ensuring temporary holding vessels are properly sized to fully submerge fish; and
- Taking special care to minimize any unsubmerged time when air temperatures are below freezing. Suspending sampling activity should be considering in such conditions (U.S. Fish and Wildlife Service, 2012).

If sample processing cannot occur immediately, fish should be kept in instream holding cages or aerated live-wells or tanks. If holding tank water cannot be continuously aerated, it should be changed frequently to prevent a stressful or lethal drop in dissolve oxygen level and holding time should be minimized. Some species, such as salmonids, are more sensitive to changes in water temperature and chemistry and parameters such as temperature, pH, salinity, and dissolved oxygen needs to be kept within fish tolerances in holding vessels. Ranges for holding water parameters should be specified in the SAP.

2.5 FIELD MEASUREMENTS AND DOCUMENTATION

Fish measurements associated with tissue sampling that are to be taken in the field will be dictated by the SAP and are likely to include the following parameters: species identification, length, weight, and examination for any external abnormalities. Some measurements, such as length and weight, may be performed by the personnel who capture fish specimens (collection team); alternatively, the personnel who take tissue samples (sampling team) may be the first project participants to take these metrics or it may be appropriate for this work to be duplicated by the sampling team. The SAP may specify a variety of sizes/ages of fish to be sampled depending upon the nature of the work (e.g. determination of a correlation between fish size or age and analyte concentration in fish tissue). If fish will not be returned to the water, euthanizing specimens (**Section 2.4.1**) prior to taking measurements may make field activities more efficient.

Sample collection activity is typically documented via a field logbook, a field sampling form, and a COC form. All field activities and observations should be documented in the field logbook. General information to be recorded in the logbook includes team member names and their affiliations, date of activity, daily activity start and end times, daily weather conditions, sample range or reach, and general accounting of field activities. Additionally, photograph descriptions, names and affiliations of visitors and

time of visit, and any deviations from the SAP and reasons for such deviations should be included in the field logbook. Typical logbook-keeping practices should be followed, including the following:

- Entries are made during activity or as soon after activity as possible;
- Entries are dark and indelible;
- Entries are factual and objective, subjective writing is rarely appropriate;
- Each new day's activities begin on a new page;
- Each page is dated and initialed by the author of the entries on it;
- Corrections are made by drawing a single line through the entry in error, allowing it to remain legible, and the corrector's initials and date of correction are included;
- No pages are removed from the logbook, regardless of legibility or accuracy;
- Field logbooks must be retained in accordance with DEQ Records and Information Management policies; and
- Generally, multiple projects should not share the same field logbook. An exception might be individual sampling tasks that are part of a larger project, in which case, the beginning and end points of the tasks should be clearly denoted.

Detailed sample metrics (see the following sections) will be documented on **Attachment B** – Fish Tissue Sample Form of this SOP. This information should be recorded as soon as possible following sample collection. A completed COC form must accompany each sample shipment package. The COC form may be provided by the sampling laboratory; however, a generic COC form is provided in **Attachment E**. See **Section 3.6** for more information on COCs.

2.5.1 Taxonomy

Ideally, fish will be identified to the species level (and possibly subspecies level, in accordance with the SAP) immediately following collection from the waterbody. Accurate identification is likely to be critical to data quality objectives, particularly if the SAP specifies composite sampling of certain species. Taxonomic identification should be performed by trained personnel knowledgeable of the local fish species. Taxonomic reference books and keys that contain species descriptions, ranges, illustrations, and photographs should be used to make field identifications. The nomenclature used to identify fish species should follow *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* (latest edition), American Fisheries Society, unless otherwise specified in the SAP.

T/E species (unless included in the SAP), non-target species, or fish that do not meet size requirements should be immediately returned to the waterbody upon capture. Collection of a voucher specimen may be appropriate for each species type sampled (see **Section 3.4** for more information on vouchers). Obtaining a voucher is particularly important for any fish that are sampled but for which a positive identification has not been made. Adequate preservation and fixation (see **Section 3.3**) is critical when the laboratory must be relied upon for positive identification of the taxon for a sample.

2.5.1.1 Hybrids

Hybridized subspecies are known to exist among Montana fishes, particularly among the trout species. In such cases, an authoritative determination made by a qualified fisheries biologist or ichthyologist should be made if at all possible. Vouchering will be necessary if a qualified individual is not available in the field. If taxonomy is controversial, nomenclature should default to *Common and Scientific Names of*

Fishes from the United States, Canada, and Mexico (latest edition), American Fisheries Society, unless otherwise specified in the SAP. Genetic sampling may be appropriate if confirmation of hybridization cannot be determined through visual cues or if data regarding the degree of introgression is required.

2.5.2 Length

Total, fork, or standard length (see **Figure 2-1**) of fish specimens should be measured in accordance with the SAP and recorded on **Attachment B – Fish Tissue Sample Form**. To be consistent with the metric used by most U.S. fisheries biologists, total length should be used (U.S. Environmental Protection Agency, 2000).

The following procedures for measuring fish length using a measuring board are adapted from U.S. Geological Survey, 2002.

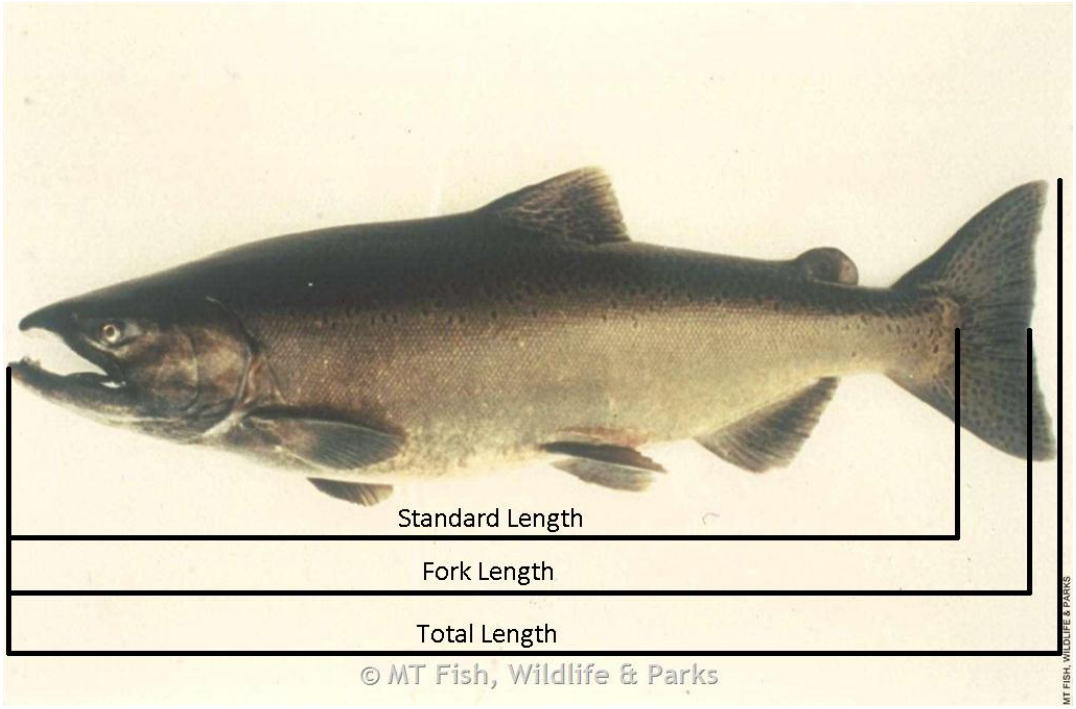


Figure 2-1. Measuring Fish Length.

Determining total length:

1. Place the fish with the body positioned on the right side, the head facing the observer's left, and the mouth closed.
2. Push the anterior end (snout) against the measuring board stop.
3. Measure total length as the distance from the closed mouth to the extreme tip of the caudal fin (see **Figure 2-1**), when the lobes of the caudal fin are compressed dorsoventrally, i.e. "un-splayed." Note: the caudal fin in **Figure 2-1** has not been compressed dorsoventrally.
4. Record total length to the nearest millimeter on **Attachment B** – Fish Tissue Sample Form.

The measuring board should be rinsed with ambient water between uses. It may be efficient to take photo vouchers while specimens are positioned on the measuring board as described in the steps above,

providing the benefit of visual representation of length as part of the voucher. Size requirements should be specified in the SAP; however, when grouping specimens into similar sizes, a general rule is that the smallest individual is at least 75% of the length of the largest individual.

2.5.3 Weight

Fish weights are to be recorded individually for larger fish; however, small species or juveniles may at times be weighed in batches; refer to the SAP for instructions. The following procedures for weighing fish specimens are adapted from U.S. Geological Survey, 2002.

Obtaining weights of individual fish:

1. Level, calibrate, and tare the specimen tray/pan and any covering (e.g. aluminum foil) on the scale.
2. Place the specimen on the scale. If the specimen overhangs the tray, ensure no parts of the specimen are supported by anything other than the balance platform.
3. Record the weight of the fish to the nearest gram on **Attachment B** – Fish Tissue Sample Form.

Obtaining composite/batch weights of fish:

1. Level, calibrate, and tare the specimen tray and any covering on the scale.
2. Place all individuals for a particular composite or batch sample on the scale.
3. Record the count (number of individuals weighed or contributing to the composite) and the total weight (batch weight) to the nearest gram on **Attachment B** – Fish Tissue Sample Form.

Scale calibration must be checked at regular intervals. Use of multiple weighing trays or trays of different sizes may aid weight measuring. Covering the scale with plastic wrap will help protect the instrument from weather and detritus. If samples will be filleted in the field, fillet weights should be measured and recorded as above, whether individually or in batches.

2.5.4 External Anomalies

External anomalies include deformities, erosions (typically in the fins), lesions, and tumors/neoplasms; often referred to as DELTs. DELTs may indicate the presence of sub-lethal environmental stresses, intermittent stresses, behavioral stresses, or chemically contaminated substrates (U.S. Geological Survey, 2002). Documentation of DELTs may be used to indicate a possible correlation between external anomalies and tissue contaminant concentrations; however, development of a statistical relationship may not be intended, appropriate, or possible.

All fish included in the sampling effort should be examined for DELTs and any observed external anomalies should be recorded on **Attachment B** – Fish Tissue Sample Form. Examination should occur while taking fish measurements to minimize handling fish. If possible, photographic record of extreme anomalies should be made. Injuries known or suspected to have occurred during collection (e.g. blackening or exophthalmia from electrofishing (Joy, 2013)) should not be included in records of DELTs for the specimen.

Attachment C – DELTs Examination has been adapted from U.S. Environmental Protection Agency, R10, 2005 and provides external features of fish that should be examined for anomalies. Consult U.S.

Geological Survey, 2002 for more information on external anomalies, including photographic examples of DELTs.

3.0 SAMPLE MANAGEMENT

Sampling of fish tissue involves a comprehensive process beginning with project planning and continuing through the field activities of the collection and sampling teams to the point of shipping to a laboratory for chemical analysis. During sampling activity, samples may pass through many processing stages and the hands of several personnel. The goal of sample management is the maintenance of sample integrity and identity through each stage of these activities, which is paramount to ensuring data quality objectives are met.

3.1 SAMPLE SELECTION

Sample selection criteria should be fully described in and followed strictly from the SAP. Typically, a minimum number of species examples of a certain length and/or weight will be required by the SAP, and species to be sampled may be divided into primary, secondary, or further tiers of targets. For example, all or certain target species of a sampling tier might be divided into three size classes: 1. <15 cm, 2. ≥15 cm – ≤30 cm, and 3. >30 cm. Particularly for composite sampling, the SAP may specify a minimum number of specimens to include in the composite (e.g. five) with enough mass to produce a sample for laboratory analysis (e.g. 500 g). The SAP should include a contingency section with instructions for instances when field conditions do not allow for achievement of the minimum sample selection requirements. Selection of specimens for sampling and categorization in accordance with the SAP is done during identification and field measurement of specimens to maximize sampling efficiency and to minimize handling of live fish. Field measurement procedures are included in **Section 2.5**.

3.2 SAMPLE PREPARATION AND HANDLING

Measures to prevent cross-contamination during sampling procedures are critical to meeting data quality objectives. All sample preparation beyond whole fish collection, non-lethal biopsy punch, or egg collection (i.e. filleting, organ harvesting, and sample compositing) should be performed in a laboratory cleanroom-type environment, free of metals or organics, and utilizing positive pressure filtered air, such as an offsite trailer designated and prepped specifically for sampling purposes. Certain factors such as parameter(s) of concern, large specimen sizes, limited cooler capacity, or lack of suitable staging sites may indicate collection methods such as filleting may be appropriate to perform in the field. However, rationale indicating how data quality objectives will be met under field conditions must be documented in the SAP, and any deviations from the SAP made during field activities must be recorded. Handling, labeling, storing, shipping, and disposal methods applicable to fish tissue samples are included in **Sections 3.4** through **3.6** and must be followed closely. Failure to follow these procedures may result in unusable data results or failure to meet project objectives such as development of translators.

3.2.1 Filleting

The SAP should specify if fillets are to be prepared skin-on or skin-off, which may be dependent upon the chemicals being sampled for. Removal of skin may lower reported concentrations of organic chemicals while retention of skin may lower results for other analytes such as mercury (California

Environmental Protection Agency, 2005). Other factors such as cultural culinary preferences may influence the appropriateness of retention or elimination of other tissue or organs (e.g. fatty belly flap) or sampling specimens whole. Additional rationale for removal or retention of skin and other tissue on fillets can be found in U.S. Environmental Protection Agency (2000).

Fish should be resected into fillets under laboratory clean-room conditions within 48 hours of collection and ideally without freezing of tissue prior to resection. Freezing can cause rupture of neighboring anatomy, potentially contaminating the target tissue. If fillets are to be resected from frozen fish, fish should be thawed only to the point of allowing incision (U.S. Environmental Protection Agency, 2000). Specimens are ready to be filleted when the deepest portions of the target tissue still contain crystals but are no longer fully frozen (Kansas Department of Health and Environment, 2013).

The following steps must be taken in the filleting process:

1. Prior to any resection, all work surfaces (i.e. cutting-boards and tables) and cutting utensils must be washed with a mild detergent solution (phosphate- and scent-free) followed by multiple rinses with deionized water. Cutting boards must then be covered with unused aluminum foil with the dull side exposed.
2. Personnel must don new, nitrile, disposable gloves prior to any contact with specimens.
3. A decontaminated stainless steel, ceramic, glass, or titanium utensil will be used for filleting each specimen.
4. Specimens should be filleted using the procedures in **Attachment D**.
5. Avoid puncturing the gut and internal organs which could contaminate the fillet sample. If such puncturing does occur or the fillet comes in contact with any non-sterilized surface, immediately rinse the fillet with deionized water and record the event on **Attachment B** – Fish Tissue Sample Form.
6. Remove any bones remaining in the fillet following resection unless otherwise directed by the SAP.
7. The aluminum foil surface must be replaced between each specimen and cutting utensils must be decontaminated through deionized water rinsing and detergent use if necessary.
8. Depending on the requirements in the SAP, fillets may be:
    - weighed individually or per fish;
    - separated into routine samples and duplicate samples;
    - immediately homogenized for compositing purposes; and/or
    - wrapped in aluminum foil, labeled (see **Section 3.6**) and dated, placed in a waterproof plastic bag, and frozen at ≤-20°C for later processing (U.S. Environmental Protection Agency, 2000).

3.2.2 Internal Organs

To meet data quality objectives, harvesting of internal organs for sampling purposes can typically only be done in a laboratory clean-room environment. Fish should be dissected as quickly as possible and no more than 48 hours after collection if stored only cooled and not frozen. To remove organs for sampling, steps one through five under **Section 3.2.1** above should be followed carefully, leaving the intestinal tract and internal organs intact. At that point, the internal organs should be exposed. During removal of the organs to be sampled specified in the SAP, the gonads should be examined to determine or confirm the sex of the fish and gross pathology should be noted on **Attachment B** – Fish Tissue Sample Form.

The amount of time tissue samples are exposed to air should be minimized to reduce the chance of contamination and moisture loss.

Figure 3-1 includes a generalized representation of salmonid interior anatomy for reference. Detailed imagery of internal fish organs and methods of dissection can be found in the *Illustrated Field Guide for Assessing External and Internal Anomalies in Fish* (Smith et al., 2002).

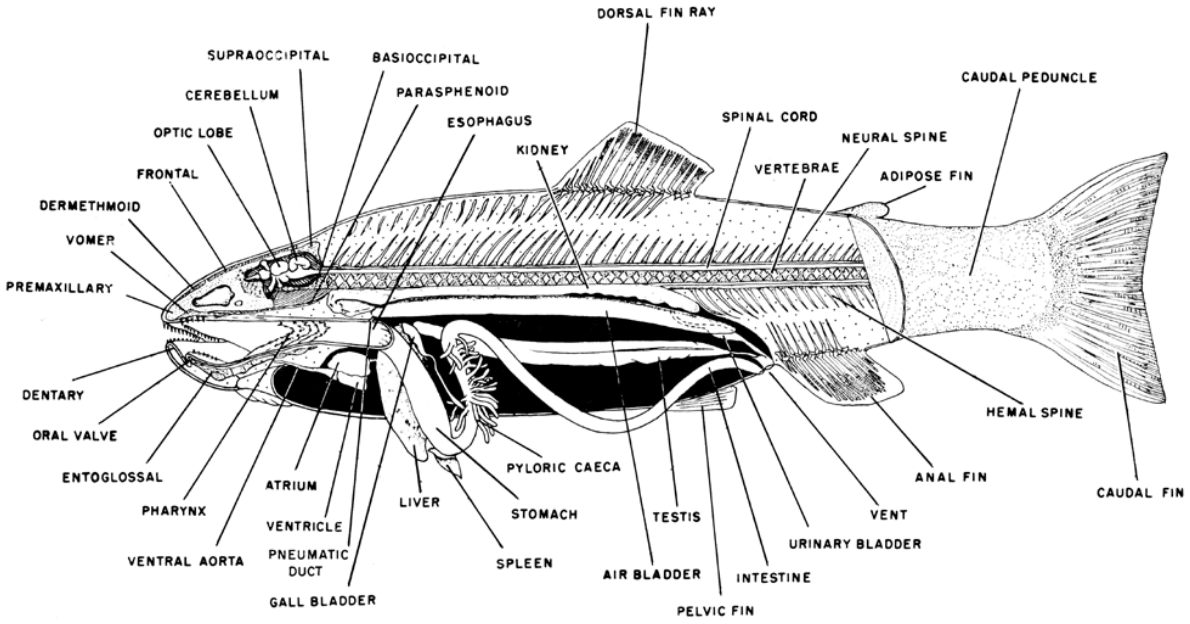


Figure 3-1. Salmonid Anatomy. (Leitritz, 1959)

Following removal and resection, steps seven and eight of **Section 3.2.1** should be followed for organ tissue or as otherwise specified in the SAP.

3.2.3 Eggs

Collection of fish eggs for sampling purposes may be desired for projects investigating concentrations of certain parameters. Field collection activities must coincide with the spawning times of the project's target species. Spawning times for species typical to Montana can be found in "Spawning Times of Montana Fishes," (Skaar, 2001). It may be difficult to achieve sufficient sample mass from small species or immature populations; therefore, SAPs should contain contingencies for these events.

If fish will be sacrificed, eggs can be harvested during dissection for collection of other tissues through the procedures for filleting and organ harvesting described above. Eggs are located along the gonads and typically form in rows. Manual stripping or spawning of eggs from ripe females should provide sufficient mass of eggs for sampling purposes and allow return of fish to the environment. Spawning activities should be performed in a manner that minimizes handling of fish and anesthetics should be used to reduce stress (Piper et al., 1982). Eggs themselves are delicate and can be damaged if fish are handled and/or spawned too roughly (Shrable et al., 1999).

Manually strip eggs as follows:

1. Anesthetize fish if appropriate (see **Section 2.4.2**).
2. Ready clean sample glassware. One large container to collect eggs in and distribute them to smaller containers may be appropriate.
3. Rinse fish in ambient water, particularly in the area of the vent (see **Figure 3-1)** to remove any foreign matter and potential contaminants.
4. Hold the fish about the head with one hand while positioning the vent over the receiving container and tilted slightly downward. Two individuals may be necessary to sample very large specimens–one person holding the fish and one person performing the stripping.
5. Apply gentle pressure with the other hand to the ventral side beginning well behind the pectoral fin and moving slowly toward the vent. Direct the resulting stream of eggs into the sampling container.
6. Room must be left in final sampling glassware for sample expansion upon freezing (Murphy, 2012).
7. Label the samples in accordance with **Section 3.6**, complete **Attachment B** – Fish Tissue Sample Form, freeze the samples as quickly as possible, and store at ≤ -20°C.

If the eggs are not easily stripped with gentle pressure, the fish is likely not ripe. Do not attempt to force stripping with excess pressure as harm to the fish and damage to the eggs may occur. Under this circumstance, the fish should be returned to the water without sampling or it will need to be sacrificed to obtain eggs.

3.2.4 Compositing

Compositing samples allows for cost-effective estimation of average concentrations of analytes in target species and helps ensure adequate sample mass for analysis of a suite of target analytes. However, composite samples by nature will not allow for discovery of the extremes of analyte concentrations in target media. The following guidelines should be followed when compositing fish tissue specimens (whole fish, fillets, biopsy punch, etc.); more details and any deviations from these procedures should be detailed in the SAP:

- Specimens should typically be all of the same species due to species-specific bioaccumulation potential, and of the same sex, if possible.
- Specimens should be collected during the same sampling event and no longer than one week apart.
- Specimens should be of legally-harvestable and at least consumable size and weight when sampling for human health purposes.
- Size of specimens should be within a range so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and samples should be classed according to any length-based requirements in the SAP (see **Section 3.1**).
- Ideally, each composite sample for a specific species will contain the same number of individual fish – a minimum of three, and preferably, five individual specimens.
- Enough sample mass (usually 200-500 grams) must be generated for the target analyte suite and equal amounts of mass should be used from each individual specimen in the composite.

General procedures for compositing samples include the following steps which should be performed in a laboratory clean-room environment. Modification of these procedures may be necessary depending on SAP requirements, available equipment, and site-specific conditions.

1. Work surfaces should be prepared in accordance with the procedures in **Section 3.2.1**.
2. Samples should be partially thawed as described in **Section 3.2.1** to facilitate homogenization.
3. Fillets or other tissue to be homogenized should be reduced to cubes of a size (e.g. ~2.5 cm) able to be easily handled by the available homogenization apparatus. Additional manual processing may be necessary for whole fish compositing.
4. Fish tissue should be homogenized through use of a food-grade grinder, high-speed processor, or a laboratory homogenizer.
5. Repeated cycles through a homogenizer and/or hand-mixing may be necessary to achieve final consistency.
6. Final consistency of the homogenate should resemble a fine paste of uniform color and texture (U.S. Environmental Protection Agency, 2009) with no remaining structure greater than 1 mm in size (U.S. Environmental Protection Agency, 2005).
7. Samples should be containerized in pre-cleaned glassware with lids lined with non-reactive materials.
8. Label the sample in accordance with **Section 3.6** and complete **Attachment B** – Fish Tissue Sample Form for the sample.
9. Composite samples must be frozen as quickly as possible and stored at ≤ -20°C.

Ideally, parts of the homogenizing apparatus that contact the sample (e.g. blades) will be made of titanium or tantalum as stainless steel has been found to be a potential source of nickel or chromium contamination due to high speed abrasion. Additionally, it may be helpful to chill these contact points prior to use (chips of dry ice may serve this function) to prevent the sample from sticking to the apparatus (U.S. Environmental Protection Agency, 2000).

3.2.5 Biopsy Punch

As described in **Section 2.4.1**, biopsy punch sampling methods provide a no-kill sampling alternative to lethal techniques. When biopsy punch sampling is implemented, the following steps should be taken. These procedures are more easily accomplished by two or more personnel so fish and tissue sample handling tasks can be performed concurrently.

1. If anesthesia will be used, fish should be anesthetized in accordance with **Section 2.4.2** and their welfare maintained as described in **Section 2.4.3**.
2. Work surfaces should be prepared in accordance with the procedures in **Section 3.2.1**.
3. Specimen measurements, particularly weight, should be obtained prior to biopsy punch sampling.

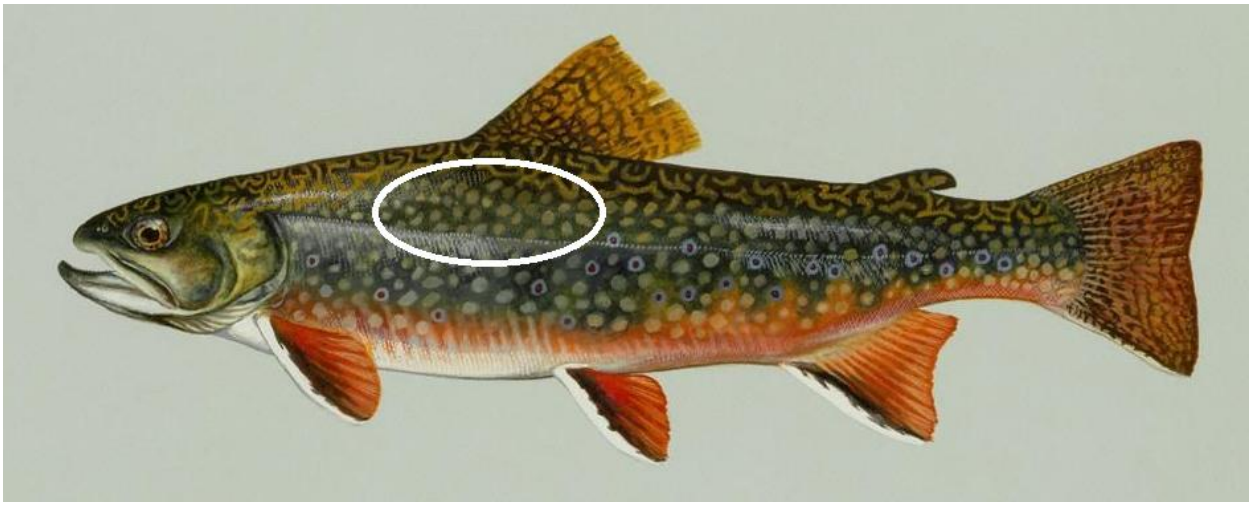


Figure 3-2. Biopsy Punch location.

4. Using a pre-cleaned utensil, a small area of scales (if present) must be cleared from one side (typically the left) of the dorsal area. The white oval on **Figure 3-1** represents the area within which to make the incision. It may be possible to also remove the skin of the specimen in the area to be biopsied at this time.
5. Insert the biopsy punch through the skin at the descaled area with a slight twisting motion to the prescribed depth of the punch, penetrating the skin (if remaining) and muscle tissue.
6. Using a slight tilting motion to sever the tissue at the end of the punch while leaving the sample intact, remove the punch.
7. Place a laboratory pipette bulb on the opposite end of the biopsy punch from the tissue sample. Squeeze the bulb quickly and firmly, to discharge the tissue sample onto unused aluminum foil if it must be further processed (e.g. removal of skin or compositing) or directly into pre-cleaned sample glassware. If necessary, use clean forceps to remove the tissue from the punch.
8. If the tissue plug comes in contact with any non-clean surface, immediately rinse it with deionized water and record the event on **Attachment B** – Fish Tissue Sample Form.
9. Liberally apply antibiotic salve to the biopsy area on the fish and return it to the waterbody as quickly as possible, first allowing a safe recovery location if anesthesia was used.
10. Label the sample in accordance with **Section 3.6** and complete **Attachment B** – Fish Tissue Sample Form for the sample.
11. Freeze samples as quickly as possible and store them at ≤ -20°C.
12. Do not attempt to reuse a biopsy punch that has contacted a specimen or any non-clean surface.

3.3 VOUCHERS

Vouchers may or may not be called for in the SAP. Voucher specimens may not be necessary when species to be sampled are well known and easily identifiable. Alternatively, voucher specimens may be critical when dealing with visually similar species and/or hybrids, making taxonomic identification in the field difficult. Vouchering is accomplished either via direct collection and preservation of individual fish, or through photographic record. Vouchering may be imperative for quality assurance/quality control

(QA/QC) purposes and may have important additional functionality beyond the scope of the project such as documentation of the existence of invasive or exotic species in a waterbody.

Specimen Vouchers

Where species size and abundance allow, the SAP may call for preservation of individuals of the project sample species. Also, if T/E, or otherwise protected species expire during sampling activities, they should be preserved as vouchers. The following steps should be taken in preparing specimen vouchers:

1. Take and record appropriate field measurements (see **Section 2.5**) and euthanize the specimen humanely (see **Section 2.4.1**).
2. Place the specimen in an appropriately-sized sample container with a 10% solution of buffered formalin (see **"Voucher Preservation"** and **"Caution"** below). Multiple individuals of the same species or suspected species may be placed in the same container; however, ensure that specimens are not too tightly containerized – anatomy should not be contorted and fish biomass should not be greater than 40% of the container contents by weight (U.S. Environmental Protection Agency, 2006a). Use enough preservative to completely submerge all specimens.
3. Specimens larger than 10 cm should receive a small incision along the right ventral area to aid preservation.
4. Complete a label with appropriate information (see **Section 3.6**) and affix it to the voucher bottle securely. The SAP may require multiple labels or identification (ID) tags for voucher specimens and may require placement inside sample containers. Vouchers must be clearly identified so they are not mistakenly used for sampling purposes.
5. Two to seven days in the solution are required to ensure proper preservation of the voucher (U.S. Geological Survey, 2002).

Voucher Preservative

A buffered formalin preservative solution can be prepared by the addition of 400 g of borax to a 20 L container of 100% formalin and checking to ensure a pH of 6.5 to 7.0 (U.S. Environmental Protection Agency, 2006b; Lazorchak, 2000). A smaller volume of the solution can be prepared by adding 100 mL of formaldehyde to 900 mL of water, separately mixing 3 g of borax with 10 mL of water, and then mixing the two solutions together (U.S. Geological Survey, 2002).

Caution: Formalin and formaldehyde are caustic, potentially carcinogenic, hazardous substances and must be handled in accordance with Federal regulations found at 40 CFR 1910.1048. Wear proper personal protective equipment (e.g. gloves and safety glasses) when handling these chemicals. Reserve solution should be stored in a safety cabinet or cooler lined with absorbent materials and transported outside the passenger area of vehicles if at all possible (Lazorchak, 2000).

Photographic Vouchers

Photographic vouchers may be the appropriate or only course of action such as in cases when examples of a target species are too large for specimen preservation or represent T/E species. The following guidelines should be followed when using photographs as vouchers:

- The equipment used should have sufficient macro capability to capture details of external characteristics when photographing small species or anatomical features.

- Fish should be photographed on a measuring board or with a rule or some other measure appearing in the photograph for scale. Conventionally, the left side of the fish is photographed (Walsh and Meador, 1998).
- Multiple shots of the same specimen should be taken if necessary or helpful in characterizing/identifying the species (e.g. a full-body, a lateral shot, and a close-up(s) of features inherent to the species). Some staging, if possible, (e.g. spreading and pinning of fins of fixed specimens) may aid in the use of the photograph for identification purposes.
- It is recommended that a tag with at minimum a voucher ID number be included in the image.
- A log of the voucher photographs containing information such as the following must be maintained:
    - project and/or site ID
    - date of photograph
    - known or suspected species name and/or common name
    - image file name
    - description of the specimen or other notes regarding the photograph.
- Whether or not a project target species, invasive/exotic and T/E species should be photo-documented, provided the welfare of T/E species can be maintained. FWP may prohibit return of some invasive/exotic species to the waterbody.
- Backup image files as soon as possible.

3.4 SAMPLE LABELING AND CUSTODY

All collected samples must have labels. The method of identifying and labeling samples should be provided in the SAP and strictly followed. At a minimum, sample labels should include the following information:

- The collecting organization name;
- The project name/number;
- The sample ID number;
- The date the sample was taken; and
- The time the sample was taken.

Additional information that may be recorded on labels includes but is not limited to:

- Sample location information (e.g. reach code);
- The sampler's name and/or team member identification number;
- Species name or code (see **Attachment F** for codes, common names, and abbreviations for fish species used by FWP);
- Analysis to be performed;
- Additional sample ID numbers (e.g. composite or individual specimen numbers); and
- Preservative used (e.g. formalin)

Further guidance on sample labeling can be found in Appendix D of the *Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring* (Montana Department of Environmental Quality, 2012).

All samples must be maintained, stored, and transferred under chain of custody (COC). COC procedures begin when the sample is collected and remain the responsibility of a sampling team member until the samples are relinquished to a laboratory or disposed of. In general, to maintain chain-of-custody, samples must remain in the physical possession (i.e. being carried by or within view) of the COC form signatory unless COC-sealed and locked in a restricted access location. Use of a COC form ensures that samples are traceable from the time of collection through processing and analysis until final disposition (Parametrix, Inc., 2009) and may be required by the analyzing laboratory. The COC form originates with the personnel who performed the sampling and remains with the samples (typically sealed in a plastic bag attached to or in the sample cooler) as it passes to other personnel for additional processing, shipping, etc. A generic COC Form suitable for use for fish tissue samples is provided as **Attachment E**.

3.5 SAMPLE HOLDING, STORAGE, AND SHIPPING

Unless further resection and/or sample analysis will occur within 24-48 hours, samples should be frozen as quickly as possible after collection and processing using dry ice or other means of cooling samples to ≤-20°C. Samples should be shipped to the laboratory within 48 hours and preferably, 24 hours from time of collection. Once frozen, actual maximum laboratory holding times may range from 28 days to one year (U.S. Environmental Protection Agency, 2000) and samples should not be allowed to thaw until time of analysis. If samples cannot be frozen while being stored in the field, they should be stored on wet or blue ice and shipped to the laboratory within 24 hours of collection. Samples must be packaged such that they will not come into contact with each other or preservative melt water. This should be accomplished through use of an appropriate combination of aluminum foil, plastic bags, and plastic tubing depending on how the samples have already been packaged or containerized. If sample compositing will be performed by the laboratory, individual specimens composing a composite should be held, stored, and shipped together. As stated in **Section 3.4**, when not in the physical possession of a sampling team member, samples should be locked in a restricted access location such as a trailer, vehicle, or building room.

During storage and prior to shipping, it must be ensured that an adequate amount of wet, blue, or dry ice covers and is layered between samples to prevent degradation. Sample volume in shipping coolers/containers should be 60-70% with the remainder being coolant, depending on ambient conditions. If voids remain within coolers after all samples and coolant have been added, the additional space should be filled with clean material (e.g. bubble wrap or paper) to prevent the samples and coolant from shifting. Following packing, coolers/containers should be taped shut securely and COC seals should be added.

Dry ice has the potential to off-gas and build up pressure inside a tightly sealed cooler and it will damage skin if handled without protection such as gloves. A Class 9 Dangerous Goods label is required for shipments containing dry ice and a cooler with vents or drains which will remain open during shipping should be used to prevent pressure build-up. The weight of dry ice included in the shipment will need to be known for completion of the information required on this label. Special containerization, labeling, and shipping forms may be required for shipments containing formalin if not in conformance with the small quantity exemptions found in 49 CFR 173.4. It may be appropriate to line the bottom of the cooler/container with absorbent material, particularly if preservatives are used, to prevent hazardous materials from leaking from shipments.

The most current shipping requirements for both the U.S. Department of Transportation and shipping carrier, if being used, should be consulted when shipping samples. When using a carrier for shipping, it may be helpful to inform the laboratory of the carrier name and anticipated arrival date and time for the samples. If the shipment contains multiple parcels, the carrier should also be alerted as to how many cooler/containers to expect and they should be labeled appropriately (i.e. “1 of 3,” “2 of 3,” etc.). The sampling schedule should be set to ensure the laboratory is prepared to process sample shipments immediately (e.g. weekend delivery may need to be avoided).

3.6 SAMPLE DISPOSAL

It is the sampling team members’ responsibility to prevent the release of hazardous substances into the environment from sampling activities. Sampling-derived waste must be disposed of in accordance with federal, state, and municipal regulations. Recyclable and non-recyclable materials should be sorted into their appropriate waste streams and transported offsite for processing or disposal, respectively. Disposable sharps such as scalpels and biopsy punches should have cutting edge guards replaced, if so equipped, and be disposed of in a safe manner. All chemical wastes must be disposed of properly; preservatives and dry ice are potentially hazardous and should not be discharged in the field. Acids should be properly neutralized prior to disposal.

Caution should be taken in the collection and disposal of offal from field sampling activities such as filleting due to the attraction of other wildlife such as bears. It may be acceptable to dispose of whole fish carcasses in the waterbody following puncture of the swim bladder. FWP should be consulted regarding when, where, and how fish remains can be left in the field.

4.0 QUALITY CONTROL

Quality control (QC) procedures for field activities primarily consist of precautionary measures that should be taken to prevent contamination of samples and equipment, and preparation of replicate and blank samples. The following subsections describe these QC procedures, but sampling team members should also be familiar with and refer to the QC section(s) of the SAP and the Quality Assurance Project Plan (QAPP) for project-specific QC tasks requirements.

In addition to the measures described below, the following basic QC tasks should be performed during field sampling:

- Measurement units should be used consistently as specified in the SAP or recommended in **Section 2.5**.
- Taxonomic references must be standardized and documented across sampling teams and for the life of the project. The services of a qualified taxonomist may be necessary and vouchers should be obtained.
- Holding times must be adhered to as specified in the SAP or recommended in **Section 3.5**.
- Sample integrity must be maintained through preservation, packaging, storage, and COC procedures.
- Field logs, sample and COC forms, labels, and any other project records must be completed promptly, thoroughly, and accurately.

Sections 4.1 and **4.2** describe the typical QC sampling that most projects will include; QC sampling requirements should be detailed in the SAP. The purpose of QC sampling is to:

1. Verify samples were not contaminated during the sample collection process;
2. If found, determine the origin of contamination; and
3. Assess the accuracy and precision of the methods of analysis (U.S. Environmental Protection Agency, 2014c).

More information on replicate and blank sampling can be found in **Sections 5.4** and **5.5** of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring," (Montana Department of Environmental Quality, 2012).

4.1 REPLICATE SAMPLES

Collection and retention of replicate (duplicate) samples, even when resources do not allow immediate analysis, preserve the possibility of QC review at a later time (U.S. Environmental Protection Agency, 2000). Wherever sufficient numbers of fish are available, replicate samples should be collected at a rate of ≥ 10% (i.e. at least one replicate sample for every 10 samples collected) unless otherwise specified in the SAP. Likewise, for composite samples, at least every 10^{th} sample should be split into a routine and a replicate sample; therefore, it may be prudent to factor available tissue mass into the decision of which composite sample to duplicate. A minimum of one replicate sample should be collected regardless of the number of sampling locations or composite samples prepared. Replicate samples should be collected in the same manner as routine samples, without deviation, and may be collected as blind replicates (i.e. labelled in a manner such that the laboratory is unaware of which routine sample they are a duplicate of) if called for by the SAP. Unless otherwise specified in the SAP, replicate samples should be taken from the same matrix preparation (i.e. taken from the same fillet as the routine sample to be replicated or the same composite should be divided into a routine and a replicate or a biopsy plug sample should be split into two samples provided enough sample mass will be available in both halves).

4.2 BLANK SAMPLES

Blank samples taken in the field generally consist of the following:

- Rinsate blanks – samples of unused rinsate and
- Process blanks – samples of rinsate used on equipment, utensils, containers, supplies, etc. which are already in a "clean" state.

Blank samples are processed, stored, handled, and shipped just as routine samples. The purpose of field blank samples is to evaluate sources of contamination introduced to the sample through field sampling methods. Detection of an analyte in a blank sample could indicate that routine sample results are biased-high. Blank samples should be prepared once at the beginning of a sampling event and then at a rate of 5% (i.e. at least one blank sample per every 20 samples processed) or one with every batch of samples, whichever is more frequent (U.S. Environmental Protection Agency, 2000), unless otherwise specified in the SAP. It may also be appropriate to prepare another blank at the end of a sampling event or end of each day. Being a solid medium, fish tissue is not easily contaminated by most analytes, and it may be difficult to quantify a magnitude of a contaminant that has been transferred to a tissue sample from rinsate, containers, supplies, or equipment (Idaho Department of Environmental Quality, 2008).

4.3 INTERFERENCES AND PRECAUTIONS

Interferences such as cross-contamination and improper sample selection can result in biased, inaccurate, or erroneous data results. Several precautions to avoid these interferences are built into the procedures and methods described above. The following precautionary measures should be kept in mind during sampling activity:

- Use wetted, gloved hands to prevent contamination transfer to fish.
- Ensure entries on forms, labels, COCs, and in logbooks are accurate and legible to prevent transfer of erroneous values to electronic systems or sample misidentification.
- Use standardized units of measure, measuring tools, and taxonomic references across sampling locations and for the duration of the project.
- Follow the sample species, size, age, sex, and mass requirements specified in the SAP precisely.
- Follow all calibration methods and frequencies specified in equipment manuals or the SAP.
- Follow the steps for decontamination of surfaces and equipment as described in **Section 3.2.1**.
- Ensure vouchers are properly preserved in accordance with the procedures in **Section 3.3**.
- Check sample integrity regularly while holding (e.g. ensure samples do not come into contact with melt water, frozen samples do not thaw prior to lab delivery, etc.).
- Maintain chain-of-custody at all times.

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ATTACHMENT A – EQUIPMENT AND FIELD SUPPLIES

Qty	Item	√
1	First aid kit	
1	Cold weather kit (seasonal)	
1	Collection permit (if required)	
1	Sampling and Analysis Plan (SAP) copy	
1	Quality Assurance Project Plan (QAPP) copy	
1	Health and Safety Plan (HASP) copy	
1	Fish Tissue Sampling Standard Operating Procedure (SOP) copy	
1	Project field logbook	
1	Taxonomic key	
NA	Field sample collection form	
NA	Sample labels (set)	
NA	Chain of custody (COC) (one for each sample cooler plus two extra)	
NA	COC seals (one for each sample cooler plus two extra)	
NA	Carrier airbills/shipping forms (one for each sample cooler plus two extra)	
1	Field clipboard/form case	
3	Indelible writing device (e.g. pencil, Rite-in-the-Rain® pen)	
2	Chemical splash goggles	
1	Nitrile gloves (100ct. box)	
2	Paper towels (roll)	
2	Anesthetization/holding vessel (e.g. live-well or bucket)	
1	Inert or foil-wrapped blunt instrument (for euthanizing fish)	
1	Live cage (for containing fish in surface waterbody)	
1	Minnow net (for collecting small species from livewell/bucket)	
2	Holding tray	
2	Inert (e.g. glass) cutting board	
1	Fish measuring board (min. precision – ±1mm)	
1	Measuring tape (mm increments)	
1	Portable balance (min. precision – ±1g)	
1	Calipers (min. precision – ±1mm)	
2	Heavy duty aluminum foil (roll)	
4	Plastic Bags (2 gallon, box of)	
4	Plastic bags (1 gallon, box of)	
4	Plastic bags (1 quart, box of)	
6	Plastic bags (33 gallon)	
1	Plastic wrap (roll)	
2	Packing/strapping tape (roll)	
NA	Cooler/ice chest	
NA	Wet/blue/dry ice	
NA	Bubble wrap, absorbent packing material	
2	Forceps	
2	Fillet knife	
2	Scaling knife or scalpel	
2	Pliers	
2	Scissors or utility knife	

Qty	Item	√
3	Deionized water (gallons)	
1	Camera – macro-capable, electronic storage media, extra batteries (for voucher photographs)	
NA	Preservative/fixative[1] (typically 10% borax-buffered formalin or 37% buffered formaldehyde, enough volume for samples & vouchers)	
NA	Reagents - acetone, hexane, methanol, 20% nitric acid (volumes sufficient for cleaning equipment and blank samples)	
2	Food-grade poly tubing (rolls)	
1	Plastic cable ties (30-50 count package)	
Additional Equipment/Supplies for Composite Sampling		
2	Stainless steel bowl (4 or 6 quart, for holding/hand-mixing samples)	
1	Homogenization apparatus (blender, grinder, laboratory homogenizer)	
NA	Lab sample glassware (for composite samples)	
Additional Equipment/Supplies for No-Kill Biopsy Sampling		
NA	Class 9 Dangerous Goods diamond placard (dry ice shipments greater than 2.5kg/5.5lbs)	
NA	Sterile disposable biopsy punch (Acu-Punch® or equivalent brand in boxes of 25 or 50)	
2	Pipette bulb (for discharge of sample from biopsy punch)	
NA	Antibiotic salve (volume enough to cover all planned biopsy punch sampling)	
NA	Scintillation vial (or equivalent sterile laboratory glassware, for punch sample)	
NA	Anesthetizer (e.g. package of Alka-Seltzer® or equivalent CO_2 tablets, clove oil)	

1. NA = not available. Refer to the SAP.
2. Formalin and formaldehyde are potentially human carcinogens and should be handled only in well-ventilated areas while wearing chemical-resistant gloves and approved eye protection.

ATTACHMENT B – FISH TISSUE SAMPLE FORM

Affix sample label here

Date: _____	**Time:** _____	**Personnel:** _____		
Waterbody: _____		**Location:** _____		
Station ID: _____	**Visit No:** _____	**HUC:** _____		**County:** _____
Lat: __ __.__ __ __ __ __	**Long:** __ __ __.__ __ __ __ __	**Datum:** _____	**Elevation:** _____ m ft	**Geo Method:** _____

Sample ID No.				**Composite Sample**	☐	**Composite No.**	
Genus species				**Min Length** (mm)		**Max Length** (mm)	
Collection Method				**Min Weight** (g)		**Max Weight** (g)	
Sample Type (circle) **Whole Fish** **Fillet** **Biopsy Punch** **Eggs** **Voucher** **Organ(s) _____**		**Length** (mm**) TF FL SL**		**Batch** (Total) **Weight** (g)		**Count**	
		Weight (g)		**DELTs**			
		Size Class					
		Sex (M or F)		**Preservative:** wet/blue ice dry ice other _____			

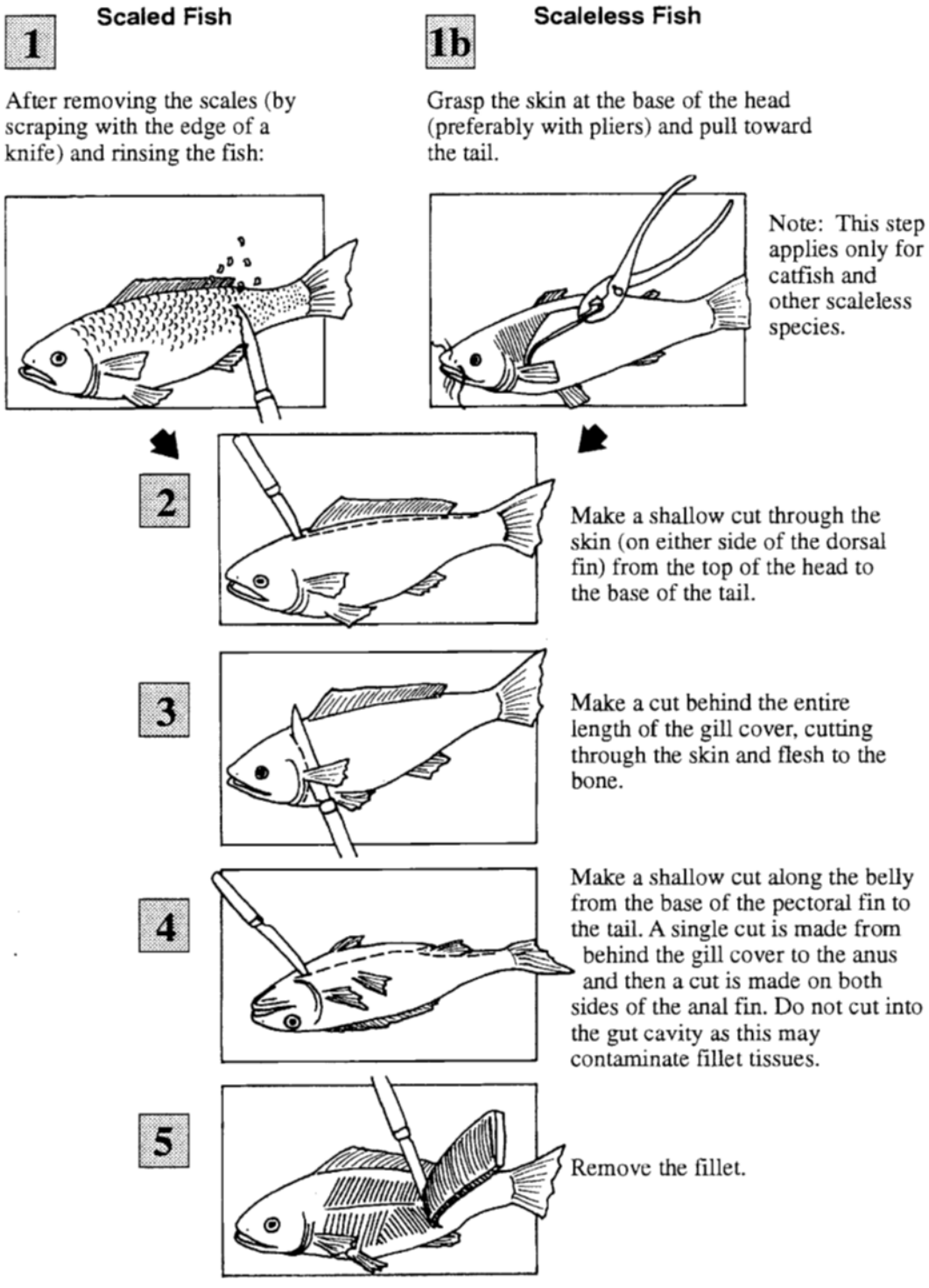
Comments:

ATTACHMENT C – DELTS EXAMINATION

Adapted from US Environmental Protection Agency, R10, (2005)

External Feature	Condition	Description
Body Structure	Deformities	Skeletal anomalies of the head, spinal vertebrae, and fins
Fin Erosion	No active erosion	Normal-appearing fins
	Mild active erosion	Active erosion, no hemorrhage or secondary infection
	Severe active erosion	Active erosion with hemorrhage and/or secondary infection
	Other	Any other observations of special significance
Eyes	Normal	No aberrations evident – clear eye
	Exophthalmia	Swollen, protruding, bulging
	Hemorrhagic	Bleeding in the eye
	Blind	Opaque
	Missing	Less than two
	Other	Any abnormalities not included above
Skin	Normal	No apparent aberrations (discolorations, blisters, cysts, tumors, scale anomalies)
	Mild	Mild aberrations present
	Moderate	Moderate aberrations present
	Severe	Extreme aberrations present
Gills	Normal	No apparent manifestations in gills
	Frayed	Erosion of tips of gill lamellae – ragged appearance
	Clubbed	Swelling of tips of gills
	Marginate	Light discolored margin along distal tips of lamellae or filaments
	Pale	Very light in color
	Pseudobranchs	Obviously convex in aspect rather than flat or concave, aberrations
	Other	Any abnormalities not included above
Thymus	Hemorrhage	None, mild (two or three spots), severe (many pin-point spots, some coalescing, swollen appearance
Opercles	Normal	No shortenings, gill completely covered
	Slight shortening	Small portion of the gill exposed
	Severe shortening	Considerable portion of gill exposed
Parasites	Infection	None, mild (few), moderate (some), numerous (many) – copepods, lice, leaches, grubs, fungus

ATTACHMENT D – FISH FILLET PROCEDURES



Source: USEPA, 1991

ATTACHMENT E – CHAIN-OF-CUSTODY FORM

Affix Chain-of-Custody Label here.

Project Name/Number	**Collecting Agency** (name, address, phone)	**Sample Date**
Sampling Personnel (print and sign)		Container _____ of _____
Waterbody (name)	**Location** (reach code and/or lat/long)	**Station ID**

Sample ID No.	**Composite No.**	**Sample Time**	**Chemical Analysis**						**Comments**

Delivery/Shipment □ hand □ shipped		**Deliver/Ship to:** (org., address, phone)		**Date/Time Shipped**	
Relinquished by: (*sig.*)	**Date/Time**	**Received by:** (*sig.*)	**Relinquished by:** (*sig.*)	**Date/Time**	**Received by:** (*sig.*)
Relinquished by: (*sig.*)	**Date/Time**	**Received by:** (*sig.*)	**Relinquished by:** (*sig.*)	**Date/Time**	**Received by:** (*sig.*)
Account/Contract No.		**LAB USE ONLY** **Delivery temp.** Wet Ice: _____ Dry Ice: _____		**Remarks:**	

ATTACHMENT F – MONTANA FISH, WILDLIFE & PARKS
FISH SPECIES CODES AND ABBREVIATIONS

Code	Common Name	Abbr.	Code	Common Name	Abbr.
001	Rainbow Trout	RB	046	Sturgeon Chub	ST CH
002	Cutthroat Trout (unk. sub. sp.)	CT	047	Emerald Shiner	EM SH
003	Brook Trout	EB	048	Sand Shiner	SD SH
004	Brown Trout	LL	049	Redside Shiner	RS SH
005	Bull Trout	BULL	050	Creek Chub	CR CH
006	Lake Trout	LT	051	Pearl Dace	P DC
007	Golden Trout	GT	052	Fathead Minnow	FH MN
008	Kokanee	KOK	053	Golden Shiner	G SH
009	Coho Salmon	SS	054	Sicklefin Chub	SF CH
010	Arctic Grayling	GR	055	River Carpsucker	RC SU
011	Rainbow x Cutthroat Hybrid	RBxCT	056	Longnose Sucker	LN SU
012	Westslope Cutthroat Trout	WCT	057	White Sucker	W SU
013	Yellowstone Cutthroat Trout	YCT	058	Largescale Sucker	LS SU
014	Whitefish (unspecified)	WF	059	Blue Sucker	B SU
015	Lake Whitefish	L WF	060	Bigmouth Buffalo	BM BUF
016	Sculpin (unspecified)	COT	061	Smallmouth Buffalo	SM BUF
017	Largemouth Bass	LMB	062	Shorthead Redhorse	SH RH
018	Bass (unspecified)	BASS	063	Mountain Sucker	MT SU
019	Sunfish (unk. centrarchid)	SUN	064	Stonecat	S CAT
020	Yellow Perch	YP	065	Black Bullhead	BL BH
021	Crappie (unspecified)	CR	066	Yellow Bullhead	YL BH
022	Sauger/Walleye	SAWE	071	Brook Stickleback	BR SB
023	Northern Pike	NP	072	White Bass	W BS
024	Channel Catfish	C CAT	073	Smallmouth Bass	SMB
025	Bullhead	BLHD	074	Bluegill	BG
026	Burbot	LING	075	Pumpkinseed	PUMP
027	Sturgeon	STRG	076	Green Sunfish	G SUN
028	Paddlefish	PF	077	Black Crappie	BL CR
029	Peamouth	PEA	078	White Crappie	WH CR
030	Goldfish	GDF	079	Rock Bass	R BS
031	Sucker (unk. catostomid)	SU	081	Sauger	SGR
032	Common Carp	CARP	082	Walleye	WE
033	Northern Pikeminnow	N PMN	083	Iowa Darter	IOWA
034	Goldeneye	GE	085	Mountain Whitefish	MWF
035	Utah Chub	GILA	086	Pygmy Whitefish	PWF
036	Freshwater Drum	DRUM	087	Chinook Salmon	CK SAL
037	Minnow (unk. cyprinid		088	Splake (Brook x Lake Trout)	SPLK
038	Shortnose Gar	GAR	089	Salmon (unspecified)	SAL
039	Longnose Dace	LN DC	090	White Sturgeon	W STRG
040	Buffalo (unspecified)	BUFF	091	Pallid Sturgeon	P STRG
041	Redbelly/Finescale Dace	NRB/F DC	092	Shovelnose Sturgeon	S STRG
042	Brassy Minnow	BR MN	099	Rainbow Smelt	RB SM
043	Western Silvery/Plains Minnow	WS/P MN	100	Trout-perch	TR PR
044	Flathead Chub	FH CH	103	Plains Killifish	PKF
045	Lake Chub	LK CH	106	Mosquitofish	MQF

Code	Common Name	Abbr.	Code	Common Name	Abbr.
108	Sailfin Molly	SFM	134	Spoonhead Sculpin	SP COT
109	Shortfin Molly	SHM	135	Rocky Mountain Sculpin	RM COT
110	Rainbow x Westslope Cutthroat	RBxWCT	136	Clark Fork Sculpin	CF COT
111	Rainbow x Yellowstone Cutthroat	RBxYCT	137	Columbia Slimy Sculpin	CSL COT
112	Variable Platy	VPF	140	Western Silvery Minnow	WS MN
113	Rainbow x Yellowstone x Cutthroat	RBYCTWCT	141	Plains Minnow	PL MN
115	Green Swordtail	GST	142	Finescale Dace	FC DC
118	Trout (unspecified)	TRT	143	Northern Redbelly Dace	NRB DC
119	Trout/Salmon (unk. salmonid)	TR SAL	144	Peamouth x N. Pikeminnow	PEAxNPMN
120	Rainbow x Golden	RBxGT	145	Spottail Shiner	SP SH
121	Upper Missouri Cutthroat	UMCT	146	Peamouth x Redside Shiner	PEAxRSSH
122	Native Rainbow Trout	NRB	147	Redbelly x Finescale Dace	NRBxFCDC
123	Cutthroat x Golden	CTxGT	148	Northern Pike x Muskie	NPxMK
124	Brook x Bull	EBxBULL	149	Sauger x Walleye	SGRxWE
125	Cisco	CIS	150	Golden x Rainbow x Cutthroat	GTxRBxCT
126	Atlantic Salmon	AL SAL	152	Sunfish Hybrid	SUN HY
128	Native Redband x Westslope	NRBxWCT	153	Central Mudminnow	CM MN
129	Native Redband x Rainbow	NRBxRB	154	Brook x Brown	EBxLL
130	Mottled Sculpin	M COT	155	Striped Bass	ST BS
131	Slimy Sculpin (unspecified)	SL COT	156	Gizzard Shad	GZ SHAD
132	Torrent Sculpin	T COT	157	Redbelly Pacu	REDB P
133	Shorthead Sculpin	SH COT	158	Grass Carp	GS CARP

Appendix C

File: PE 107517 – Lake Koocanusa Working Group

Date 20 October 2017

Mike Sokal, Impact Assessment Biologist, ENV
102 Industrial Place, Penticton
BC V2A 7C8

Dear Mike,

RE: Selenium Loading to Lake Koocanusa

This memo, and the analysis and results presented here-in, were prepared in response to a request by the Lake Koocanusa Working Group (LKWG) for an estimate of, and comparison between, selenium (Se) loadings discharged to Lake Koocanusa from the Elk River and Kootenay River. This information is of interest to the LKWG as regional context of relative Se loading rates to Lake Koocanusa from the five coal mines operated by Teck Coal Limited (Teck) in the Elk River watershed compared with all other regional Se loadings to the lake ("background" loadings). This information and analysis includes several assumptions, discussed in the following text, and is intended to support environmental impact and management discussions within the working group. The analysis was also conducted using only 2015/2016 data to provide a snapshot of current Se loading conditions.

Analysis

Both water quantity (m^3/s) and Se concentration (ug/l) are required to calculate Se load (mass). Ideally, water quantity and Se concentration would be measured concurrently and at the same location to minimize uncertainty in calculated load. Water quantity data recorded by the Water Survey of Canada in the Elk River at Fernie and the Kootenay River at Fort Steele were selected for use in the calculations, as they represent active water quantity monitoring stations on their respective rivers nearest to both Lake Koocanusa and available Se concentration data. Selected Se concentration data were recorded by Teck in the Elk River at Elko and jointly by Environment Canada and the Province of British Columbia in the Kootenay River at Fenwick. Metadata for each of these monitoring locations is summarized in Table 1.

Ministry of Environment and Climate Change Strategy
Mining Operations
Environmental Protection Division
Mailing Address:
401-333 Victoria Street
Nelson, B.C.
V1L 1G2
Telephone: 250-354-6358
Facsimile: 250-354-6332
Website: www.gov.bc.ca/env

Table 1: Monitoring Location Metadata

Station Name/Location	Station ID	Data Used	Location		Watershed	Drainage Area Adjustment	
			Latitude	Longitude	Area (km^2)	Value	Description
Elk River at Fernie	08NK002	Water Quantity	49°30'12" N	115°04'12" W	3,090	1.15	Scale Water Quantity to Elko
Elk River at Elko	08NK001/E294312	Se Concentration	49°16'50" N	115°05'55" W	3,550	-	
Kootenay River at Fort Steele	08NG065	Water Quantity	49°36'43" N	115°38'07" W	11,500	1.03	Scale Water Quantity to Fenwick
Kootenay River at Fenwick	08NG0009	Se Concentration	49°31'40" N	115°32'57" W	11,850	1.39	Scale "Background" Se Load to Border
Kootenay River at Newgate (Canada/US Border)	08NG042	Watershed Area	49°00'52" N	115°10'27" W	20,000		"Background" area is 16,450 km^2

Prior to calculating Se load, the water quantity data were adjusted to the applicable Se concentration measurement location to account for additional inflows to the rivers between monitoring locations. This adjustment was accomplished by scaling the water quantity data by the difference in drainage area between the monitoring locations, the values of which are presented in Table 1. This adjustment method inherently assumes that the flow per unit watershed area (i.e. $m^3/s/km^2$) is the same upstream and downstream of the water quantity monitoring location. While there is likely to be some difference in the actual flow per unit watershed area, this adjustment is considered reasonable given both the proximity of the quantity and concentration measurement locations (i.e. small adjustment values), and the comparative purpose of the analysis. Only the quantity data recorded nearest in time to the concentration data were used in the analysis, with the discrepancy in timing between all quantity and Se concentration measurements being less than one day.

All five Teck coal mines in the Elk Valley are all located upstream of Elko, and therefore the Se load values for the Elk River at Elko are representative of total Se contributed to the system from the mines; however, the Fenwick values are only representative of the Kootenay River watershed upstream of Fenwick, not of the entire "background" watershed area contributing to Lake Koocanusa. To adjust the results to be representative of the total "background" contributing area, the Se load values were also scaled by the relative difference in "background" watershed area between Fenwick and Lake Koocanusa at the Canada/US border. This is a simplifying assumption but is considered reasonable given the comparative purpose of the analysis and the similar monitored Se concentrations within the Kootenay River watershed, as shown on Figure 1. The Wardner Se concentrations are nearly identical and the station is nearer to the inlet of Lake Koocanusa than Fenwick, and is downstream of the Bull River but upstream of Kikomun Creek, which has anomalously high Se concentrations. Exclusion of the Kikomun Creek Se load isn't expected to meaningfully alter the results and conclusions of the analysis, as its watershed is only 0.4% of the total "background" watershed of Lake Koocanusa. Considering the relative size of the creek and the Se concentrations presented on Figure 1, Se load in Kikomun Creek likely contributes less than 4% additional "background" Se load to Lake Koocanusa, which is within the range of uncertainty of this analysis (see Result and Discussion Section for more details on uncertainty).

In addition to calculating Kootenay River "background" and Elk River Se load for each concentration measurement, the daily mean Se load was also calculated for each set of values. To minimize potential bias associated with differences in the timing and number of samples, mean daily load was calculated using only "concurrent" values. The discrepancy in timing of concurrent values was up to two weeks during the less frequently monitored August through February period, and 1-2 days during the more frequently monitored March through July period.

Figure 1: Se concentrations measured August 17, 2016

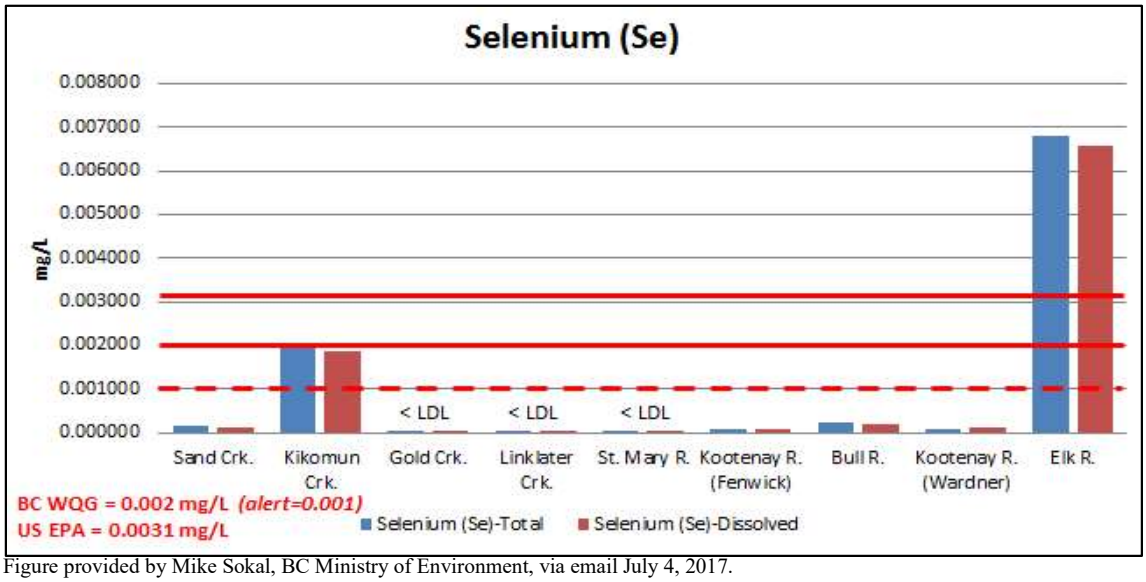


Figure provided by Mike Sokal, BC Ministry of Environment, via email July 4, 2017.

Results and Discussion

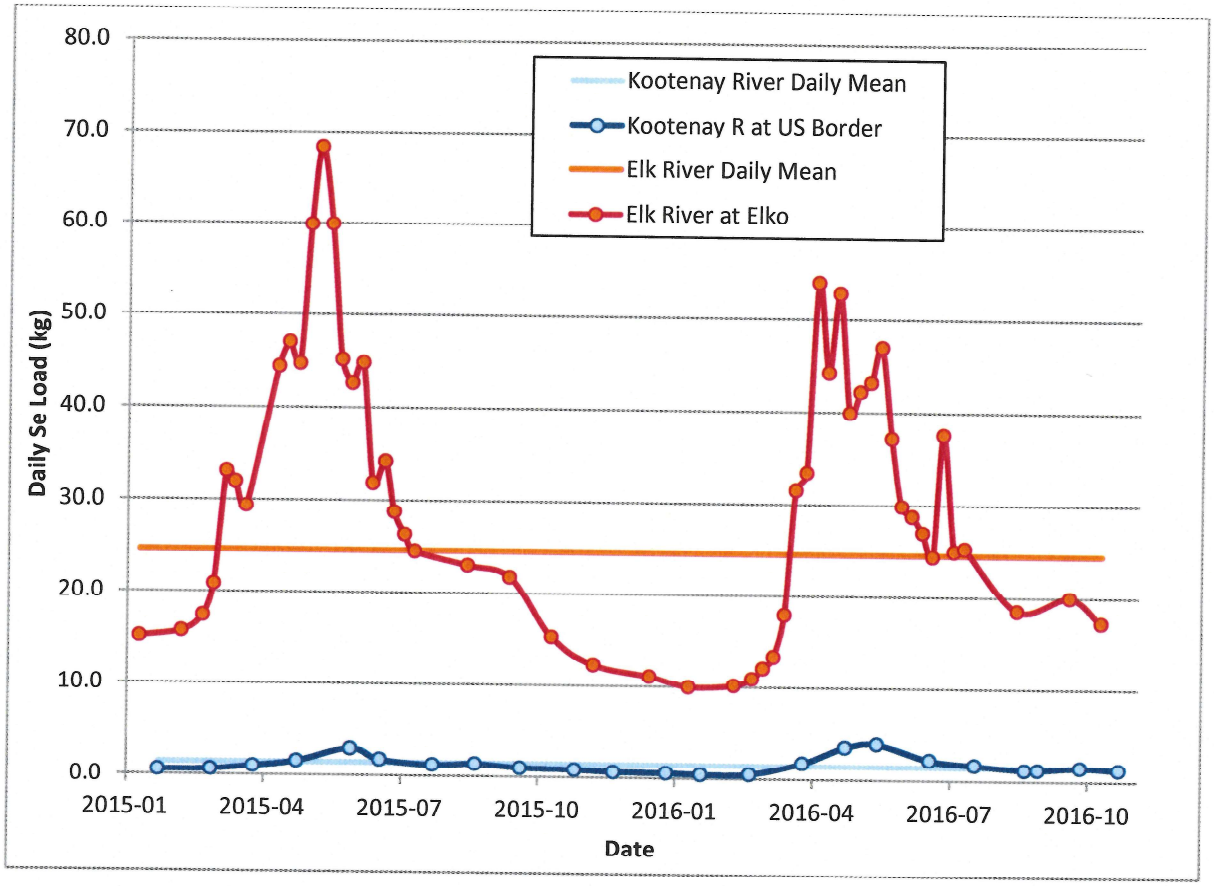
All calculated Se loading results are presented below in Figure 2. The figure provides a comparison between Se loadings (kg/day) to Lake Koocanusa from the entire "background" Kootenay River watershed above the Canada/US border, and the Elk River at Elko, for each Se concentration sample date in 2015 and 2016 as well as the concurrent mean Se daily load.

The results clearly show that Se loadings to Lake Koocanusa are dominated by the Elk River. On average during 2015-2016, the Elk River contributed approximately 17 times more Se to the lake than did the entirety of the remaining Kootenay River watershed. This result also makes sense intuitively; the "background" Kootenay River watershed at the border is approximately 4.5 times larger than the Elk River at Elko, a relationship that can be used to approximate the relationship between mean water quantity, but the mean concentration of Se is approximately 80 times lower. Therefore it would be expected, as proven out by this analysis, that Se load should be in the range of 17 (~ 80/4.5) times lower in the Kootenay River than the Elk River.

As discussed in the Analysis section, the results incorporate several assumptions that add to the overall uncertainty. The magnitude of uncertainty in the analysis was assessed by varying the water quantity and load scaling assumptions as follows:

- Water quantity and Se load (Kootenay River only) generated from the scaled portion of the Kootenay and Elk River watersheds was half of the upstream monitored value, and
- Water quantity and Se load (Kootenay River only) generated from the scaled portion of the Kootenay and Elk River watersheds was 50% greater than the upstream monitored value.

Figure 2: Lake Koocanusa Se Loading Results



The resultant uncertainty is less than +/- 20% for the Kootenay River Se load values at the Canada/US border, and less than +/- 10% for the Elk River Se load values at Elko. The magnitude of the uncertainty is two orders of magnitude less than the difference between the Kootenay and Elk River Se loading results, and as such there is very high confidence in the conclusion that Se loading to Lake Koocanusa is primarily driven by loading from the Elk River.

I trust that this memo meets the Lake Koocanusa Working Groups needs for a comparison of Se loadings to Lake Koocanusa from the Elk and Kootenay Rivers. Please address any questions or comments to the undersigned.

Sincerely,

PROFESSIONAL
PROVINCE OF
K. T. C. TERRY
39897
BRITISH COLUMBIA
GEOSCIENTIST

Kyle Terry, P.Geo.
Hydrologist

Appendix D

FWP/DEQ Fish Tissue Quality Assurance Project Plan for Lake Koocanusa

Date: 4/30/2018
Number: WQSMQAP-02
Approvals:

Trevor Selch, Project Manager, Toxicologist, FWP — Date 5/1/18

Terri Mavencamp, Project Manager, WQPB, SeTSC Co-Chair DEQ — Date 4/30/18

Myla Kelly, Water Quality Standards Section Supervisor, WQPB, DEQ — Date 5/7/18

Eric Urban, WQPB Bureau Chief, MRWG SC, DEQ — Date 5/1/18

Michelle Hauer, QA/QC Officer, WQPB, DEQ — Date 5/2/18

Michael Sokal, BCMOE SeTSC Co-Chair — Date 4/30/2018

Michael Pipp, Information Management and Technical Services, IMTS, DEQ — Date 5/1/2018
IMTS or designee

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REVISION TABLE

Revision No.	Date	Modified By	Sections Modified	Description of Changes

1.0 PROJECT MANAGEMENT

This Quality Assurance Project Plan (QAPP) describes the planning, field collection, quality assurance and control and the analysis of fish tissue collected in Lake Koocanusa to measure the selenium tissue concentration of fish in Lake Koocanusa and to inform a site-specific selenium criterion/objective for Lake Koocanusa. Fish tissue samples prepared under this QAPP will be analyzed for selenium and percent moisture, and will be temporally (w/in 30 days) and spatially (within approximately 10 miles) matched to dissolved selenium water column concentrations, benthic macroinvertebrate and surface macroinvertebrate selenium concentrations and possibly to concentrations of selenium in the suspended sediment (funding pending) (DEQ, 2018a and DEQ 2018b).

This QAPP was prepared in accordance with the EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001). This QAPP is a dynamic document that is subject to change as activities progress. When/if changes occur in the planning/collection/analysis components, the QAPP will be revised accordingly and the revision noted in the revision table and circulated for approval, and forwarded to all project participants listed in the distribution list (Section 1.2).

1.1 PROJECT ORGANIZATION

Montana Fish Wildlife and Parks (FWP) and Department of Environmental Quality (DEQ) are working together to collect and analyze fish tissue on Lake Koocanusa. The general project organization is outlined in Figure 1 and shows the project managers, Selch and Mavencamp who are responsible for developing and updating the QAPP with input from the Selenium Technical Subcommittee (SeTSC) and Monitoring and Research Committee (MRC). Jim Dunnigan and Trevor Selch are the field leads and will report and deviations from the QAPP to Mavencamp and the DEQ quality assurance/quality control (QA/QC) officer, Michelle Hauer. Michelle Hauer, has the role of approving the QAPP and ensuring that it is adhered to as well as determining if QAPP modifications are necessary. DEQ Data management has the responsibility of entering the data into DEQ's Montana EQuIS Water Quality Exchange database (MT-eWQX) and of performing QC on data received from the labs (please see attachment C for the QC checklist).

FWP has the responsibility of

- Planning field activities with input from the MRC/SeTSC (project manager, Selch and field lead Dunnigan)
- Overseeing field activities, including field QC and addressing any deviations from the QAPP (project manager, Selch and field lead Dunnigan)
- Overseeing tissue preparation and QC from the field to the lab (Selch)
- Coordinating with DEQ to by using the appropriate forms and aiding in the creation of QAPP so that the data can be entered as a DEQ project into DEQ's (MT-eWQX) (FWP all parties indicated in Figure 1 will follow the data processes set out in this QAPP)
- Communication of results to the SeTSC (Selch)

The FWP field project lead and field team are responsible for collecting fish, delivering them to the lab, following the procedures described in this QAPP, and documenting their activities using the correct site visit forms (Attachment A).

DEQ has the responsibility of

- Coordinating and communicating the needs of the SeTSC with the field planning (Mavencamp
- Coordinating with FWP to ensure data are collected in a way that can be entered into MT-eWQX (site visit forms, site visit stickers, etc.)
- QAPP preparation in collaboration with FWP and MRC/SeTSC
- Coordinating with the laboratory (and setting up lab contracts) to ensure proper methods, method detection limits and processes are being followed.
- DEQ QA/QC Officer has the responsibility of reviewing and approving this QAPP.
- DEQ Data Management, Information Management and Technical Services (IMTS),Michael Pipp/Jolene McQuillan, is responsible for data validation of received data from the lab and entering the data into DEQ's MT-eWQX and subsequently sending the data to EPA's National WQX Warehouse where the data will be available for use by the public and other agencies through EPA's Water Quality Portal.

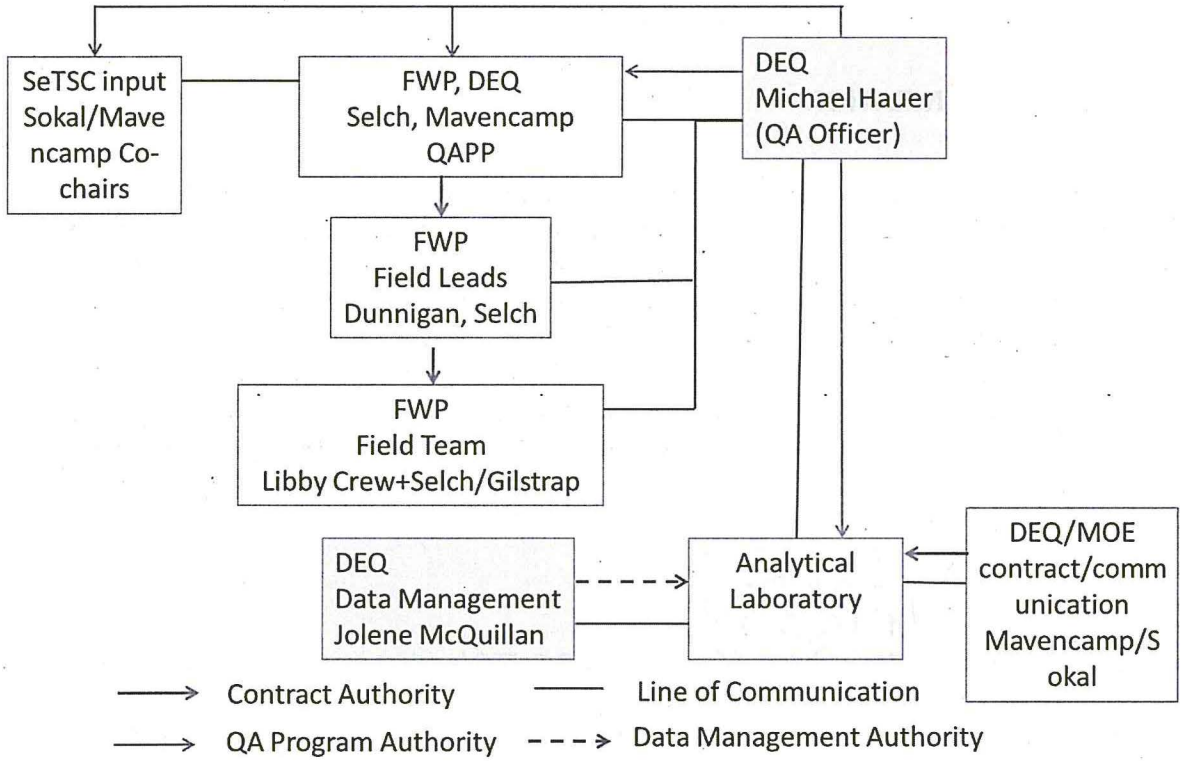


Figure 1. Project Organization Chart. Blue boxes indicate data quality/management and green indicates an independent analytical laboratory. Lines of communication, green, flow both ways.

1.2 Project Team and Responsibilities

Field Personnel:
James Dunnigan: Field Manager, FWP
Monty Benner, Conservation Technician, FWP
Thomas Ostrowski, Conservation Technician, FWP

Jared Lampton, Conservation Technician, FWP
Neil Benson, Conservation Technician, FWP

FWP fish processing in Helena/ FWP Project Manager: Trevor Selch

DEQ Project Manager, QAPP preparation, logistics and laboratory contracts: Terri Mavencamp

Fish processing assistance: Lindsay Gilstrap/Terri Mavencamp (FWP/DEQ)

QA/QC Michelle Hauer/Jolene McQuillan (DEQ)

Data handling and processing for WQX and STORET: Jolene McQuillan (DEQ)

1.3 Distribution List

All persons on the distribution list will receive electronic copies of the final QAPP and any subsequent revisions.

Trevor Selch, FWP, Helena, MT
Jim Dunnigan, FWP, Libby, MT
Terri Mavencamp, DEQ, Helena, MT
Myla Kelly, DEQ, Helena, MT
Darrin Kron, DEQ, Helena, MT,
Michelle Hauer, DEQ, Helena, MT
Michael Pipp, DEQ, Helena, MT
Jolene McQuillan, DEQ, Helena, MT
Eric Urban and Robyn Roome, DEQ and ENV, Steering Committee, Monitoring and Research Working Group (MRWG)
Selenium Technical Subcommittee
Monitoring and Research Committee

1.4 Problem Definition

Selenium is indirectly discharged into the international waterbody, Lake Koocanusa, from steelmaking coal mines owned by Teck Coal Limited (Teck Coal) in the Elk Valley, British Columbia (B.C.). Selenium concentrations in Lake Koocanusa range from approximately .6 µg/L to 1.5 µg/L, or higher in certain areas of B.C.

Teck Coal submitted the Elk Valley Water Quality Plan (approved in July, 2014) to address increasing selenium and nitrate water concentrations in B.C.- while maintaining concentrations within Lake Koocanusa at or below Provincial water quality guidelines, the Plan does not address U.S. waters of Lake Koocanusa. As a result, an unmatched dataset exists for parameters in Lake Koocanusa on the Canadian and U.S. sides.

The U.S. has pursued routine water column monitoring via multiple programs, including U.S. Geological Survey (USGS) water quality monitoring and more recently the U.S. Army Corps of Engineers (USACE). Since 2008, in a collaborative effort between FWP and DEQ, fish tissues have been collected and analyzed for a suite of metals, including selenium. FWP has fish monitoring stations at three locations throughout the reservoir, the Canadian portion near the Elk, Rexford, near the Tobacco River and Tenmile. In 2008, tissue analysis was performed on fish from the Canadian and Rexford stations; and in 2013, the effort included the analysis of fish from Tenmile (McGillivray) and Rexford in the spring and Rexford and Canada in the Fall. The study will be repeated in 2018, including the Tenmile site in the spring, Rexford in the spring and fall and the Canadian site in the fall.

1.5 Project/Task Description

DEQ/FWP are collaborating to collect and analyze fish tissue in Lake Koocanusa. Fish will be collected by the FWP fisheries division, and sent to Energy Laboratory in Helena, MT for analysis of selenium and percent moisture.
The following are key design components of fish collection in Lake Koocanusa:

3 Fish identified as being critical to the development of a site-specific selenium criterion/objective will be selected from the gill netting efforts conducted by FWP to assess population trends in Lake Koocanusa and analyzed for selenium in multiple tissues (filet, whole body, and/or egg/ovaries) as available.

4 Fish will be caught and analyzed for selenium from the Canadian site in the fall, Rexford in the spring and fall and Tenmile (McGillivray) in the spring.

1.6 Quality Objectives and Criteria

The overall quality objective for the analysis of fish tissue for selenium is to continue baseline monitoring of fish tissue selenium in Lake Koocanusa, evaluate concentration trends (compare to 2008 and 2013 as well as other data collected in Canada) and help inform a site-specific selenium criterion/objective for Lake Koocanusa. Fish tissue values can be used for Se model validation, derivation of conversion factors and trophic transfer factors and/or in bioaccumulation factor (BAF) model derivation. Please see Section 2.0 for technical details of the project design.

To have usable data to meet the above objectives, the following measurement quality objectives have been identified: the laboratory reporting limits (RLs) must be low enough to yield usable results. Based on past fish tissue concentrations we do not anticipate that RLs will be a concern, and are set at 0.5 µg/g (dry weight) for selenium. Accuracy and precision are important and must lie within the guidelines outlined in section 6 of this QAPP and in the analytical methods. Completeness is defined as the percentage of samples collected in the study for which usable analytical results are produced. The goal for completeness is 90% and is calculated at the sample-analyte level. For baseline monitoring and trends analysis, we would like to collect a minimum of five fish of each identified species and sampling event (location and time) (identified in Section 4).

To use these results to develop site-specific criterial we need temporal coordination (collected within the same year, for this project we are aiming to collect matched data within a 30-day time frame) and

spatial coordination (the reservoir has been functionally split into three sections, the lower, mid and upper sections or Forebay/McGillivary; Rexford/International Border; and Canada), spatially, we would like matched samples collected within a maximum of 10 miles of one another.

We propose to use the performance based measurement system (PBMS) concept, meaning that as long as the data quality objectives (performance or acceptance criteria) are defined, the data quality indicators (DQIs defined in section 6) are identified and the appropriate measurement quality objectives (MQOs) are met, the data should be appropriate to use for the project objectives.

1.7 Special Training/Certification

All FWP employees have been trained or mentored in the field sampling methods described in the QAPP. All FWP employees who use electrofishing, are trained and attend an electrofishing course. FWP fishing methods/trainings are covered under different documents, i.e., FWP processes, Bonneville Power Administration(BPA)Mitigation document (Dunnigan et al. 2014).

Staff relevant to this project are trained and experienced in proper sampling, field analysis and boat safety. Training for field procedures under this QAPP will be performed by FWP project leaders. Sampling personnel are experienced fisheries technicians knowledgeable in local fish species, proficient in field sampling techniques and familiar with this QAPP and Fish Tissue Standard Operating Procedure SOP (DEQ, 2015).

Laboratories analyzing samples under this QAPP are responsible for providing personnel qualified for the methods requested and adhering to their laboratory Quality Assurance Plan LQAP. Laboratories MT DEQ uses for analyzing samples are either certified through the State of Montana, accredited under national programs, or their quality system is known and meets DEQ's requirements (DEQ QMP, 2015).

2.0 Introduction and Background

Lake Koocanusa is a managed reservoir. Water levels in Lake Koocanusa are generally lowest in late winter/early spring (i.e., February through April) and highest in summer/early fall (Minnow Environ. Inc., 2014). Several biological sampling programs have occurred in Lake Koocanusa (Dalbey et al., 1998; Dunnigan et al., 2016; Montana FWP, 2013; Minnow, 2017).

This QAPP describes the fish sample collection by FWP in 2018/2019 in Lake Koocanusa, fish handling and lab analysis. The sampling is being done to support baseline monitoring and fish tissue concentration trends, specifically selenium, and support the development of a site-specific selenium criterion/objective for Lake Koocanusa.

This QAPP is a collaborator vie effort with FWP population studies on Lake Koocanusa that falls under the Bonneville Power Administration mitigation grant program that funds FWP population studies, including the fish catch efforts referred to in this QAPP https://www.cbfish.org/Project.mvc/ProjectDocuments/1995-004-00 .

In 2008 and 2013, Bull Trout, Longnose Sucker, Northern Pikeminnow, Kokanee, Peamouth, Rainbow Trout, and Westslope Cutthroat Trout, were targeted for tissue and opportunistic egg/ovary sampling. Egg/ovary samples were taken if ovaries were with eggs, but the stage of development was not noted. Sites sampled were the Canadian portion of the reservoir around the mouth of the Elk River, Rexford around the confluence of the Tobacco River and Tenmile or the lower 1/3 of the reservoir (also referred to as McGillivray).

Figure 2 shows results of 2013 tissue collection across the aforementioned sampling sites: Canada, Rexford and McGillivray (Tenmile).

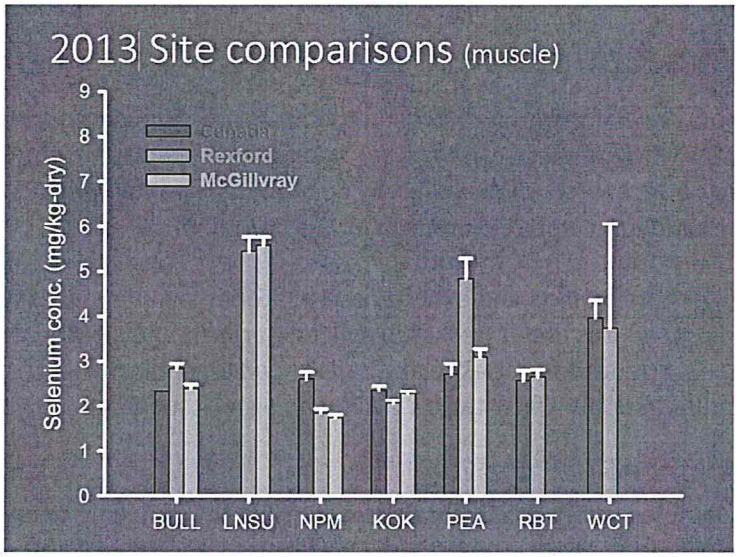


Figure: 2 Muscle selenium concentrations from the 2013 FWP/DEQ fish tissue monitoring effort. Figure from Selch presentation, Kalispell, 2017.

Selenium muscle tissue, whole body and egg values will be compared to current BC Ministry of Environment (MOE) and the EPA recommended selenium fish tissue criterion (EPA 2016 Se Criteria).

Aquatic Life Selenium Guidelines / Criterion			
Agency	Fish Tissue (Whole Body)	Fish Tissue (Egg-ovary)	Fish Tissue (muscle)
EPA suggested [1]	8.5 mg/kg dw	15.1 mg/kg dw	11.3 mg/kg dw
MT current	None	None	none
MOE (current guidelines)[2]	4 mg/kg dw	11 mg/kg dw	4 mg/kg dw

Table 1: Aquatic Life Selenium Guideline/Criterion

[1] EPA Fact Sheet, Aquatic Life Criterion https://www.epa.gov/sites/production/files/2016-06/documents/se_2016_fact_sheet_final.pdf

[2] BC MOE Companion Document to Ambient Water Quality Guidelines for Selenium Update, Water Protection and Sustainability Branch, Environmental Sustainability and Strategic Policy Division, 2014.

Lake Koocanusa is in the Middle Kootenai 4th level HUC 17010101 and has been assigned a B-1 beneficial use classification (ARM 17.30.609). A B-1 beneficial use classification is applied to waters that are suitable for domestic use after conventional treatment, growth and propagation of salmonid fishes, associated aquatic life and wildlife, and agricultural and industrial uses. Lake Koocanusa is considered threatened by MT DEQ because of rising selenium levels from sources outside state jurisdiction or borders and is impaired for aquatic life by other flow regime alterations due to impacts from Libby Dam (Montana 2016 list of impaired waters http://deq.mt.gov/Portals/112/Water/wqpb/CWAIC/Reports/IRs/2016/App_A.pdf).

3.0 Objectives and Sampling Design

3.1 Objective

The goal of this project is to continue the baseline monitoring of fish tissue selenium in Lake Koocanusa, evaluate concentration trends and use the data to inform the development of a site-specific selenium criterion/objective.

The objective of this sampling project is to collect fish at three locations in the reservoir to examine the species, spatial and temporal variations in selenium fish tissue concentrations. The objectives will be met by coordinating with FWP's fish population monitoring effort of Lake Koocanusa outlined in (BPA) project reports for the 'Mitigation For the Construction and Operation of Libby Dam' found here https://www.cbfish.org/Project.mvc/ProjectDocuments/1995-004-00 .

3.2 Selection of Sites

FWP has split the reservoir into three study areas. For 2018/2019 field fish sampling, there will be three main sites where FWP will set gillnets. The sites around Tenmile are considered the lower 1/3 of the

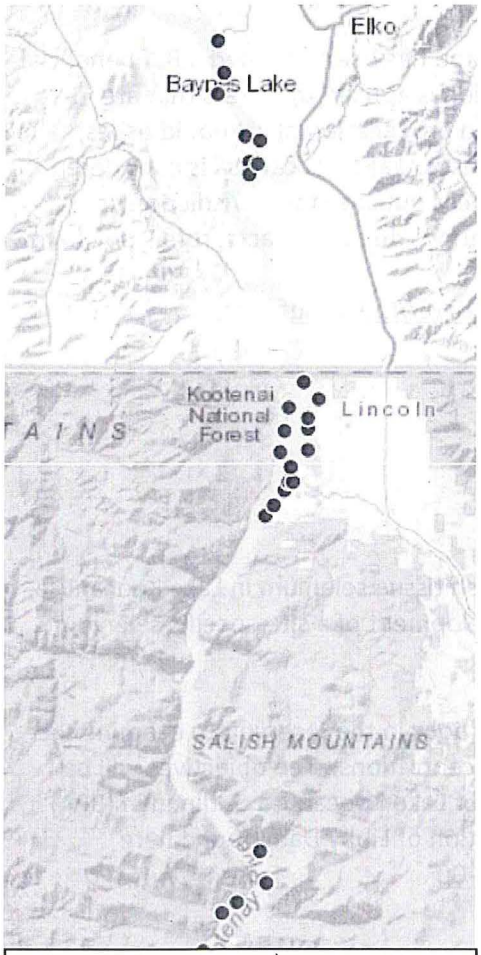


Figure 3: Map of FWP Gillnet Sites for Long term species/trend information. The exact location of each net will be recorded on the SVF.

reservoir, the middle 1/3 is considered the sites around Rexford and the upper 1/3 of the reservoir is considered "Canada" where nets are set near the Elk River confluence. The areas are affected differently by dam operation and reservoir morphology (Chisholm, 1987). These geographic areas were chosen based on reservoir morphometry, effects of reservoir drawdown and political boundaries (Chisholm, 1987).

Sites for 2018/2019 are the same as previous years for the Canada and Rexford sites. For the Tenmile site, net locations have been spread out from the Tenmile site to the Forebay to give a more complete spatial representation of this area. Figure 3 shows the Gillnet locations for 2018. Gillnets are often set near the mouth of incoming tributaries and the locations of each net set (lat, long) will be recorded. The Tenmile site will be fished in the Spring and the Canada site in the Fall. Rexford will be fished in the Spring and Fall. At Rexford, 14 nets are set in the spring and 7 in the fall. Please see attachment B for a list of the lat/longs.

3.3 Sampling Timeframe

Sampling will run from approximately May 2018 through the end of September, 2018. The sampling time may be extended, if necessary. Reasons that the sampling may be extended are if trout or another target species become available and may supplement our catch numbers. If this occurs, the QAPP will be addended or modified.

4.0 Field Sampling Methods

4.1 Fish Sample Collection

Fish identified by the SeTSC for the focus of fish tissue collection are as follows:
Cutthroat Trout; Rainbow Trout, Peamouth Chub, Red Side Shiner, Northern Pikeminnow and Longnose/Largescale Sucker.

Burbot, Kokanee and Bull Trout (for trends and baseline analyses will also be collected for selenium analysis).

Species	Tenmile -spring Catch rate	Rexford Spring catch rate	Rexford Fall catch rate	Canada Fall Catch rate	Target catch rate / season/species	Actual Catch prediction	Stomach analysis	multiple tissue analysis	Spawning time
Bull Trout (Salvelinus confluentus)	11	50	1	1	5	5/5/1/1			
Kokanee (Oncorhynchus nerka)	3	24	74	124	5	3/5/5/5			Nov-Dec
Largescale sucker (Catostomus macrocheilus)	70			20	10	10/0/0/5	Y	Spring eggs / filet/whole body	April-May
Longnose sucker (Catostomus catostomus)	25	15	0	.4	10	10/10/0/1	Y	Spring eggs / filet/whole body	April-early July
Mountain White Fish (Prosopium williamsoni)	11			8					
Northern Pikeminnow (Ptychocheilus oregonensis)	73	290	160	143	10	10/10/10/5		Spring eggs / filet/whole body	May-Early July
Peamouth (mylocheilus caurinus)	192	886	160	180	10	10/10/10/5		Spring eggs / filet/whole body	May-June
Yellow perch (Perca flavescens)	1	14	1	11					April-May
RB Trout (Oncorhynchus mykiss)		11	4	4	10	0/10/5/5		Spring eggs / filet/whole body	Late April-early July "spring"
Westslope Cutthroat Trout (Oncorhynchus clarki lewisi)		2	1	5	10	0/5/5/5		Spring eggs / filet/whole body	Late April-early July "spring"
Burbot** (Lota lota)				.1	5	5			

Redside Shiner* (Richardsonius balteatus)	5	5	5		5/5/5	5/5/5	Yes focus	Spring eggs / filet/whole body	May-early August
Predicted Total analyses						186		250 analyses	

*Redside Shiners will be fished via electrofishing.

' fish per sampling event. An event is setting the gillnets overnight at each location outlined in the QAPP (attachment B).

**Burbot are occasionally caught in gillnets, in the case that they are found, they will be analyzed for selenium.

Table 3. Previous catch rates (catch rates from Jim Dunnigan, email communication, 2018). Fish for SeTSC focus are shaded in gray. The target catch rate/season/species column is the maximum number of fish we will pull from the nets/event for 2018 sampling. Based on past fishing efforts, the next column "Actual Catch prediction" estimates how many of each species/event we will catch for planning and cost estimates. The fish where stomach analysis will be performed on are indicated as are spawning times when eggs may be present.

All fish, excluding the Redside Shiner will be collected from annual gill-netting efforts conducted by FWP in 2018 during fall and spring on Lake Koocanusa (see attachment B for net locations).

All fish will be measured (total length (mm), weight (g)) and sampled in the field as described in "Mitigation for the Construction and Operation of Libby Dam, Annual Report 2012" (Dunnigan et al., 2014)- excerpt below. Fish will be sampled by lethal means (fish in nets are largely expired). If eggs are available in key species (see Table 3) they will be collected as described in the Fish Tissue Sampling Standard Operating Procedure, DEQ, 2015 (attachment D).

Gillnets: Sinking gillnets will be deployed in May at 14 locations (for past locations, see Table D1). Floating gillnets will be deployed at seven locations in the fall (Attachment B) in both the Montana and British Columbia portions of Lake Koocanusa. FWP retrieves the nets after soaking overnight by pulling them back into the boat and storing them in individual tubs marked by location. Tubs are then transported to shore, where a team of biologists and technicians remove fish from the nets, weigh, and measure each fish by net site. Dunnigan et al. (2014) provides additional methodology details related to gill net sampling on Lake Koocanusa. Once the nets are pulled from the reservoir and brought to shore, all fish in the nets will be measured, weighed and recorded by the FWP Libby crew for their population studies and reported on in their annual mitigation reports. Targeted fish for selenium analysis will be sorted out and processed as described below.

4.2 Fish Sample Preparation

Sources of field contamination to tissue should be identified and eliminated or minimized before the sampling event begins.

When fish are processed in the field, care should be taken to avoid contamination (away from exhaust in a clean area). Personnel must wear new, nitrile, disposable gloves prior to any contact with the

specimen. The sampling site, date, species, length (mm-TL) and weight (g) are measured and recorded in the field. If a clean area has been set up in the field, the filet can be extracted (or this can be done in a clean lab) from the right-hand side of the fish using a sterile (standard) fillet knife and cutting board and this should be noted on the site visit form (SVF) that the extraction was done in the field. For Se analysis, the filet is prepared skin off. Upon extraction, the muscle fillet sample is either placed in labeled tinfoil and placed in zip-lock style bag, or placed directly into a sterile amber glass jar with Teflon lid and placed in cooler on ice for submission to the laboratory for analysis. Between samples, the fillet knife is cleaned with phosphate free lab soap and water. The cutting surface is kept clean by frequent rinses, or by placing tinfoil on the surface of the board between samples. The sampling volumes, containers, preservation and holding time requirements for all samples collected are summarized in Table 6. The samples will be frozen at -20°C until the time of analysis.

Where eggs are found, they will be removed in the field or lab. The eggs will be collected as described in the DEQ 2015 Fish Tissue SOP (DEQ, 2015), as follows:

"Collection of fish eggs for sampling purposes may be desired for projects investigating concentrations of certain parameters. Field collection activities must coincide with the spawning times of the project's target species. Spawning times for species typical to Montana can be found in "Spawning Times of Montana Fishes," (Skaar, 2001). It may be difficult to achieve sufficient sample mass from small species or immature populations; therefore, SAPs should contain contingencies for these events.

If fish will be sacrificed, eggs can be harvested during dissection for collection of other tissues through the procedures for filleting and organ harvesting described above. Eggs are located along the gonads and typically form in rows. Manual stripping or spawning of eggs from ripe females should provide sufficient mass of eggs for sampling purposes and allow return of fish to the environment. Spawning activities should be performed in a manner that minimizes handling of fish and anesthetics should be used to reduce stress (Piper et al., 1982). Eggs themselves are delicate and can be damaged if fish are handled and/or spawned too roughly (Shrable et al., 1999).

Manually strip eggs as follows:

- Anesthetize fish if appropriate (see **Section 2.4.2**).
- Ready clean sample glassware. One large container to collect eggs in and distribute them to smaller containers may be appropriate.
- Rinse fish in ambient water, particularly in the area of the vent (see **Figure 3-1)** to remove any foreign matter and potential contaminants.
- Hold the fish about the head with one hand while positioning the vent over the receiving container and tilted slightly downward. Two individuals may be necessary to sample very large specimens–one person holding the fish and one person performing the stripping.
- Apply gentle pressure with the other hand to the ventral side beginning well behind the pectoral fin and moving slowly toward the vent. Direct the resulting stream of eggs into the sampling container.
- Room must be left in final sampling glassware for sample expansion upon freezing (Murphy, 2012).
- Label the samples in accordance with ***Section 7.2***, complete **Attachment A** – Fish Tissue SVF, freeze the samples as quickly as possible, and store at ≤ -20°C.

If the eggs are not easily stripped with gentle pressure, the fish is likely not ripe. Do not attempt to force stripping with excess pressure as harm to the fish and damage to the eggs may occur. Under this circumstance, the fish should be returned to the water without sampling or it will need to be sacrificed to obtain eggs."

For whole body analysis, the remaining carcass or whole fish will be wrapped in tinfoil, put in a large zip lock bag and placed on ice. Processing of whole body fish will be done in the FWP lab in Helena or Energy Labs in Helena, for details, please see below in section 5.

Lab Measurement	Container	Holding Time	preservation
Moisture			
Digestion for metals analysis			
Selenium (& Cd, Cu, Pb, As)	Whole fish or filets in aluminum foil and in zip top bag on ice Eggs in screw top jar (glass/HDPE) or whirl pac	6 months*	-20°C

* U.S. EPA, 2000

Table 4. Fish tissue containers/packaging, preservation and holding times

4.2.1 Lethal Method

If necessary, fish will be euthanized by a sharp, forceful blow to the head using a blunt, clean instrument. The force of the blow should be similar (slightly less) to that needed to drive a nail into wood (Erway et al., 2004). Fish from gillnets are typically already expired.

4.3 Deviations from QAPP

Any deviations from the QAPP will be reported to the field lead as soon as the deviation is identified. The field lead will inform the project managers, Selch, Mavencamp and the QA/QC officer, Hauer. The deviations will be recorded in writing and appended to the QAPP file (hard copy and on-line). If the deviations will affect laboratory processes and data, both the lab, the DEQ data manger, Jolene McQuillan, and the DEQ QA/QC officer, Michelle Hauer, will be notified. A plan to address the deviation will be sent to the appropriate individuals.

If changes to the QAPP are needed the QA/QC officer will determine if they significantly impact the technical and quality objectives of the project. If they do, Terri Mavencamp will modify the QAPP to document the change and submit the revision for approval. Following approval, the changes may be implemented.

5.0 Laboratory Analytical Measurements

Lab Measurement	Method	Reporting Limit (mg/kg)
Moisture	D2974A (modified dry 60-65C)	0.20 %
Digestion for metals analysis	EPA 3050	n/a

Selenium (Cd, Cu, Pb, As also using same method)	SW6020	0.50

Table 5. Laboratory methods and required reporting limits.

Fish Tissue moisture determination: The following excerpt is from the EPA Technical Support for Fish Tissue Monitoring for Implementation of EPA's 2016 Se Criterion # EPA820-F-16-007, Sep, 2016.

> *Egg and tissue samples should be thawed, and wet weight recorded for each individual sample. To prevent cross contamination between samples, a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is required to record the moisture content for each individual sample in order to express analytical data on a dry weight basis. Trace element (e.g., selenium) analysis is routinely performed using hydride generation atomic absorption spectrophotometry (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry-weight basis (Janz and Muscatello, 2008).*

Whole Body Fish Analysis: Whole body fish will be homogenized in a high-power blender before digestion and analysis either in the FWP lab or at the analytical laboratory. Fish may be cut into smaller 2.5 cm cubes with high-quality stainless steel or titanium knives prior to homogenization. Fish should be ground until homogenous in appearance. Percent moisture will be determined for the whole-body fish as performed for filets (see above). If the filet/eggs were extracted for separate analysis, the final whole-body percent moisture and selenium concentrations will be determined from the mass percent and concentrations the filet will be added back to the whole fish during homogenization and included in the analysis.

6.0 Quality Assurance and Quality Control Requirements

Data quality indicators (DQIs) need to be defined to ensure the quality of the data for decision-making. DQIs, which include precision, accuracy, representativeness, completeness, comparability, and sensitivity, are quantitative and qualitative criteria established for the data acquired within this design to assure it is of sufficient quality for its intended use. The DQIs for this project are defined below.

6.1 Precision

Precision is the degree of agreement between or among independent measurements of a similar property (often reported as relative percent difference [RPD]). This indicator relates to the analysis of duplicate laboratory or field samples. Duplicates document the effect of sample homogeneity and matrix limitations on method performance. This project will rely on analytical and field duplicates to assess precision based on their RPD.

$$\text{RPD (as \%)} = ((\text{Sample Result} - \text{Duplicate Result})/((\text{Sample Result}+\text{Duplicate Result})/2)) \times 100$$

For samples that come back at the RL or 5 times the MDL, the pairs should agree within 30%. For samples that come back at less than five times the RL the pairs should agree within +/-50%. If duplicates

fail the above criteria, all associated sample results will be qualified with a "J", which means estimated (for qualifiers, please see appendix C).

In addition to these duplicate composite splits, all labs are to perform and report on the standard suit of QC measures.

Performance criteria are based on overall data quality and not achieving the DQI for a single precision measure does not imply the data are unacceptable for use in the study.

Analytical Precision (Laboratory Duplicates)

Precision quality control (QC) for all laboratory methods will follow the frequency specified in the analytical method or as described in a laboratory quality assurance plan (LQAP). Precision for laboratory duplicates will be assessed by ensuring that the RPD is ≤30%.

Field Precision (Field Duplicates)

Field duplicates shall be collected for 10% of all samples collected. Precision for field duplicates will be assessed by ensuring that the RPD is ≤30%.

Fish duplicates will be taken either by two biopsies in one fish or by splitting the whole (sacrificed) fish into two samples.

If duplicates fail the above criteria, all associated sample results will be qualified with a "J" flag.

6.2 Accuracy

Accuracy is the degree of agreement of a measurement with a known or true value. To determine accuracy, a laboratory or field value is compared to a known or true concentration. Measures of accuracy include calibrations (accuracy over a range of values), laboratory control samples (LCS) and sample specific controls such as matrix spikes (MS).

Laboratories are responsible for method accuracy in initial and continuing calibrations in accordance with the analytical method requirements. LCS and MS are common measures of accuracy in analytical laboratories. LCSs are prepared by spiking reagent water with a known concentration of an analyte. The results are compared to the known value to determine a percent % Recovery.

$$\% \text{ Recovery (LCS)} = (\text{Analytical Result}/\text{True Value}) \times 100\%$$

Matrix Spikes are prepared by spiking a sample with a known concentration of an analyte. The results are compared to the known value to determine a percent % Recovery.

$$\% \text{ Recovery (MS)} = ((\text{Spiked Sample Result} - \text{Sample Result})/\text{Amount Spiked}) \times 100\%$$

Matrix spikes, calibration and continuing calibration and certified reference material measures should fall within the acceptance criteria specified for each method. If they do not, they should be rerun until compliance is achieved or results may be rejected from the lab and affect lab completeness and payment.

6.3 Representativeness

Representativeness is the expression of the degree to which data accurately and precisely represent an environmental condition in time and space. The selection of the sampling design (e.g., sample location, number of samples, and collection period) affects the monitoring project's representativeness. For this project, representativeness will be achieved by ensuring that spatial and temporal components are properly selected to adequately characterize the environmental condition and that this QAPP and field collection standard operating procedures (SOPs) are followed.

6.4 Completeness

Any loss of data due to site access issues, QC failures, or laboratory mistakes may result in no trend analyses, calculations of CFs, TTFs, or use in Se model development. To calculate completeness, compare the number of valid measurements completed (samples collected or samples analyzed) with those you originally planned to take. Our goal is to get 5 fish of each target species at each location and time collected. Because catches are variable and certain species prefer various locations in the reservoir, this goal may not be attainable. The completeness goal for this monitoring project is at least 90% of this goal collected and passing QC evaluation.

6.5 Comparability

Comparability is the extent to which data from one study can be compared directly to data from another study. To achieve a comparable result, both the field collection method and the analytical method must be comparable. This is achieved through the use of standardized sampling and analytical methods and by adhering to this QAPP, project sampling plans and SOP (DEQ, 2015).

6.6 Sensitivity

For this study, the MDL and ML will be used to define the sensitivity of each measurement process. The MDL is the minimum concentration that can be measured with 99% certainty that the analyte concentration is greater than zero. The procedures are given in 40CFR 136, Appendix B. The ML is the lowest concentration on the calibration curve or approximately the MDL x 3.18. The measurement quality objective for this fish tissue study is that the MDL and MLs are sufficiently sensitive to meet the reporting limits in Table 5. If the results fall between the MDL and ML, the results will be "J" flagged as estimates.

Analytical Sensitivity QC (Method Blanks)

Sensitivity quality control (QC) for all laboratory methods will follow the frequency specified in the analytical method or as described in a laboratory quality assurance plan and include continuing calibration verification standards (CCV) run at intervals during an analytical run. These should be the same standards as the calibration standards to be able to detect sensitivity changes during analysis. All data obtained while an instrument is out of control is not reportable and all samples must be reanalyzed or flagged with the appropriate qualifier and reported along with a narrative describing why the instrument was out of control and when/how corrective action was taken.

7.0 Data Analysis, Recordkeeping, and Reporting

7.1 Sample Handling and Custody

Field crews are responsible for the integrity of samples from the time of collection until shipment or drop-off to a laboratory. This responsibility includes proper preservation, labeling, sample custody documentation, and storage.

Samples will be hand delivered from the FWP field office in Libby to Helena by FWP staff. Once in Helena, the chain of custody section of the SVF will be updated and the fish stored in the freezer in the FWP laboratory. Samples will then be processed and hand delivered to Energy labs with the SVFs.

7.2 Sample Handling Procedures

After samples are collected and labeled, samples are placed in a clean cooler on ice. This temperature will be maintained until received by Energy Labs. The laboratory will keep samples at -20°C (or frozen) until the time of analysis.

7.3 Sample Labeling

All samples must have DEQ site visit stickers, unique Sample IDs, and should be clearly linked to the information on the SVF (attachment A). When multiple samples are made from one fish (i.e., filet, eggs, whole body (remaining carcass), the sticker must have a unique suffix for each tissue type, Filet = F1;F2, Egg = EGG and Whole body = WB.

7.4 Sample Custody

Custody documentation (SVF) will accompany all samples from the field to the laboratory (see Attachment A for the SVF with chain of custody information at the bottom). Field personnel will initiate custody documentation before samples are stored in the cooler and maintain the custody forms until the samples are shipped. The project lead will sign the custody documentation and inspect the integrity of the samples and documentation before shipment. Any missing information or discrepancies will be communicated to the field crew (if applicable). Once the laboratory or other recipient receives the samples, the recipient will sign the custody documentation. The custody will be tracked until the sample reaches Energy Labs and is analyzed. Each handler of the samples will inspect the integrity of the samples and documentation during the sample receipt. Any issues or discrepancies identified by the laboratory will be communicated to the project leads and field tech/laboratory coordinator.

7.5 Data Analysis

Data will be analyzed to determine the variation in selenium concentrations (muscle, egg/ovary, whole body) in each species and will be compared to past years. Typical statistical analysis will be run on the data (regression analysis, goodness of fit (R^2 analysis)) to determine the relationship between selenium concentrations in different body tissues, i.e., muscle and ovary, ovary and whole body, etc.

Conversion factors for each species will be determined where there are enough data and where the data can be plotted on a linear plot. A statistical relationship between egg-ovary and whole body (or muscle) or between filet and whole body will be determined for each species using ordinary least squares (OLS) linear regression of the matched pairs of measurements as described in the EPA aquatic life selenium criteria document on page 77 (EPA, 2016). Conversion factors that may be calculated from the data may be seen in Table 6.

Type of Analysis	Determination	Conversion Factor (s)
Fillet	Percent moisture, Se	Muscle to WB
Whole Body	Percent moisture, Se	muscle to whole body Egg/ovary to whole body
Egg/ovary	Percent moisture, Se	Egg ovary to whole body Egg ovary to muscle

Table 6. Possible conversion factors that may be calculated from the data.

In addition, the fish may be compared to Fish Consumption Advisories. For more details on this, please see the FCA Draft QAPP, 2018.

Fish meals/month	Fish tissue concentrations (ppm wet weight)	Fish tissue concentrations (ppm dry weight) using a 75% moisture
Unrestricted > 16	• 1.5	0-6
16	>1.5-2.9	>6.0- 11.6
12	>2.9-3.9	>11.6-15.6
8	>3.9-5.9	>15.6-23.6
4	>5.9-12	>23.6-48
1	>23-47	>92-188
None (<0.5)	>94	>376

Table 7. Selenium fish tissue concentration guidelines (EPA, 2000b) and approximate conversions between fish tissue selenium wet and dry weight for quick reference.

7.6 Reports to Management

A brief report with the results of this study will be submitted to DEQ management. In the report, a brief project quality audit/assessment from the audit of data quality will be submitted; and if any of the DQOs were not achieved, a corrective action will be outlined describing how failure to meet the DQOs may be avoided in future study plans.

Results of this study will be presented to the SeTSC/MRC by FWP in the next face-to-face meeting planned for October 2018, currently scheduled to take place in British Columbia. The data will also be available on the wiki site either included in the selenium data pull or as a reference to where the data is available.

7.7 Data Management

DEQ-funded data are entered into MT-eWQX where it is subsequently entered into the USGS/EPA Water Quality Portal for public access https://www.waterqualitydata.us// This project is to follow the WQPB "internal process." This internal process consists of writing a sampling and analysis plan (SAP) or quality assurance project plan (QAPP) and submitting it to the DEQ QA/QC officer. Once the sampling plan has been approved and filed, and the sampling is being undertaken, appropriate Site Visit Forms (SVFs) and any additional field forms need to be filled out so that laboratory results can be processed by WQPB staff. One SVF will be filled out for each site/net collection, and multiple Site Visit Codes will be placed on each SVF. A unique Site Visit Code sticker will be used for each fish collected. If multiple samples are created from one fish, the same Site Visit Code sicker will be used and a unique suffix will distinguish between the multiple samples (such as C1200-F1 and C1200-F2). If the samples are to be brought back to the FWP lab for further processing, please fill out the SVFs at the time the samples are packaged for the labs.

When samples are dropped off at the Energy Labs, each SVF is signed and handed over to the lab personal who takes the temperature of the sample and signs the sample in. The lab will make copies of the SVFs for their records and will return the original SVFs to the FWP/DEQ staff that delivered the samples. The copies need to be filed with WQPB administration (currently Deanna Tartum), who will enter the forms into our electronic records system.
For site visit forms, tissue sample forms and fish ID summaries, please see attachments or find files here G:\WQP\6_DataMgmt\3_EQuIS\EQuIS_Resources\EQuIS_Field_Forms\Masters.

9.0 References

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10.0 ATTACHMENTS

10.1 ATTACHMENT A: SITE VISIT FORM

Fish Tissue – Multiple Samples
Site Visit Form

Project ID: _____

Date: _____ Time: _____ Personnel: _____

Waterbody: _____ Location: _____

Station ID: _____ HUC: _____ County: _____ AUID: _____

Latitude: . Longitude: . Elevation: _____ ft m

Sample Information (for all samples collected unless otherwise noted in comments)	
Lab Analysis:	
Preservation:	Ice/-20C Freezer Other: _____
Extraction:	FIELD LAB

Site Visit Comments

Chemistry Lab Information		
Lab Samples Submitted to:	Account #:	Term Contract Number:
Invoice Contact:		
Contact Name & Phone:		EDD ☒ Format: MT-eWQX Compatible
1) Relinquished By & Date/Time:	1) Shipped By: Hand ☐ FedEx/UPS ☐ USPS ☐	1) Received By & Date/Time:
2) Relinquished By & Date/Time:	2) Shipped By: Hand ☐ FedEx/UPS ☐ USPS ☐	2) Received By & Date/Time:

Lab Use Only - Delivery Temperature: Wet Ice _____ °C Dry Ice _____ °C _____

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Fish Tissue – Multiple Samples
Site Visit Form Continued

Samples Collected	**Use one Site Visit Code sticker for each fish. Use the same Site Visit Code sticker for all the samples created from each fish.								
Species Codes: BULL=Bull Trout; KOK=Kokanee; LING=Burbot; LN SU=Longnose sucker; LS SU=Largescale sucker; N PMN=Northern Pikeminnow; PEA=Peamouth; RB=Rainbow Trout; RS SH=Redside Shiner; WCT=Westslope Cutthroat Trout									
Site Visit Code	**Sample Collection Method**	**Species**					**Length (mm)**	**Weight (g)**	**Sex**	**Eggs Present**	**Developmental Stage**
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	
	WHOLE FILLET EGGS	BULL KOK LING LN SU LS SU N PMN PEA RB RS SH WCT							M F	Y N	

10.2 Attachment B: Fish Sampling Locations from FWP for 2018

		Canada Gillnet Locations	**seasons**
Lat	Long	Site Description	Fall only
49.23524	-115.2718378	West of Ramp	
49.1991414	-115.2356747	North of Cabin (Campground)	
49.17813013	-115.2317702	Cabin	
49.16665998	-115.2311636	South Point Elk	
49.17628068	-115.2208864	North Point Elk	
49.19489758	-115.2164987	Across from Campground	
49.25347538	-115.2652918	South of Bridge	
49.27933877	-115.2725245	North & West of Kikomun (Sinker)	
		Tenmile Gillnet Locations	**Spring only**
		Site Description	
48.59060674	-115.2138805	South Point Ten Mile	
48.56416657	-115.2070315	Big Bend	
48.54680961	-115.2448403	North Point Bristow (Floater)	
48.53870172	-115.2649008	South Point Bristow	
48.505188	-115.288477	North Point Barron	
48.499751	-115.266774	South Point Warland	
48.468669	-115.310307	North Point Jackson	
48.422685	-115.300418	South Point Canyon	
		Rexford Gillnet Locations	**Fall and spring**
		Site Description	
48.87599549	-115.2103134	South Black Lake	Spring
48.88590903	-115.1993054	North Black Lake	Spring
48.89789834	-115.1853524	South of Far South Tobacco	Fall and Spring
48.90495353	-115.1797746	Far South Tobacco	Fall and Spring
48.9062048	-115.1741338	South Tobacco	Spring
48.91839349	-115.1757507	North Point Tobacco	Fall and Spring
48.93303993	-115.1554178	North of North Point Tobacco	Spring
48.95015213	-115.1538033	North Point Murray	Fall and Spring
48.960534	-115.1554967	South Sophie	Spring
48.97593677	-115.1398817	Middle Sophie	Spring

48.99008499	-115.1598875	North Sophie	Fall and Spring
48.96958238	-115.1785839	North Point Young Creek	Fall and Spring
48.94934256	-115.1855495	Sandhill	Fall and Spring
48.9308041	-115.1908913	North Point Dodge Creek	Spring

10.3 ATTACHMENT C: QUALITY CONTROL CHECKLIST

EDD - Activity

__ Lab EDD Activity IDs match the SVF/SUDS Activity IDs.

__ Lab EDD Activity Start Date and Time match the date/time the samples were collected.

__ Field duplicates and field blanks are clearly identified by an Activity Comment, Result Comment, and appropriate Activity Type.

Lab Report

__ Reporting detection limits meet the project-required detection limit defined in SAP.

__ Laboratory blanks/duplicates/matrix spikes/lab control samples were all within the required control limits defined within the SAP/QAPP. If any samples exceeded the control limits, the associated data is "J" flagged.

1.0 Lab Qualifier "S" (Spike recovery outside of advisory limits) = apply "J" flag with Result Comment "MS/MSD failed [high or low] (xx/xx%), expect [high or low] bias." Apply flag and comment to all associated results (in the same batch) that were detected above the LRL.

__ All method blanks are less than the project-required detection limit. If a method blank has a detect level at or above the reporting limit (LRL), then samples up to or equal 10x the detected value are "B" flagged with a Result Comment "Method blank contamination, results <[x.xx] mg/l are B flagged." The actual blank is not "B" flagged. Non-detects are not flagged.

EDD - Results

__ Holding times met. If any data exceeds the holding time, an "H" flag and Result Comment are added (such as "Sample exceeded EPA 7 day holding time.")

__ Ensure the Analytical Method ID matches the laboratory report and followed the method defined in the SAP.

__ Ensure approriate Result Value Units are used. Ensure that the Result Values and the Result Value Units correlate.

__ Ensure Characteristic ID, Characteristic Name, Method Speciation Name, and Sample Fraction are entered approriately and correctly. Refer to Appendix C in the data manager desk manual.

__ If the result value is between the MDL and the LRL, the result is "J" flagged and has a Result Comment "Result between MDL and LRL, J flagged as estimate."

__ All field blanks are less than the project-required detection limit. If a field blank has a detect level at or above the reporting limit (LRL), then samples up to or equal 10x the detected value are "B" flagged with a Result Comment "Field blank contamination, results <[x.xx] mg/l are B flagged." The actual blank is not "B" flagged. Non-detects are not flagged.

__ Field duplicates were all within the required control limits specified in the SAP/QAPP (usually if result value >5x LRL, then duplicates should be within 25% of each other). If any field duplicates exceeded the project-required control limits, all associated results are "J" flagged with a Result Comment "Field duplicate RPD >25% (xx%)." Or "Associated field duplicate RPD >25% (xx%)."

__ All samples requiring dilutions have a "D" result qualifier and the associated detection/reporting limits have the dilution factor applied.

__ For STREFPRO State Lab cations with a sample fraction of "Free Avail", add a Result Comment: "Analyzed directly from acid-preserved bottle without digestion." Only project allowed to have Free Avail sample fraction is STREFPRO.

__ All samples that have result qualifiers in the laboratory report have the appropriate qualifier, or equivilent qualifier, in the Result Qualifier field.

__ Ensure that Total Nitrogen results are greater than both Nitrate+Nitrite and Ammonia (within 10% is ok). If TN is less than the total, apply "J" flag and Result Comment describing why the Result Value is estimated.

__ Ensure that Total Phosphorus results are greater than SRP/Orthophosphate (within 10% is ok). If TP is less than SRP, apply "J" flag and Result Comment describing why the Result Value is estimated.

__ Ensure that total recoverable metal results for a particular analyte are greater than the dissolved fraction.

- When detected at normal levels, well above the reporting limit, always go with 10% since that is typical for an RPD limit if you were comparing duplicates. However, when it is a really low level, the 10% rule doesn't work. If they were different by more than the reporting limit for a low-level result, have the lab recheck the results.

__ Look for any unusual outlier data by analyte.

Data Qualifiers and Descriptions

Result Qualifier	Result Qualifier Description
B	Detection in blank.
D	Contract Required Quantitation Limit (CRQL) not met due to sample matrix interference, dilution required.
H	Holding time exceeded.
J	Estimated: The analyte was positively identified and the associated numerical value is the approximate concentration of the analyte in the sample.
L	Lowest available reporting limit for the analytical method used.
R	Rejected: The sample results are unusable due to the quality of the data generated because certain criteria were not met. The analyte may or may not be present in the sample.
**Any combination of these qualifiers can be associated with each result value.	

Quality Control Terminology and Descriptions

FIELD QC		
Term	**Description**	**Purpose/Usage**
Trip Blanks	Prepared at the lab prior to the sampling event and kept with the collected samples throughout the sampling trip.	To determine if cross contamination occurs between samples and identify contaminants that may be introduced into samples during transit to and from the lab.
Field Blank	Prepared in the field with lab water and kept with the collected samples throughout the sampling trip.	Monitors contamination resulting from field activities and or ambient levels of analytes present at time of sampling.
Field Duplicate	Two independent samples taken under the same conditions. Water samples would be two independent samples taken at the same location at the same time.	To determine the homogeneity of the samples collected.
LABORATORY BATCH QC		
Acronym	**Description**	**Definition**
LRB/Method Blank	Laboratory Reagent Blank	An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present.

LFB/LCS	Laboratory Fortified Blank; Laboratory Control Sample	Reagent water spiked with a known amount of analyte. Ideally treated exactly like a MS/LFM. Control used to determine bias in sample spikes.
MS/LFM	Matrix Spike/Laboratory Fortified Matrix	An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations
MSD/LFMD	Matrix Spike Duplicate/Laboratory Fortified Matrix Duplicate	Determine method precision in sample concentrations are < 5X the RL.
DUP	Duplicate	Determine method precision in sample concentrations are > 5X the RL.
QCS	Quality Control Sample	A solution of method analytes of known concentrations which is used to fortify an aliquot of reagent water or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance
SRM	Standard Reference Material	Primarily used as a QCS to verify instrument calibration.

LABORATORY ANALYSIS QC

Acronym	Description	Definition
ICB	Initial Calibration Blank	Monitors instrument drift at low end of calibration curve.
CCB	Continuing Calibration Blank	Monitors instrument drift at low end of calibration curve.
ICV	Initial Calibration Blank	Monitors instrument drift at a defined concentration near the mid range of calibration curve.
CCV	Continuing Calibration Blank	Monitors instrument drift at a defined concentration near the mid range of calibration curve.
IPC	Instrument Performance Check	Monitors instrument drift at a defined concentration near the mid range of calibration curve.
MS/LFM	Matrix Spike/Laboratory Fortified Matrix	An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations
MSD/LFMD	Matrix Spike Duplicate/Laboratory Fortified Matrix Duplicate	Determine method precision in sample concentrations are < 5X the RL.

DUP	Duplicate	Determine method precision in sample concentrations are > 5X the RL.
QCS	Quality Control Sample	A solution of method analytes of known concentrations which is used to fortify an aliquot of reagent water or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance
SRM	Standard Reference Material	Primarily used as a QCS to verify instrument calibration.
IDL	Instrument detection limit	Signal just above baseline. 3-5x the STD DEV of 7 replicates of a blank. Not used for quantification.
MDL	Method detection limit	Statistical determination of the lowest concentration of an analyte with 95% certainty the analyte is present.
PQL	Practical Quantitation Limit	3-5x the MDL. Lowest level that quantification is determined
RL	Reporting Limit	Value a Laboratory reports results. Usually the PQL.

11.0 APPENDICES

11.1 APPENDIX A: LABORATORY QC SECTION, SELENIUM IN TISSUE:

A non-routine QC check was done for the following three laboratories that DEQ/FWP have used for fish tissue analysis: contract FWS lab Envirosystems Inc.; Energy Labs, Helena MT; and the Department of Public Health and Human Services lab (HHS), Helena, MT. The certified reference material, DORM-4 was purchased from the National Research Council of Canada and the specs are shown below https://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/dorm_4.html .

The material will be thawed slightly (from -20°C) and subdivided into three portions weighing 5 g each and sent on ice overnight to the FWS lab and hand delivered to Energy and Human Health and Environmental labs. Each standard sample will be delivered with approximately 4 other fish tissue samples, and the analytes shown in table D-1 are requested: The estimated cost is 500-600$/lab.

Lab Measurement	Method	Reporting Limit (mg/kg)
Moisture	D2974	0.20
Digestion for metals analysis	EPA 3050	n/a
Selenium	SW6020	0.50 dry weight
Cadmium		.088 wet weight
copper		1 wet weight
lead		.02 wet weight
arsenic		.02 wet weight
Mercury	SW7471B	0.029 wet weight

Table A1: Requested Analytes for QC check of labs and requested reporting limits.

Element	(mg/kg)
Arsenic (b,d,f)	6.87 ± 0.44
Cadmium (a,d)	0.299 ± 0.018
Calcium (d,e)	2360 ± 140
Chromium (a,d,e)	1.87 ± 0.18
Copper (a,d,e)	15.7 ± 0.46
Iron (a,d)	343 ± 20
Lead (a,b,d)	0.404 ± 0.062
Magnesium (d,e)	910 ± 80
Manganese (b,d,e)	3.17 ± 0.26
Mercury (a,c,g)	0.412 ± 0.036
Nickel (a)	1.34 ± 0.14
Potassium (d,e)	15 500 ± 1000
Selenium (a,d,f)	3.45 ± 0.40
Silver (a,d)	0.0252 ± 0.0050
Strontium (d,e)	10.1 ± 0.8
Vanadium (d,e)	1.57 ± 0.14
Zinc (a,d)	51.6 ± 2.8

Table A-2 Certified reference material, DORM-4 specs purchased from the National Research Council of Canada.

Appendix E

Derivation of a Site-Specific Water Column Selenium Standard for Lake Koocanusa.

September 2020

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EXECUTIVE SUMMARY

This document provides the Montana Department of Environmental Quality's scientific framework and recommendations for site-specific selenium water quality standards for Lake Koocanusa. The proposed standards are designed to protect fish as the most sensitive ecological endpoint, including federally listed threatened species, from effects of elevated levels of selenium. The standards described herein reflect the latest science on the toxicological effects of selenium. This document considered the United States Environmental Protection Agency's 2016 304(a) National Recommended Water Quality Criteria to develop site-specific selenium criteria, whenever feasible, and their guidance to states on developing site-specific criteria as described in Appendix K of, *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*.

The proposed criteria for Lake Koocanusa consist of a site-specific water column value and 304(a) recommended fish tissue values. The site-specific water column value is based on biodynamic selenium modeling using the United States Geological Survey Ecosystem-Scale Selenium Model. The proposed values are presented below.

Proposed Selenium Water Quality Criteria for Lake Koocanusa, Montana.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

ACKNOWLEDGEMENTS

This work was guided and directed by the collaborative state and provincial efforts of the Lake Koocanusa Monitoring and Research Working Group; in particular, the collective knowledge of the Monitoring and Research Committee and the expertise of the scientists recognized for their work in selenium who participated on the Selenium Technical Subcommittee. For the coordinated monitoring efforts, we would like to thank Montana Fish Wildlife and Parks, United States Army Corps of Engineers, United States Geological Survey, United States Fish and Wildlife Service, British Columbia Ministry of Environment and Climate Change Strategy, and Teck Resources Limited. We would like to thank the United States Geological Survey Selenium Modeling Team. We acknowledge and appreciate the British Columbia Ministry of Environment and Climate Change Strategy for the staff support and overall collaboration and participation throughout this multi-year long process. Without the joined efforts between the Department of Environmental Quality and the British Columbia Ministry of Environment staff this work would not have been possible.

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ACRONYMS

Acronym	Definition
ABMP	Area Based Management Plan
ARM	Administrative Rules of Montana
BAF	Bioaccumulation factor
BC	British Columbia
BC-ENV	Ministry of Environment and Climate Change Strategy
CWA	Clean Water Act
CSKT	Confederated Salish and Kootenai Tribes
DEQ	Department of Environmental Quality (Montana)
dw	Dry weight
EF	Enrichment factor
EO	Egg-ovary
EPA	United States Environmental Protection Agency
ESA	Endangered Species Act
EVWQP	Elk Valley Water Quality Plan
K_d	Partitioning coefficient
Kg	Kilogram
KNC	Ktunaxa Nation Council
KTOI	Kootenai Tribe of Idaho
LKMRC	Lake Koocanusa Monitoring and Research Committee
LKMRWG	Lake Koocanusa Monitoring and Research Working Group
LOEC	Low Effect Concentration
MT	Montana
ppb	Parts per billion (equivalent to micrograms per liter in aqueous solutions)
ppm	Parts per million (equivalent to milligrams per liter in aqueous solutions)
Se	Selenium
SOP	Standard Operating Procedure
SETSC	Selenium Technical Subcommittee (of the LKMRC)
SOP	Standard Operating Procedure
TAC	Technical Advisory Committee
TTF	Trophic transfer factor
TSI	Trophic state index
USACE	United States Army Corps of Engineers
USGS	United States Geological Survey
µg/L	Micrograms per liter
WQG	Water Quality Guideline
WQPB	Water Quality Planning Bureau
WQSM	Water Quality Standards and Modeling Section

1.0 INTRODUCTION

This document presents the scientific basis used for the development of the site-specific water column selenium standard for Lake Koocanusa, MT. This work was a collaborative effort between the Montana Department of Environmental Quality (DEQ), the British Columbia (BC) Ministry of Environment and Climate Change Strategy (BC-ENV), the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), and a Selenium Technical Subcommittee (SeTSC).

1.1 BACKGROUND

In 2010, a Memorandum of Understanding and Cooperation (MOUC) between BC and MT was drafted to end the decades-long dispute over transboundary mining in the Flathead Valley. The MOUC expanded collaboration on environmental protection and assessments that have bi-national significance. Coordinated efforts began between BC-ENV and DEQ to address regional transboundary water quality issues including those in the Elk Valley, BC.

In 2012, DEQ added Lake Koocanusa to the 303(d) list of impaired or threatened waterbodies, as threatened by selenium (DEQ, 2012). In April 2013, the BC Minister of Environment issued a Ministerial Order (No. M113) under the Environmental Management Act to remediate water quality effects of past mining activities and to guide environmental management of future mining activities in the Elk Valley, including the Canadian portion of Lake Koocanusa.

This Order mandated the development of an area-based management plan (ABMP) due to evidence of increasing concentrations of water quality constituents of potential concern, including but not limited to selenium (Se), from numerous sources related to mining activity in the Elk Valley watershed https://www2.gov.bc.ca/gov/content/environment/waste-management/industrial-waste/mining-smelting/teck-area-based-management-plan. As the sole operator of the five coal mines in the Elk Valley, Teck Resources Limited (Teck) was required by the Order to develop the Elk Valley ABMP according to requirements outlined in the Order. Agencies from Canada and the US participated in a Technical Advisory Committee (TAC)—established as a condition under the Order—to provide science-based technical advice to Teck during the development of the Elk Valley Water Quality Plan (EVWQP). The EVWQP was submitted by Teck to BC-ENV in July 2014 and revised by BC-ENV in 2019. The EVWQP established short, medium, and long-term water quality targets for Se (and other constituents) at specific order stations in the Elk Valley, including a station in Lake Koocanusa (LK2) where the target for Se was set at 2 µg/L (equivalent to the BC Water Quality Guideline (WQG)). These water quality targets were incorporated into enforceable limits under Permit No. 107517, issued to Teck on November 19, 2014 (**Table 1-1**).

Table 1-1. Effluent limits for Order Stations in the Elk Valley (Teck, 2014).

Management Unit	Order Stations	Selenium (µg/L)
1	FR4	57
2	FR5	40
3	ER1	19
4	ER2	19
5	ER3, ER4	19
6	LK2	2

At the conclusion of the EVWQP development process, the TAC recommended that a site-specific ecological effects assessment be completed to evaluate whether the BC WQG set at 2 µg/L for Se is protective for Lake Koocanusa. Although BC WQGs are designed to be protective of aquatic life and wildlife, WQGs do not account for site-specific factors, so the TAC recommended that an ecological effects assessment be conducted for Lake Koocanusa. The TAC concluded that Se bioaccumulation in organisms is affected by site-specific factors and has potentially irreversible consequences (i.e. extirpation of species). In addition, current concentrations of Se in Lake Koocanusa were recorded above the BC alert threshold of 1 µg/L promulgated by BC-ENV during the same time period that draft EPA 304(a) Se criteria—released in 2015 and finalized in 2016—showed that a protective Se water quality concentration for aquatic life in lentic waters was 1.5 µg/L (**Table 1-2**). EPA also provided a recommendation that site-specific Se criteria be developed whenever possible.

TAC recommendations on Lake Koocanusa and government-to-government discussions on transboundary impacts resulted in formal commitments by BC-ENV to DEQ and the EPA to establish a process to assess whether a Se target of 2 µg/L at Lake Koocanusa is protective, and to provide a forum for discussing other water quality issues relevant to Lake Koocanusa. To meet this commitment, BC-ENV proposed the establishment of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG).

As directed by the Steering Committee of the LKMRWG, a Selenium Technical Subcommittee (SeTSC) was established in 2015 with selenium experts from both the US and Canada. In 2019, technical representatives from the Ktunaxa Nation Council (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI) joined the SeTSC. The SeTSC multi-agency, multi-institutional, international team of experts worked with DEQ and BC-ENV to develop the scientific basis for a site-specific Se criterion in Lake Koocanusa. The multi-year collaborative effort included research goals and methodology, coordinated monitoring plans, Se biodynamic modeling, and Se criteria recommendations. The technical work of the SeTSC was routinely reported to the Monitoring and Research Committee (MRC) and the Steering Committee. **Figure 1-1** describes the structure of the LKMRWG.

The goal of this coordinated effort, is a MT and BC co-developed site-specific Se criteria for Lake Koocanusa detailed in this document.

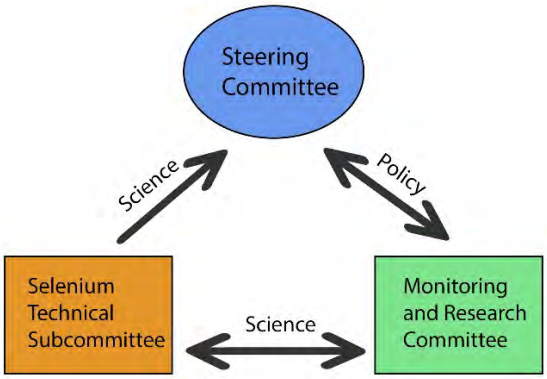


Figure 1-1. Structure and information flow of the LKMRWG.

1.2 PURPOSE

The purpose of this document is to provide the technical framework for the derivation of a site-specific selenium standard for Lake Koocanusa, MT. Montana is required under section 303(c)(2)(B) of the federal Clean Water Act (CWA) to establish water quality criteria for toxic pollutants, for which selenium is listed in section 307(a)(1). In adopting criteria, Montana is authorized to establish numeric values based on CWA Section 304(a) guidance modified to reflect site-specific conditions.

1.3 EXISTING SELENIUM WATER QUALITY STANDARDS

This section details the existing Se water quality standards for Montana, the 304(a) criteria, and British Columbia's Se WQG's defined in **Table 1-1**.

Table 1-2. Selenium water column thresholds applicable to Lake Koocanusa.

Agency	Target (µg/L)	Description
EPA	1.5	2016 updated criterion for dissolved Se in lentic systems
ENV	2	2014 Established guideline for aqueous Se
ENV	1	2014 Established Alert level for aqueous Se
DEQ	5	Chronic standard for dissolved Se established based on 1987 EPA guidance
DEQ	20	Acute standard for dissolved Se established based on 1987 EPA guidance

1.3.1 Montana's Surface Water Quality Standards for Selenium

Existing Se water quality standards are found in Department Circular DEQ -7 (June 2019 edition) which is incorporated by reference into the Administrative Rules of Montana (ARM) title 17, chapter 30, subchapter 6. The current acute (20 µg/L) and chronic (5 µg/L) water column standards were established based upon the 1987 EPA Ambient Water Quality Criteria for Selenium (U.S. EPA, 1987).

Water quality standards are designed to protect the beneficial uses of a given waterbody. The state of Montana has classified waterbodies based on the beneficial uses they are expected to support, as found in ARM 17.30.621 through 17.30.629. Lake Koocanusa is classified as a B-1 waterbody (ARM 17.30.609). State law requires that waterbodies in a B-1 use class be suitable for drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply. The most sensitive beneficial use for Se in Lake Koocanusa is growth and propagation of salmonid fishes and associated aquatic life.

Section 305(b) of the federal CWA requires states to assess waterbodies to determine whether the waterbodies are supporting their beneficial uses. In 2012, DEQ listed Lake Koocanusa as threatened by Se. Montana defines a threatened waterbody as currently meeting water quality standards and supporting the beneficial uses, but standards are likely to be exceeded and beneficial uses threatened if current trends continue.

1.3.2 National Ambient Water Quality Criteria for Selenium

The CWA section 304(a) requires the EPA to develop water quality criteria using the best available science. While the EPA requires states to consider their recommendations (40 CFR part 131) when adopting water quality standards, it is a recommendation only and the state must adopt the criterion into state water quality standards for it to become a regulation.

The science on Se toxicology has significantly advanced over the last thirty years. The EPA first issued recommended water quality criteria for Se in 1980, with revised criteria issued in 1987. In 1996, the acute criterion was updated to account for toxicity of two Se species, selenite and selenate. In 2004, EPA released the first draft update to the 1996 Se criterion which included fish tissue criteria to account for a dietary exposure pathway. In 2015, EPA released a draft update to the 2004 criteria, and it was finalized in 2016 as *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater* (U.S. EPA, 2016).

The 2016 document considers the latest scientific information and provides guidance to states and tribes to protect freshwater aquatic life from toxic effects of Se. It is based on dietary exposure and accounts for reproductive effects in fish. The 2016 recommended criteria—which are derived for the protection of 95% of species nation-wide—is comprised of four elements (**Figure 1-2**). Two elements are fish tissue concentrations and two are water column concentrations. The elements are defined below:

1) a fish egg-ovary element,
2) a fish whole-body or muscle element,
3) a water column element (one for lentic and one for lotic) and
4) an intermittent element for short term exposures

EPA's 2016 guidance document recognizes that selenium bioaccumulation and toxicity in an ecosystem are based on site-specific environmental factors, therefore, the EPA provides additional guidance and methodology in Appendix K for states and tribes to follow when deriving site-specific criteria.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg/Ovary [2]**	**Fish Whole Body or Muscle [3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. Water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and fint is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

Figure 1-2. Selenium water quality criteria (U.S. EPA, 2016).

1.3.3 British Columbia's Water Quality Guideline for Selenium

BC-ENV is responsible for developing province-wide ambient WQGs. Per BC-ENV Policy 6.10.03.02, WQG's are defined as:

"A maximum and/or minimum value for a physical, chemical or biological characteristic of water, sediment or biota, applicable province-wide, which should not be exceeded to prevent specified detrimental effects from occurring to a water use, including aquatic life, under specific environmental conditions."

BC's aquatic WQGs are science-based and intended for provincial-wide application. BC-ENV applies an appropriate assessment factor determined on a case-by-case basis, according to the toxicity and bioaccumulation potential of the constituent (B.C. ENV, 2014). The assessment factor is the best estimate of a no-effect concentration, for the protection of 100% of species during all life stages. WQGs are not legally enforceable themselves but are used to provide scientific basis for best management practices and may be incorporated into permits (such as Elk Valley Permit No. 107517).

BC-ENV updated their Se Water Quality Guidelines (WQGs) in 2014 which includes an assessment factor of 2 pertaining to Se. (**Table 1-3**).

Table 1-3. BC Provincial WQG for selenium (B.C. ENV, 2014).

Water Use		Updated 2012 BC Se WQG	2001 Approved BC Se WQG	Guideline Derivation Method/Approach
Source Drinking Water		10 µg/L	10 µg/L	*Source Drinking Water:* Adopted from Health Canada; a maximum acceptable concentration of 10 µg/L to protect against adverse effects in humans from excessive exposure.
Human Consumption Screening Values				
High fish intake (0.22 kg/day)		1.8 µg/g (ww), 7.3 (dw)[1]	None proposed	*Tissue Consumption*: Values were derived using Health Canada's recommended equation for ingestion of Se-contaminated fish and the dietary reference value's tolerable upper intake.
Moderate fish intake (0.11 kg/day)		3.6 µg/g (ww), 14.5 (dw)	None proposed	
Low fish intake (0.03 kg/day)		18.7 µg/g (ww), 75.0 (dw)	None proposed	
Aquatic Life				*Water column*: Review of previous WQG (uncertainty factor (UF) applied to toxicity threshold); weight of evidence including food web modelling and reported relationships between impacts and Se concentrations in water.
Water column freshwater & marine				
Alert concentration	1	µg/L	None proposed	
Guideline	2	µg/L	2 µg/L	
Sediment - Alert concentration		2 µg/g (dw)	None proposed	*Sediment*: Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time.
Dietary				*Dietary:* Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time. Invertebrate tissue as surrogate for aquatic dietary tissue.
Invertebrate tissue (interim)		4 µg/g (dw)	2 µg/g (dw)	
Tissue (fish)				*Egg/ovary:* Combination weight of evidence and mean of published effects data with an UF of 2 applied; *Whole-body*: previous WB guideline compared with published literature, mean of published effects data with UF (2) applied and weight of evidence; *Muscle*: WB translation to derive muscle WQG, no additional UF applied to muscle guideline.
Egg/ovary		11 µg/g (dw)	None proposed	
Whole-body (WB)		4 µg/g (dw)	4 µg/g (dw)	
Muscle/muscle plug (interim)		4 µg/g (dw)	None proposed	
Wildlife				The *water column* guideline for aquatic life (fish) is adopted for wildlife since dietary accumulation is most critical. *Bird eggs* were used as surrogate for all wildlife; weight of evidence; egg Se most direct/sensitive measure; mallard EC10 with UF of 2 applied.
Water column		2 µg/L	4 µg/L (maximum)	
Bird egg		6 µg/g (dw)	7 µg/g (dw)	
Recreation and Aesthetics		None proposed	None proposed	No data
Irrigation Water				Not updated at this time
2001 guideline not updated		10 µg/L	10 µg/L	
Livestock Watering				Not updated at this time
2001 guideline not updated		30 µg/L	30 µg/L	
Industrial Water		None proposed	None proposed	No data

1.4 SELENIUM

Selenium is a member of Group 16, the chalcogen group on the periodic table of elements. Classified as a nonmetal, Se has properties of both metals and nonmetals. Selenium is considered chemically similar to other nonmetals in Group 16, for example sulfur (S) (Chapman et al., 2010). Selenium, a naturally occurring trace element essential for life, has a narrow margin between the amount necessary for proper functioning of organisms and the amount considered toxic (Janz, 2011). The toxicological potential of Se is strongly related to its chemical form (speciation).

1.4.1 Physical-chemical properties

Selenium exists in four oxidation states; elemental Se (0), selenite (+4), selenate (+6), and selenide (-2) (**Table 1-4**). Three primary transformation mechanisms occur for Se; 1) oxidation/reduction, 2) mineralization/immobilization, and 3) volatilization with Se speciation, with microbial activity and pH-redox conditions driving the kinetics of each function.

In aquatic ecosystems there exists three main fates for Se; it can 1) remain in solution, 2) be assimilated by organisms, and 3) it can be sorbed to suspended sediment. The oxyanions, selenate and selenite, are the dissolved inorganic forms of Se most commonly present in waters. It is generally understood that

selenite is less soluble, more easily transformed, assimilated by organisms, and considered to have a greater toxicity in aquatic systems than selenate. When taken up by organisms, Se can replace S in two amino acids, thought to contribute to toxicity in organisms particularly in oviparous (egg-laying) vertebrates (EPA, 2016). Finally, selenate and more commonly selenite can sorb to organic matter, clays, and oxides and hydroxides most frequently associated with the ions of iron (Fe), manganese (Mn), and sodium (Na). EPA (2016) provides significant detail on both the biochemical and the geochemical pathways of selenium in aquatic systems. It is clear that an understanding of the biogeochemical transformations of selenium is essential to understanding the fate, transport, and toxicological effects of selenium in the environment.

Table 1-4. Examples of the forms of selenium found in the environment (B.C. ENV, 2014).

Name	Valence/ Oxidation State	Forms/Se Species	Occurrence
Selenides	-II, Se^{II-}, Se^{2-}	Inorganic selenides, (Se^{2-}, HSe^-)	Found in reducing environments, sorbed onto soil/mineral particles, e.g., ferroselite ($FeSe_2$), chalcopyrite ($CuFeSe_2$)
		Hydrogen selenide, H_2Se	Unstable highly toxic gas, converts to Se^0 in H_20
		Organic selenides, R_2Se	
		Volatile organic selenides:	
		dimethyl selenide (DMSe), $(CH_3)_2Se$;	Gas, volatilization from soil/sediment bacteria and fungi
		dimethyl diselenide (DMDSe), $(CH_3)_2Se_2$;	Gas, volatilization from soil/sediment plants
		dimethyl selenone $(CH_3)2SeO_2$	Volatile metabolite, intermediate form between DMSe and DMDSe
		Biochemical intermediates, amino acids	Many forms, but most common are the amino acids selenomethionine (SeMet) and selenocysteine (SeCys)
Elemental selenium	0, Se^0		Insoluble, fairly stable, unweathered mineral form of Se, found in water, soil, sediment and biological tissue
Selenium dioxide	+II, Se^{+II}, Se^{+2}	SeO_2	Gas, not a naturally occurring form, product of fossil fuel combustion (coal, oil, gas), and smelting, soluble, forms selenous acid with water
Selenites/selenous acid	+ IV, Se^{+IV}, Se^{+4}	SeO_3^{2-}	Soluble, found in mildly oxidizing conditions in air, water, soil/sediment,
		Hydrogen selenite ($HSeO_3^-$)	Common form of selenites in soils, easily sorbed onto iron(hydr)oxide minerals $Fe(OH)SeO_3$, or other ions e.g., sodium selenite Na_2SeO_3, highly mobile and available to plants
		Selenous acid (H_2SeO_3)	
Selenates/selenic acid	+ VI, Se^{+VI}, Se^{+6}	SeO_4^{2-}	Common form of Se in surface water and soils, very soluble in water, stable in well-oxygenated water, not easily transformed biologically to more reduced forms, reduction reactions slow. In plants, selenate is actively transported against electrochemical potential gradient.
		Hydrogen selenate $HSeO_4^-$	
		Selenic acid H_2SeO_4	

1.4.2 Selenium Toxicity to Wildlife

Selenium bioaccumulates in wildlife primarily through a dietary pathway with egg-laying vertebrates determined to be the most sensitive. EPA (2016) outlines the differences in bioaccumulation between lentic (lake-like) and lotic (flowing-water) systems. Retention time, dissolved oxygen, and carbon content result in greater bioaccumulation in lentic systems. It is understood that while Se is nutritionally required in small quantities, it becomes highly toxic in slightly greater amounts with the potential to cause rippling effects through both aquatic and terrestrial food webs (Naslund et al., 2020). **Figure 1-3** describes selenium toxicity for fish and birds. Fish are considered the most sensitive ecological end point in Lake Koocanusa as determined by the SeTSC (see **Section 3.7**), therefore, fish are the focus of this report and the development of the Se standards for Lake Koocanusa.

Selenium toxicity in fish is most severe at the reproductive stage where newly hatched larval fish may experience teratogenic deformities and death while feeding off yolk sacs enriched in Se (Lemly 1993, Skorupa 1998). Extremely high concentrations of Se can be lethal to adult fish but this is not common; rather, more commonly, fish are exposed to various sublethal effects (**Figure 1-3**).

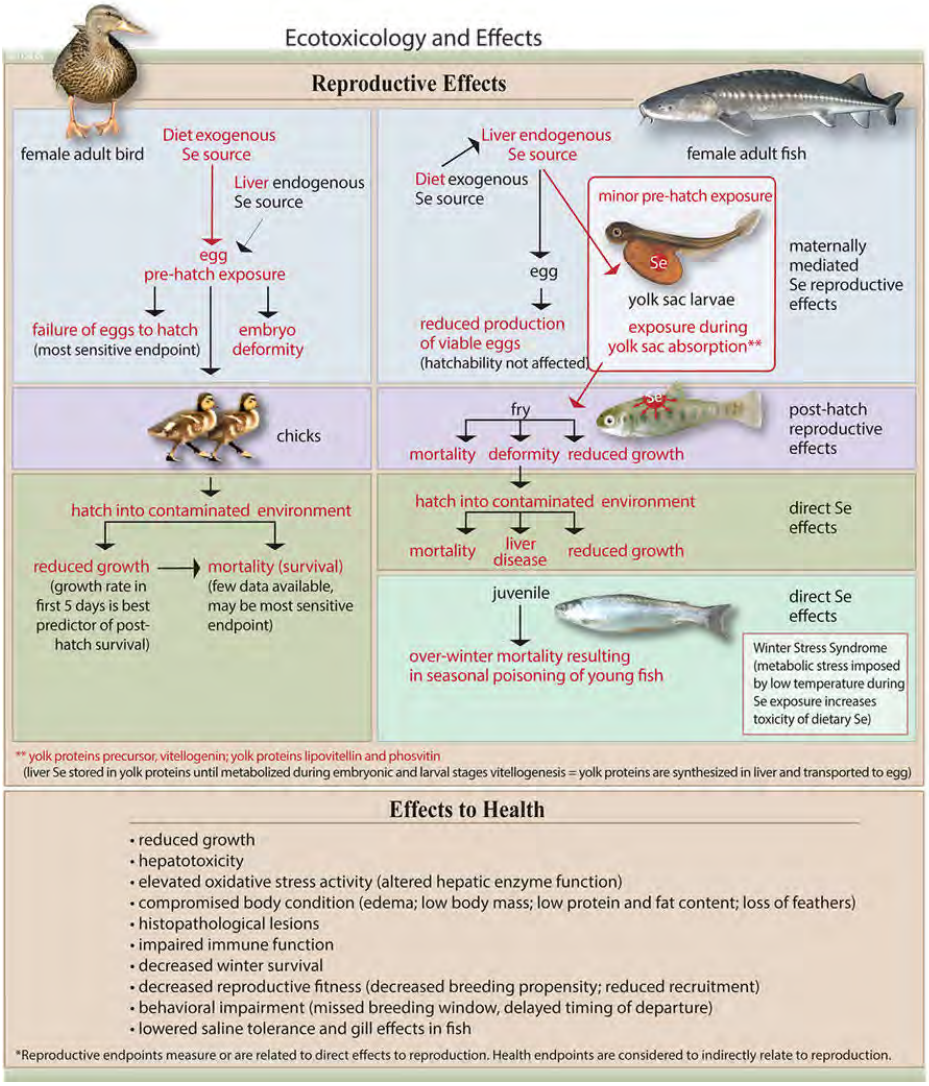


Figure 1-3. Details of general ecotoxicological pathways of selenium for fish and birds (top) and effects of concern for selenium (bottom). As represented here, birds and fish differ in how selenium is taken up in the diet and distributed among tissues (Presser and Skorupa, 2019).

1.4.3 Sources of Selenium

The primary source of Se to Lake Koocanusa is anthropogenic release to the environment from historic and present-day mining operations in the Elk Valley, BC. Coal in the Elk Valley belongs mainly to the Mist Mountain Formation of the Jurassic-Cretaceous Kootenay Group and is part of the East Kootenay coalfields (Grieve, 1952). Carbonate bedrock in the Elk Valley is excavated to access coal seams underneath for metallurgical steelmaking coal production. The excavation process creates a by-product

called overburden (waste rock) which becomes exposed to oxidation, increasing selenium mobilization through infiltration and runoff to nearby groundwater and surface water including the Elk River, BC.

Currently, Teck is the sole operator of five active coal mines in the Elk Valley and permitted under Elk Valley Permit No. 107517 (**Figure 1-4**). That permit authorizes Se concentrations at sites throughout the Valley (**Table 1-1**). **Figure 1-5** illustrates the location of Teck's existing coal mines in relation to coal bearing strata in the Kootenai (Kootenay) River Watershed.



Figure 1-4. Teck's Operations in the Elk Valley Watershed (Teck, 2017).

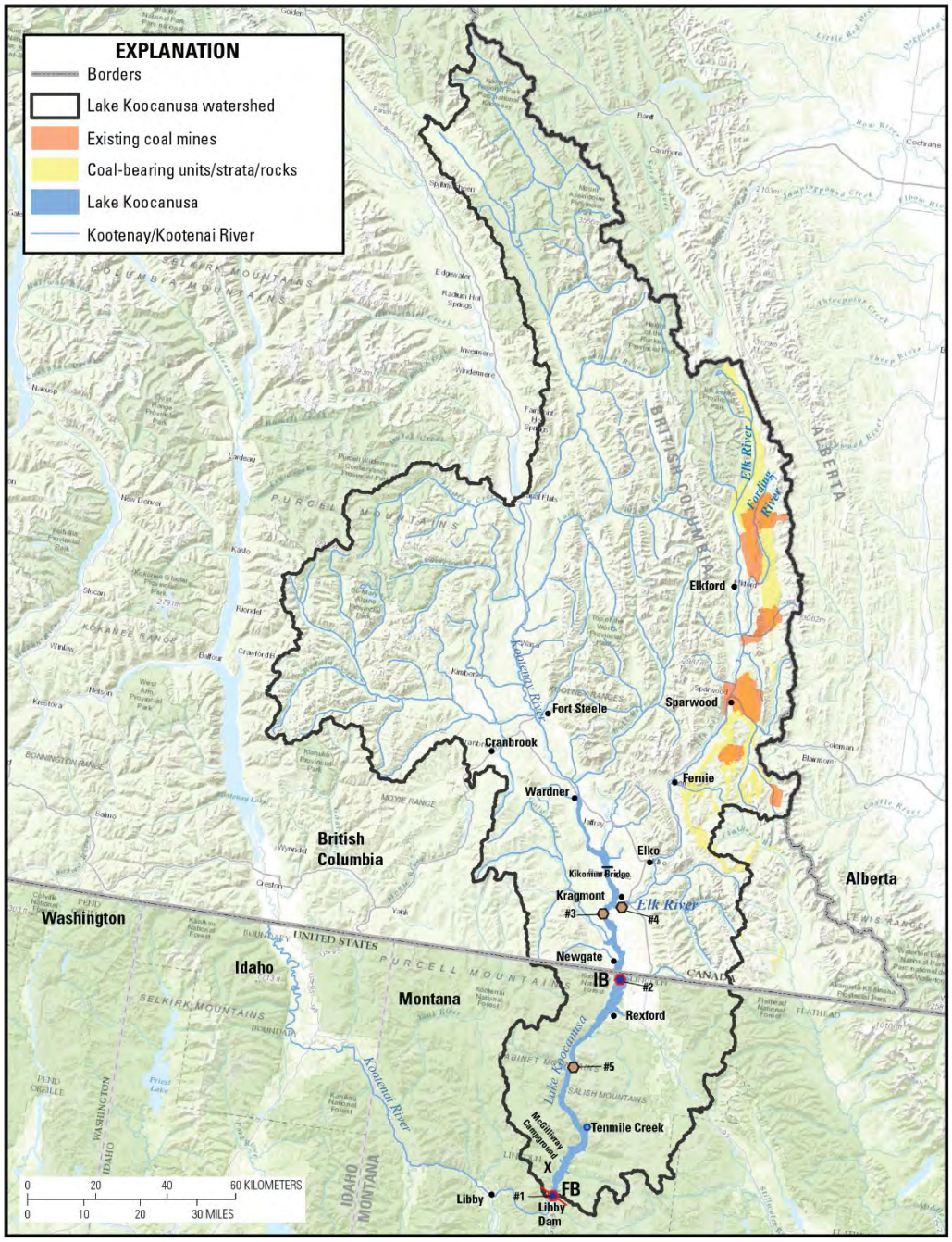


Figure 1-5. Teck's existing coal mines and location of coal bearing strata within the Kootenai (Kootenay) River Watershed (Jenni et al., 2017).

From 1984 to 2019, concentrations ranging from below detection level (DL) to greater than 8 µg/L have been recorded 2.2 miles upstream from Lake Koocanusa on the Elk River, a tributary (**Figure 1-6**). Selenium contributions from the Kootenay River are minimal and described in **Figure 1-7**, and **Table 1-5**.

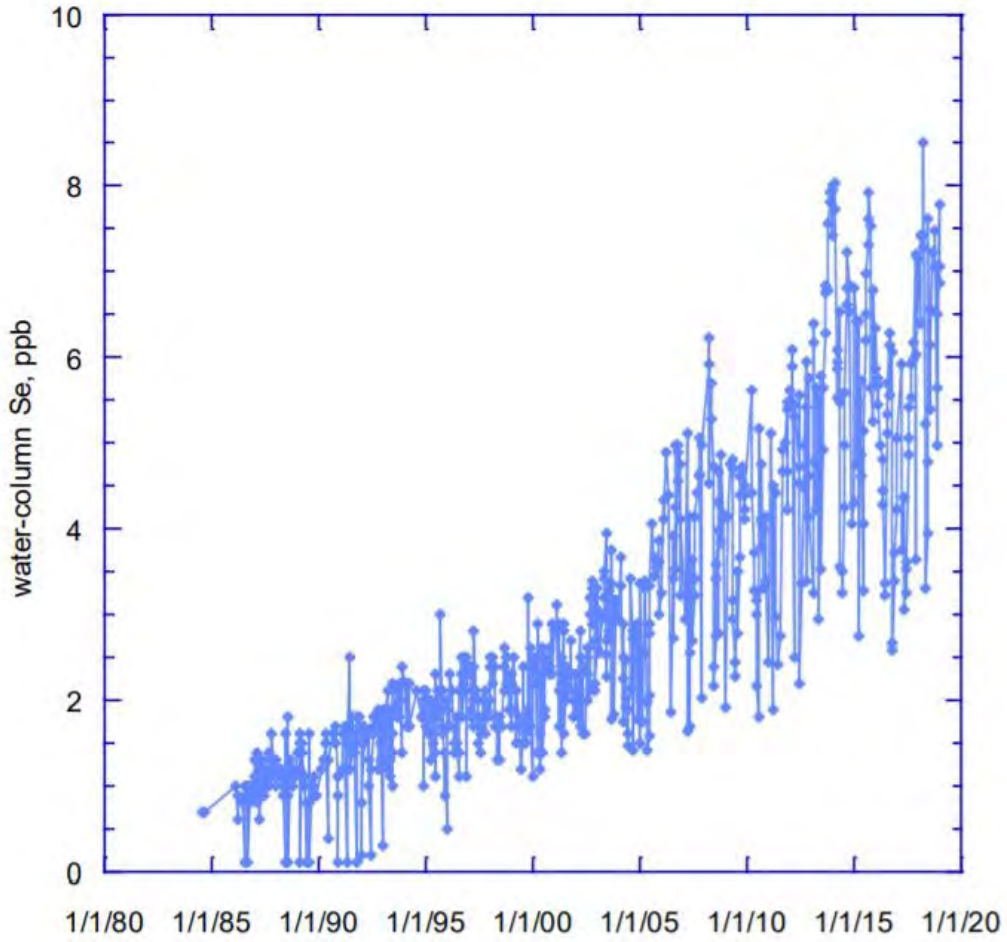


Figure 1-6. Se concentrations from 1984-2019 recorded at station BC08NK003 on the Elk River, a tributary to the Kootenay River located 2.2 miles upstream of its confluence with Lake Koocanusa (Presser and Naftz, 2020).

The Elk River contributes over 95% of the selenium to Lake Koocanusa with the Kootenay River and Bull River delivering together less than 5%. McDonald (2009) reported that in 2008 the total selenium loads were 23,720 lb/yr (10,759 kg/yr). The Elk River contributed 22,450 lb (10,183 kg) Se, the Kootenay River provided 1,078 lb (489 kg) Se, and the Bull River added only 192 (87 kg) Se. In 2012, it was determined that from 1992 to 2012, the amount of Se entering the lake each year increased fivefold, from 5,732 lb (2,600 kg) in 1992 to more than 28,660 lb (13,000 kg). **Figure 1-7** shows the comparison of Se load estimates between the Kootenay River and Elk River. The values presented in **Figure 1-7** were calculated and incorporate scaled flow values, and do include some uncertainty and are not direct measurements of load or water quality and quantity at the reported locations. **Table 1-5** describes the loading comparisons and Se concentration comparisons between upstream and downstream sites as reported in Teck's 2019 Monitoring report.

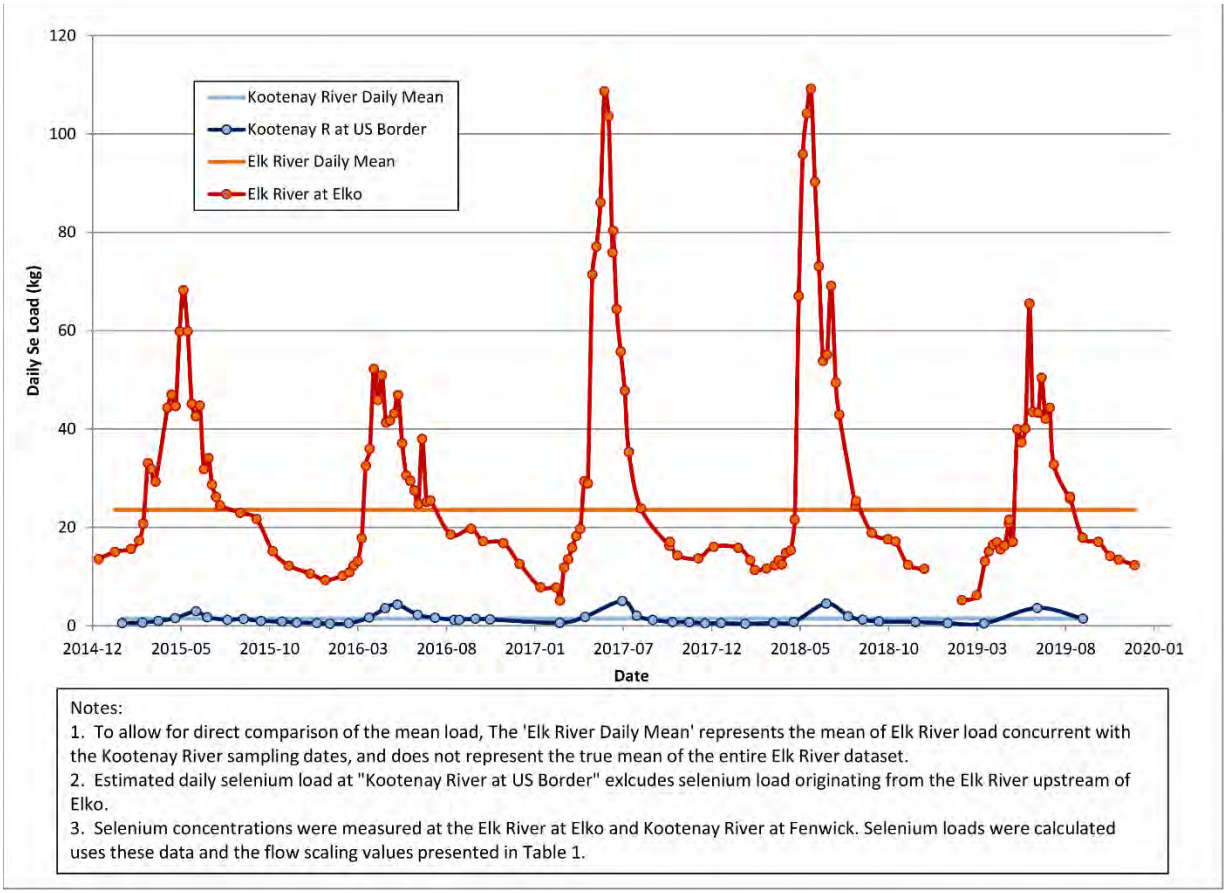


Figure 1-7. Selenium loads from the Kootenay River and Elk River (Sheldon Reddekopp, BC-ENV, personal communication, 8/4/2020).

Table 1-5. Average monthly selenium concentrations, selenium loadings, and total water volume at two Teck monitoring sites; 1) the mouth of the Elk River (RG_ELKMOUTH), and 2) the upstream site on the Kootenay River (RG_WARDB) (Minnow, 2019).

Source	Month	Average Selenium µg/L	Total Volume (m^3)	Selenium Loadings (kg/day)
Elk River (RG_ELKMOUTH)	January	6.66	41,006,976	7
	February	7.43	25,089,073	6
	March	7.21	46,239,045	11
	April	5.62	106,686,508	18
	May	3.84	276,188,619	32
	June	3.57	421,070,214	51
	July	4.64	297,267,032	46
	August	5.92	149,747,763	30
	September	6.1	98,566,165	20
	October	6.62	82,005,592	19
	November	6.54	54,291,842	14
	December	6.57	60,114,499	12
Kootenay River (RG_WARDB)	January	0.14	112,819,052	0
	February	0.14	77,486,057	0
	March	0.12	116,675,008	0
	April	0.11	267,887,635	1
	May	0.09	947,360,464	3
	June	0.08	1,370,615,445	4
	July	0.11	941,856,710	3
	August	0.11	482,055,684	2
	September	0.1	359,498,669	1
	October	0.1	267,152,361	1
	November	0.11	171,424,041	1
	December	0.15	147,008,197	1

2.0 SITE DESCRIPTION

Lake Koocanusa, sometimes referred to as Koocanusa Reservoir, is a 90-mile long transboundary body of water that lies in northwest Montana and southeast British Columbia (**Figure 2-1**). It is within the international Kootenai (Kootenay) River watershed, draining an area of approximately 19,420 square miles (50,298 km^2). Around 70% of the watershed is located within BC with 23% in Montana and 6% in Idaho (USFWS, 2006). The Kootenai River is the second largest tributary to the Columbia River in volume and third in drainage area (USACE, 1972). The total river length is 485 miles (781 km), originating in southeast British Columbia, extending through Montana and Idaho, returning back into BC where it flows through Kootenay Lake, and finally reaches the Columbia River at Castlegar, BC. (**Figure 2-2**).

Lake Koocanusa was formed by the impoundment of the Kootenai (Kootenay) River upon construction of Libby Dam, approximately 17 miles (27 km) upstream of Libby, Montana, and was completed in 1972. The reservoir occupies lands on the territories of the Ktunaxa First Nations (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI). Lake Koocanusa was created under the Columbia River Treaty (CRT) between the United States and Canada to provide power and flood control (Storm et al., 1982). Construction of the Libby dam resulted in the inundation of approximately 90 miles (145 km) of the Kootenay River and 40 miles (65km) of low-gradient tributary habitat. The reservoir operations of Lake Koocanusa are managed by the U.S. Army Corps of Engineers (USACE) outlined in the CRT and hydroelectric power is sold by Bonneville Power Administration (BPA).

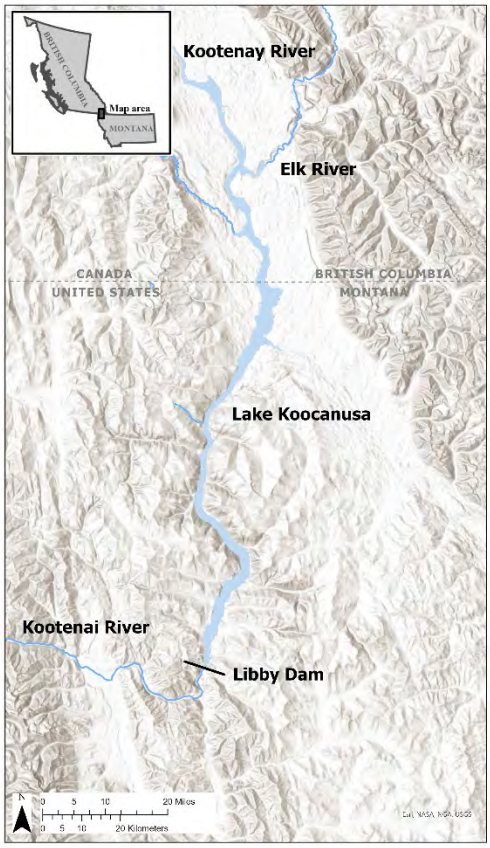
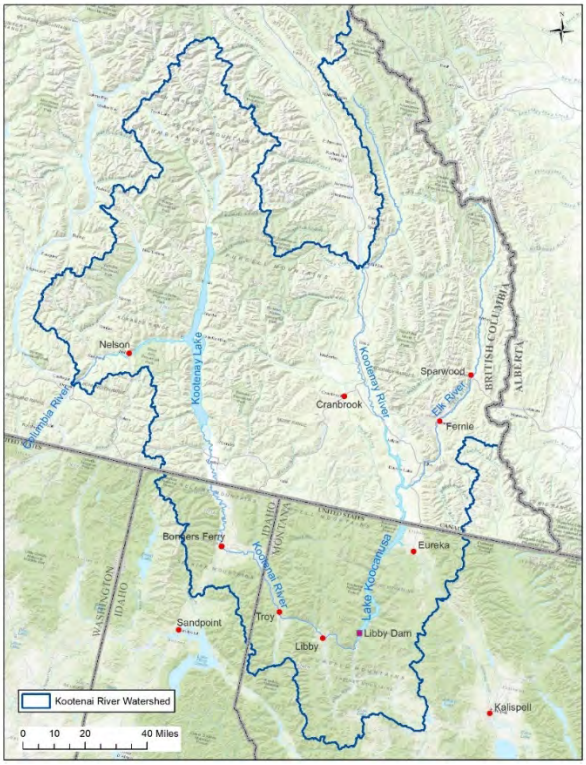


Figure 2-1. Transboundary Lake Koocanusa in northwest Montana.


Nelson
Columbia River
Kootenay Lake
Kootenay River
Cranbrook
Sparwood
Elk River
Fernie
BRITISH COLUMBIA
ALBERTA
Boundary
Boundary
Boundary

2.1 RESERVOIR HYDROLOGY

A complete hydrological description of the Kootenai (Kootenay) River watershed, Lake Koocanusa and its tributaries, hydrology, climate, and physical properties can be found in the Lake Koocanusa Data Compilation Report (Lotic, 2019).

The construction of the Libby Dam in 1972 converted the Kootenai (Kootenay) river from a lotic to a lentic system. The aquatic community, dryland ecosystems, waterfowl species, and other human uses and values were affected.

Three major rivers supply water to Lake Koocanusa. The mean annual flow contribution from the Kootenay River is 56%, the Elk River provides 22%, and the Bull River contributes 11%. The Tobacco River provides 2% while the remaining 9% coming from ungauged flows (Lotic, 2019).

Lake Koocanusa water-level elevations are managed primarily for power and flood control purposes (Storm et al., 1982). **Table 2-1** describes the hydrological characteristics of Lake Koocanusa. Maximum surface area is 46,500 acres (188 km^2) with 28 sq. miles (72km^2), or 38% within BC. Maximum depth at full pool is approximately 350 ft (107 m) and mean bulk water retention time is 6 months. Typical draw down is 98 ft (30 m) with a maximum draw down capacity of 170 feet (52 m).

Table 2-1. Lake Koocanusa hydrological characteristics (Chisholm, 1989)

Surface elevation		
	maximum pool	2,459 ft (749.5 m)
	minimum operational pool	2,287 ft (697.1 m)
	minimul pool (dead storage)	2,222 ft (671.2 m)
Area		
	maximum pool	46,500 acres (188 km^2)
	minimum operational pool	14,487 acres (58.6 km^2)
Volume		
	maximum pool	5,869,400 acre-ft (7.24 km^3)
	minimum operational pool	890,000 acre-ft (1.10 km^3)
Maximum legnth		90 mi (145 km)
Maximum depth		350 ft (107 m)
Mean depth		126 ft (38 m)
Shoreline length		224 mi (360 km)
Shoreline development		4.6 mi (7.4 km)
Drainage Area		8,985 sq. mi (23,271 sq. km)

Water levels in Lake Koocanusa are generally lowest in late winter and early spring (i.e., February through April) and highest in summer and early fall (i.e. August through October; Minnow, 2014). Power generation drawdown begins in November with maximum draws during April. Flow is dependent on hydro-related demands, where spring flows have been attenuated, winter flows are above normal, and daily and hourly flows can change drastically (FWP, 2019b). Residence times have been reported as

variable. Presser and Naftz (2020) describe reports ranging from 1.7-7.5 months during initial construction (1972-1980). While more recent reports range between 5.5 and 9 months.

The USACE manages Libby Dam with a selective withdrawal system to provide flows through Libby Dam for downstream Kootenai River fish. Specifically, flows are provided for federally listed white sturgeon (*Acipenser transmontanus*), bull trout (*Salvelinus confluentus*), and salmon (*Oncorhynchus nerka*) during spring; for salmon during summer, and for bull trout and resident fish in September (BPA, 2018). The selective withdrawal system was installed on Libby Dam to control temperature of water releases from the dam. This system is intended to maximize the probability of allowing significant white sturgeon recruitment, provide a year-round thermograph that approximates normative conditions, while also meeting flood damage reduction objectives (BPA, 2018). The ecological flow plan followed by USACE was developed to restore ecological function in support of Kootenai River white sturgeon recovery while also maintaining flood control (USFWS, 2006). These functional normative flows (ecological flows) are designed to more closely mimic pre-dam hydrographs (**Figure 2-3**). Annual variability for flow and elevation for the reservoir and river from 2006 to 2019 are shown in **Figure 2-4**.

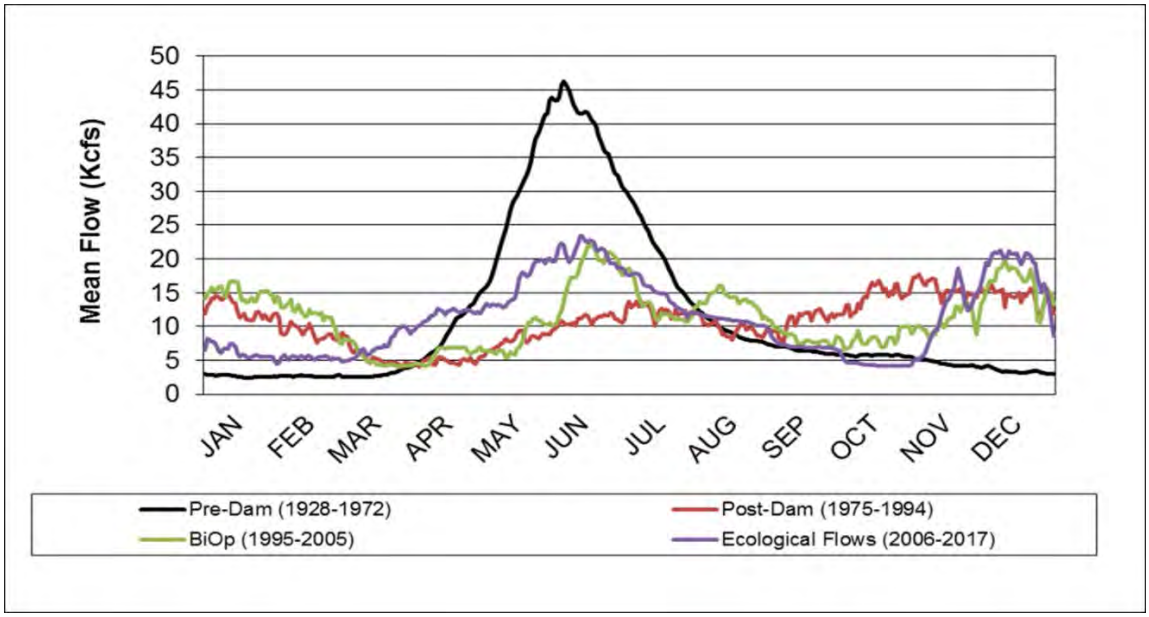


Figure 2-3. USACE stream flow management out of Libby dam from 1976-2017. Ecological flows are displayed in purple and are designed to more similarly mimic pre-dam flows displayed in black (U.S. ACE, 2019).

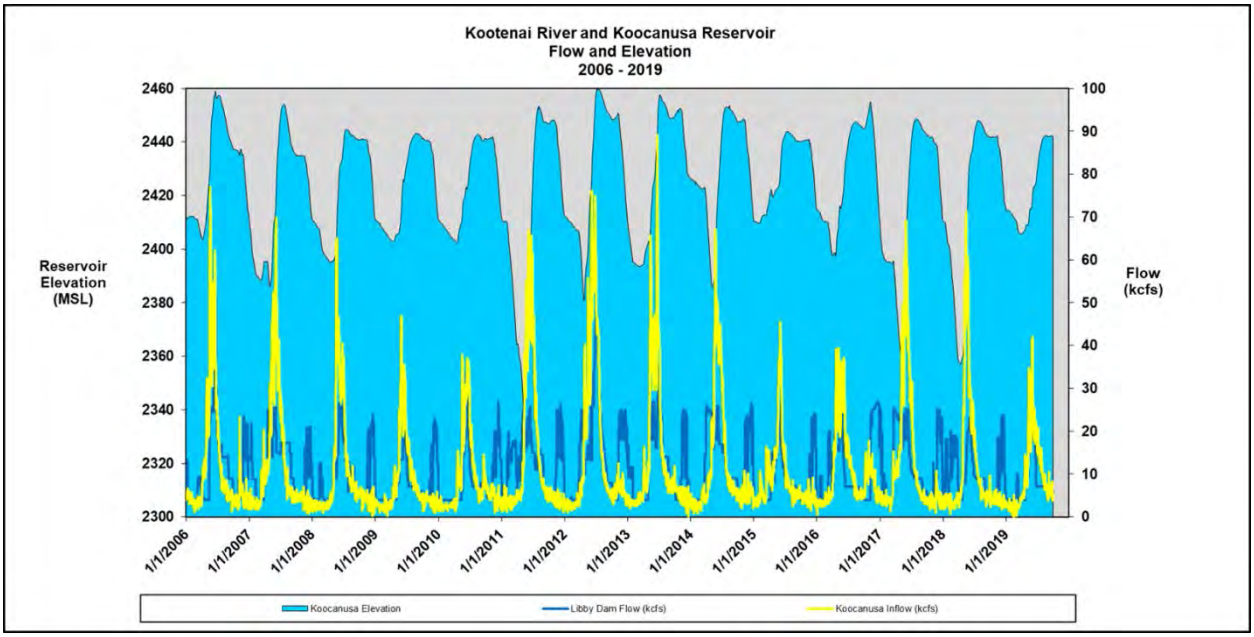


Figure 2-4. Fluctuations in reservoir elevation and flow from 2006-2018 (U.S. ACE, 2019).

The seasonal fluctuation of water levels affects the hydrology of the waterbody and associated aquatic life by impeding the establishment of riparian vegetation or aquatic macrophytes which has left the composition of the littoral zone to be that of cobble, mud, and sand substrates with limited habitat structures. Variable water levels have also affected bank stability and can impact spawning success of certain fish such as burbot (Lotic, 2019).

2.2 PHYSICOCHEMICAL CHARACTERISTICS

Routine monitoring has been conducted on the US portion of the reservoir beginning in the early 1970s with detailed summaries included in reports by USACE found at https://www.nws.usace.army.mil/About/Offices/Engineering/Hydraulics-and-Hydrology/Water-Quality/Water-Quality-Documents/ and USGS publicly available data found at https://www.waterqualitydata.us/provider/NWIS/USGS-MT/USGS-12301919/. The USACE maintains three main monitoring stations in Lake Koocanusa (**Figure 2-5**).

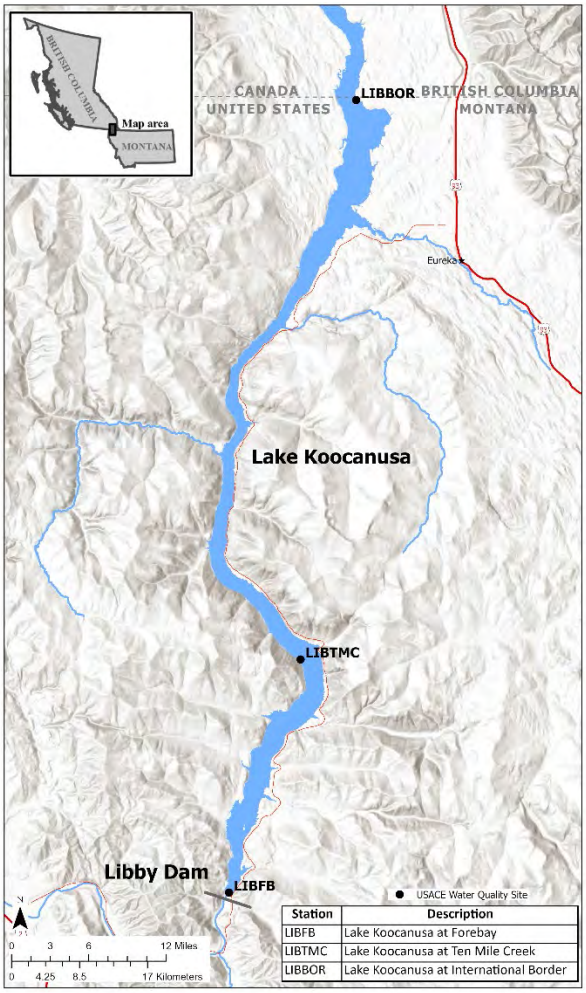


Figure 2-5. Locations of USACE's routine monitoring stations in Lake Koocanusa.

In BC, routine physicochemical monitoring is done by the federal department of Environment and Climate Change Canada (ECCC) which has a long-term monitoring station (BC08NK0003) on the Elk River approximately 2.2 miles upstream from the confluence with the Kootenay River. Additionally, under the EVWQP, Teck is required to conduct comprehensive physico-chemical and biological monitoring which first began in 2014. Monitoring sites for Teck are located both upstream and downstream of the Elk River. Monitoring reports by Teck are published annually and are vetted by BC-ENV scientists, the KNC, and independent scientists. Reports from 2014-2019 can be found on at https://www.teck.com/responsibility/sustainability-topics/water/water-quality-in-the-elk-valley/.

2.2.1 Nutrients & chlorophyll *a*

Nutrients and chlorophyll *a* routinely monitored by the USACE and Teck and are detailed extensively in USACE reports found at https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/ and Teck reports found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Briefly, Lake Koocanusa in Montana is considered oligotrophic and at times mesotrophic as defined by Lake Koocanusa's trophic State Index (TSI). TSI measures transparency and presence of nutrients and chlorophyll *a* (Carlson, 1977). The Canadian section of the reservoir experiences periods of eutrophic conditions in spring (April – June) but qualifies as oligotrophic the remainder of the year. The majority

of the phosphorus (P) entering the reservoir is P sorbed to soil particles, entering the reservoir during spring runoff which is likely not biologically available. The years with the highest runoff are associated with greater total phosphorus (TP). The P-bound sediment settles from the water column to the sediment layer as it travels through the 90-mile reservoir towards the dam. As a result, Lake Koocanusa acts as a nutrient sink, trapping an estimated 63% of incoming phosphorus (Wood, 1982). Soluble Reactive Phosphorus (SRP) is consistently lower than the 1 µg/l detection limit (DL). While low levels of TP and SRP are recorded in the reservoir, increasing concentrations of nitrogen (N) have been detected (**Figure 2-6 and Figure 2-7**). Elevated nitrate concentrations entering the reservoir are linked to blasting practices during coal production (Mahmood et al., 2017). The high N concentrations and low P concentrations have resulted in a N:P ratio far from what is considered healthy for an aquatic system and has had deleterious effects below Libby Dam by encouraging the presence of the nuisance diatom *Didymosphenia geminata* (didymo) (Dunn et al., 2015).

Chlorophyll *a* concentrations are greatest during the spring and early summer with similar concentrations recorded at all three USACE monitoring stations. Higher concentrations of chlorophyll *a* are associated with high flow years. Chlorophyll *a* measurements supported the stratification trends identified with dissolved oxygen, temperature and pH (Minnow, 2018).

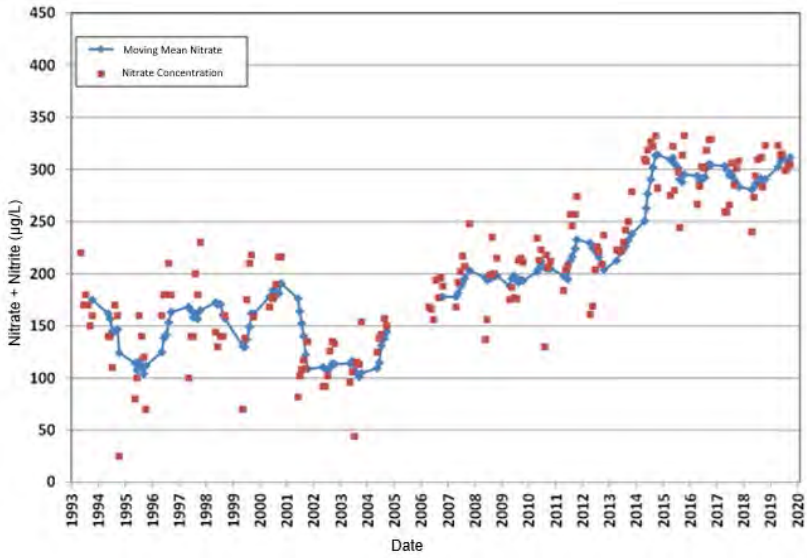


Figure 2-6. Nitrate + nitrite concentrations in the hypolimnion at the LIBFB (Forebay) monitoring station (USACE, 2018).

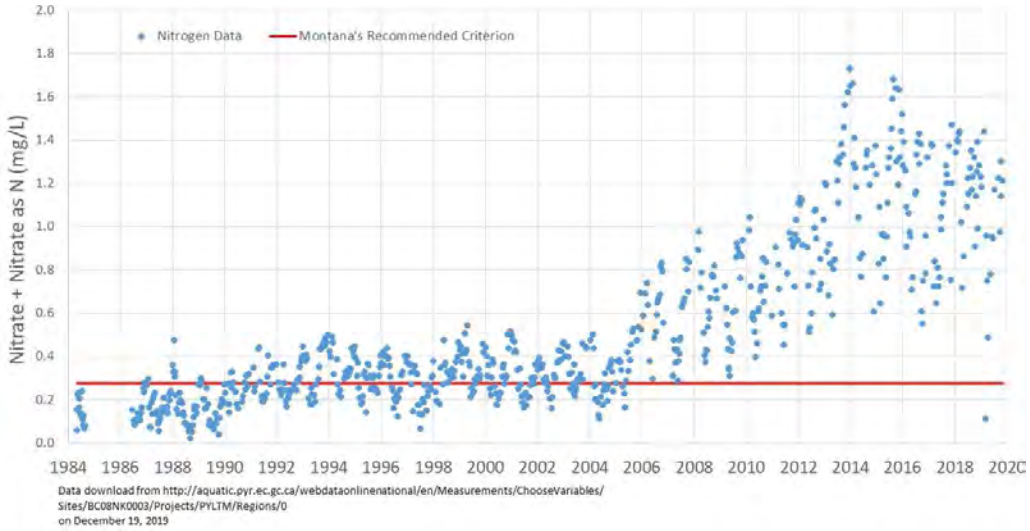


Figure 2-7. Nitrate + nitrite from 1984-2019 recorded at station BC08NK003 located 2.2 miles upstream of Lake Koocanusa (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020).

2.2.2 Metals and Metalloids

Lake Koocanusa has been monitored for a suite of major and trace metals and metalloids since the 1970s by USACE and USGS. Trend data for each metal and metalloid can be found in the USACE reports https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/. In BC, metal(loid) data are included in the Teck monitoring reports found in at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

The MT data show below DL or low levels of metals with the exception of the metalloid selenium. Selenium has been routinely monitored on the US side of the reservoir since 2013. Dissolved selenium concentrations in MT range from 0.04-2.29 µg/L from 2013-2018.

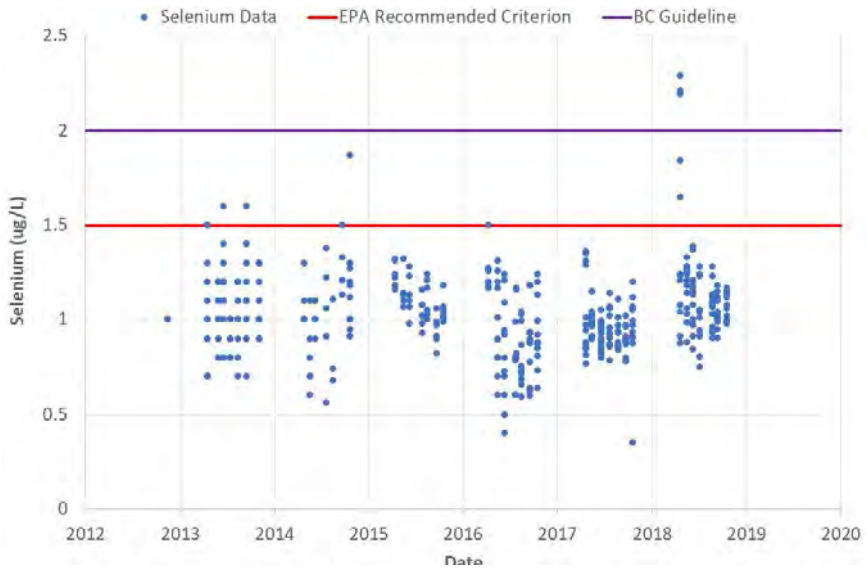


Figure 2-9. Selenium Data in Lake Koocanusa, U.S. Stations, all depths 2012-2018. (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020)

2.3 BIOLOGICAL CHARACTERISTICS

This section details baseline biological characteristics of Lake Koocanusa to provide a basic understanding of the system.

2.3.1 Phytoplankton

USACE has included phytoplankton in their routine monitoring from 2006-present. Phytoplankton densities vary from year to year and by location within the reservoir. A more detailed report of USACE's findings can be found in https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/.

Peak concentrations occur between May and August. The dominant algal group are Diatoms followed by Chrysophytes. Cyanobacteria have been measured since 2015 with *Planktolynbia* documented as the dominant type of cyanobacteria. The diatom species dominance has changed since sampling began in 2006. The most abundant diatoms in the reservoir include: *Asterionella, Cyclotella, Fragilaria, Stephanodiscus, Syndedra,* and *Nitzschia*. From 2008-2012 diatoms were recorded at low density with diverse composition (**Figure 2-10 and Figure 2-11**). In 2014 a shift was recorded in which *Cyclotella* and *Fragilaria* dominated. In 2017 USACE recorded high density and low diversity with near total dominance of *Cyclotella* and *Fragilaria*. There have been no differences in the phytoplankton community compositions between upstream and downstream monitoring locations on the Elk River (Minnow, 2019).

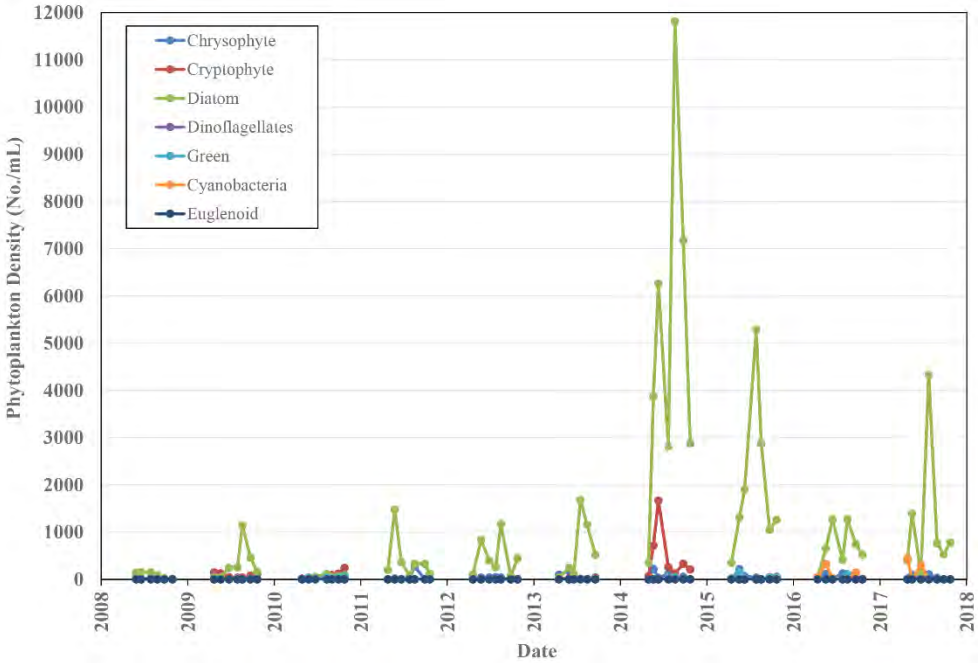


Figure 2-10. Phytoplankton density at LIBFB (Forebay) from 2008-2017 (U.S. ACE, 2019).

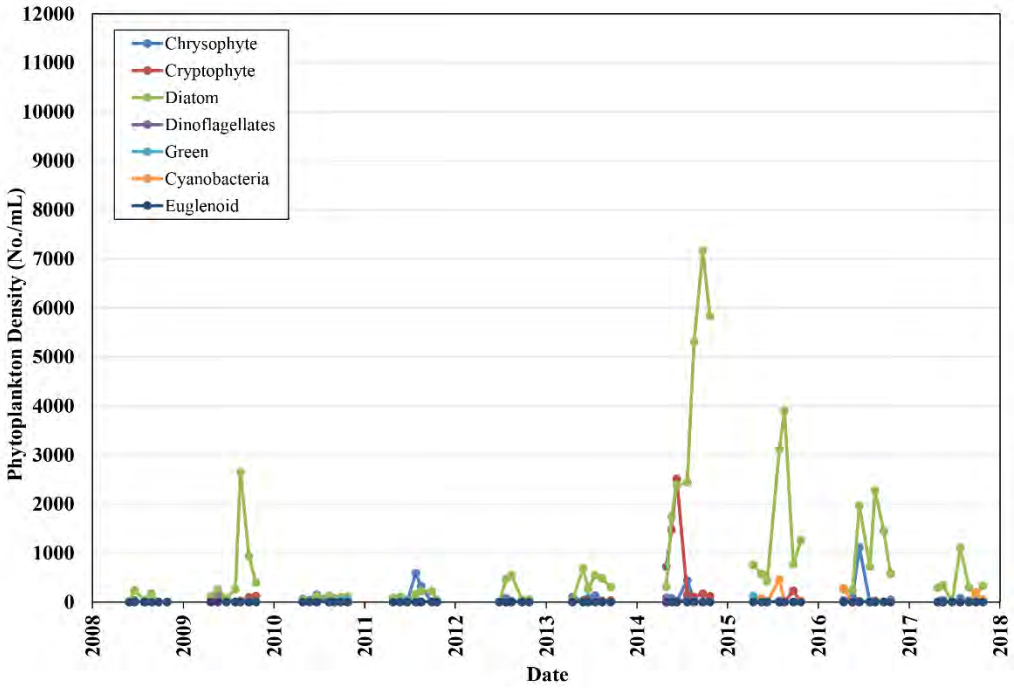


Figure 2-11. Phytoplankton density at LIBBOR (International Border) from 2008-2017 (U.S. ACE, 2019).

2.3.2 Zooplankton

Zooplankton abundance and composition recorded in Montana by USACE has been variable between the years of record 2006-2017. Copepods dominated between 2006 and 2010 while Rotifers dominated between 2011 and 2017 (**Figure 2-12 and Figure 2-13**). Within Rotifers, *Keratella* and *Keilicottia*

dominate. Cyclopoid and *Diacyclops* are the dominant copepods. *Daphnia* and *Bosmina* are the dominant Cladocerans. Seasonal succession in zooplankton community is likely in response to food source availability, changes in temperature, and grazing pressure by fish.

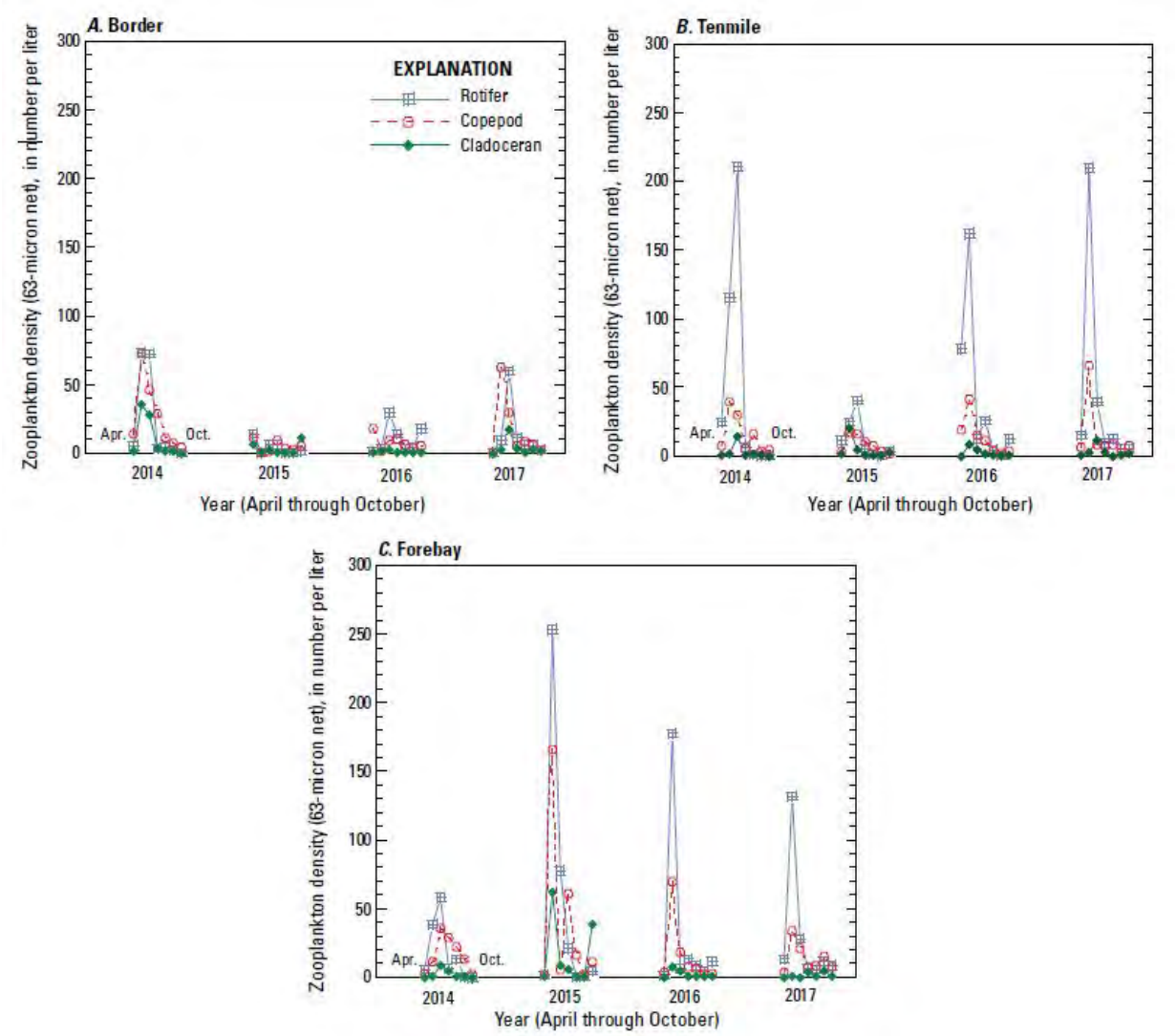


Figure 2-12. Zooplankton densities 2014-2017 (63 micron net) at sites A) Border, B) Tenmile, and C) Forebay (Presser and Naftz, 2020).

Figure 2-13 describes the Se concentrations in Zooplankton collected by Teck, USACE, and USGS between 2008 and 2019.

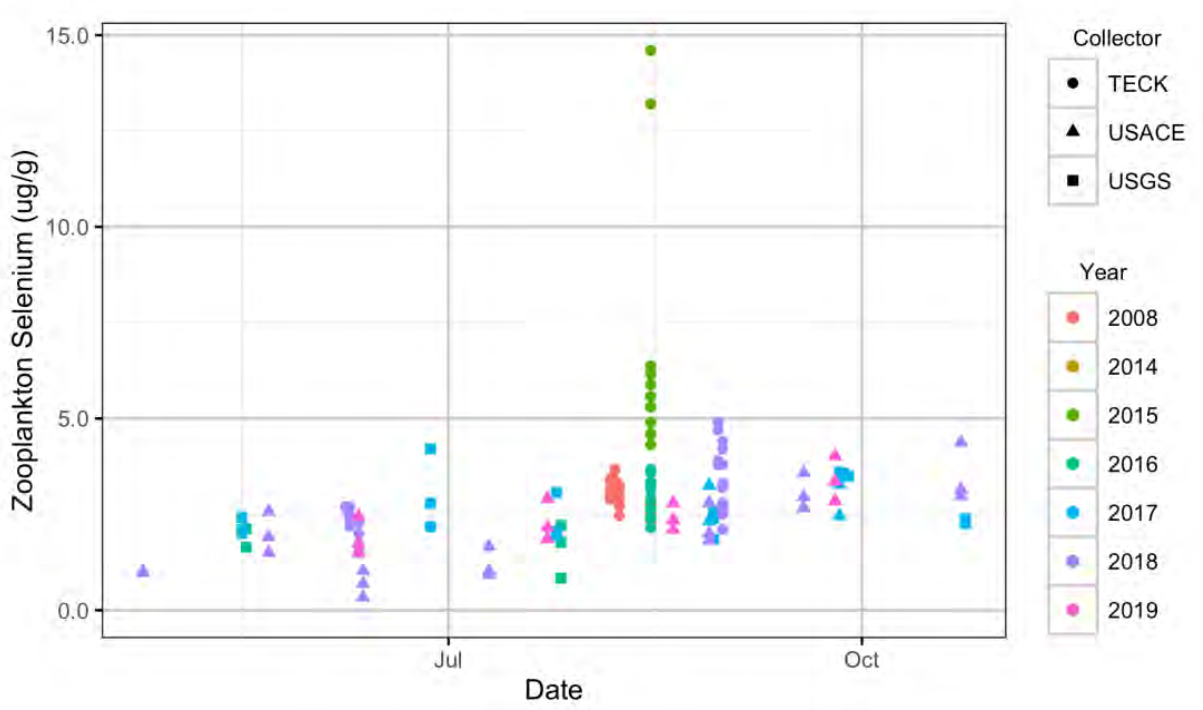


Figure 2-13. Zooplankton selenium concentrations by date, collector, and year (Thorley, 2020).

2.3.3 Macroinvertebrates

Routine data on richness and abundance for macroinvertebrates on the Montana portion of Lake Koocanusa do not exist. Montana Fish Wildlife and Parks (FWP) in collaboration with DEQ conducted benthic and surface macroinvertebrate sampling during 2018 (see **Section 3.0**). The results for Se concentration in macroinvertebrates showed chironomid dominance.

In BC, Teck conducted benthic community data collection and detailed reports on richness and abundance can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf. Dominant taxa found at their sampling sites were *Chironomus, Procladius* and *Tanytarsus*. Additionally, Teck has monitored for Se in macroinvertebrate tissues as required by their permit. Their 2019 monitoring report showed Se concentrations in benthic tissues were higher at the mouth of the Elk River compared to upstream (Kootenay River) and downstream locations. Moreover, average selenium concentrations in invertebrate tissues collected both upstream and downstream of the Elk River have oscillated between being below BC guidelines (4 µg/g dw) during spring samples and above guidelines in summer and fall samples (Minnow, 2019). Between sites, there were significant differences found in selenium concentrations from Kootenay River upstream concentrations (lower Se) to downstream concentrations (higher Se) regardless of season.

2.3.4 Fish

Numerous biological inventories have been conducted on the MT portion of the reservoir both pre-and-post dam construction. Reports detailing fish monitoring results can be located in Libby Mitigation Reports located at http://fwp.mt.gov/fwpDoc.html?id=95385 and results from fish monitoring conducted by Teck can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Figure 2-13 shows the relative abundance of the following fish species found in Lake Koocanusa: bull trout, burbot (*Lota lota*), Kokanee (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), westslope cutthroat trout (*Oncorhynchus clarkia lewisi*), mountain whitefish (*Prosopium williamsoni*), northern pikeminnow (*Ptychocheilus oregonensis*), redside shiner (*Richardsonius balteatus*), peamouth chub (*Mylocheilus caurinus*), longnose sucker (*Catostomus catostomus*), and largescale sucker (*Catostomus macrocheilus*) (Presser and Naftz, 2020). An accidental release of 250,000 kokanee fry into Lake Koocanusa from Kootenay Trout Hatchery in the late 1970s (Richards, 1997) led to the establishment of Kokanee in the reservoir. MT FWP stocks rainbow trout under their fisheries management program.

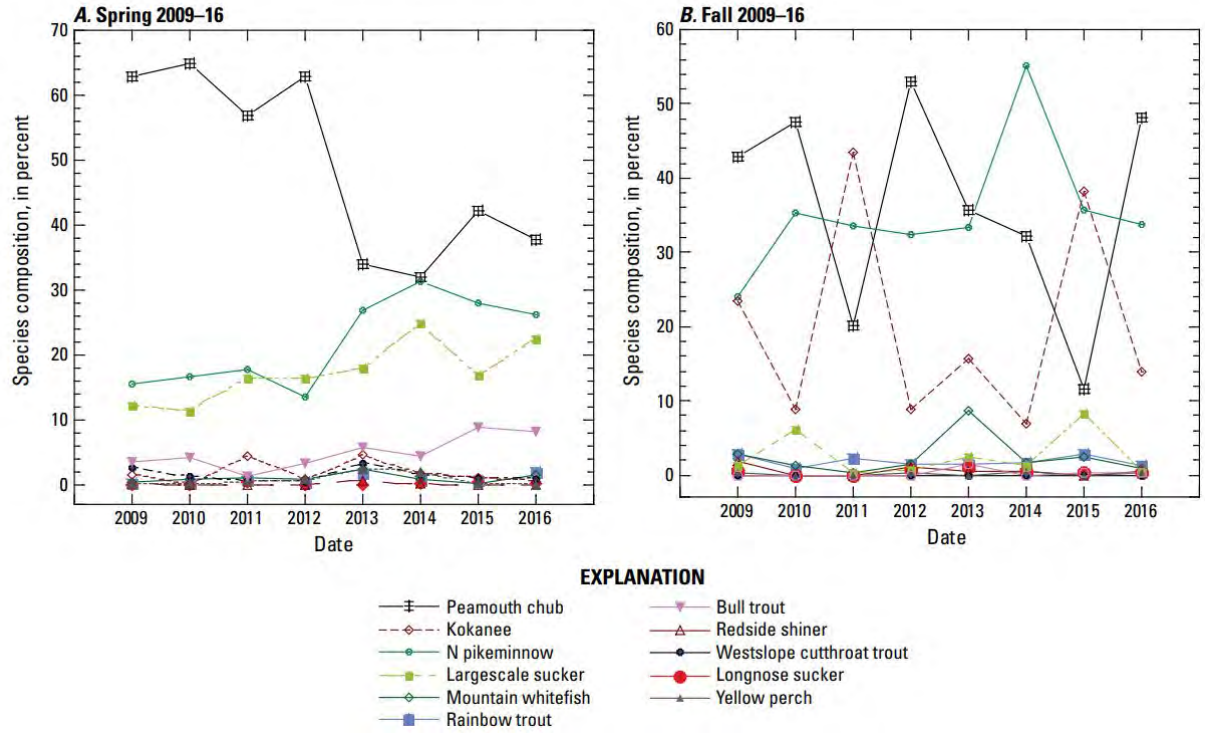


Figure 2-13. Fish species composition during A) Spring 2009-2019 and B) Fall 2009-2016 (Presser and Naftz, 2020).

Figures 2-14 and 2-15 show the results of MT FWP's 2018 fish sampling. All three redside shiner egg/ovary samples exceeded the EPA 304(a) criterion of 15.1 mg/kg dw along with one sample from peamouth chub. The other samples all remained below the EPA criterion. The 2018 fish muscle tissue samples were found to be at a comparable concentration as MT FWP's 2008 results. All samples were below the EPA fish muscle criterion of 11.3 mg/kg dw. Fish tissue samples from the BC portion of the reservoir vary in concentrations for egg/ovary ranging from 4-5 mg/kg dw for kokanee and yellow perch to higher levels for redside shiner, peamouth chub, and northern pikeminnow, ranging between 5 mg/kg to 40 mg/kg with one red side shiner reaching 80 mg/kg. The BC WQG for egg/ovary is 11 mg/kg dw.

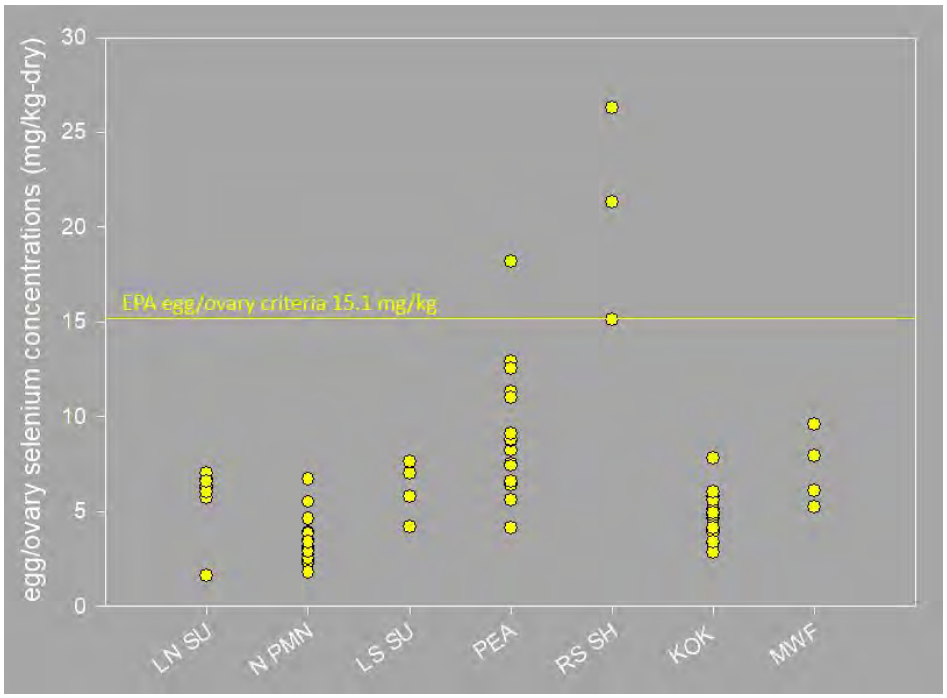


Figure 2-14. Montana Fish Wildlife and Parks 2018 egg/ovary Se concentrations found in LN SU (longnose sucker), N PMN (northern pikeminnow), LS SU (largescale sucker), PEA (peamouth chub), RS SH (redside shiner), KOK (kokanee), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

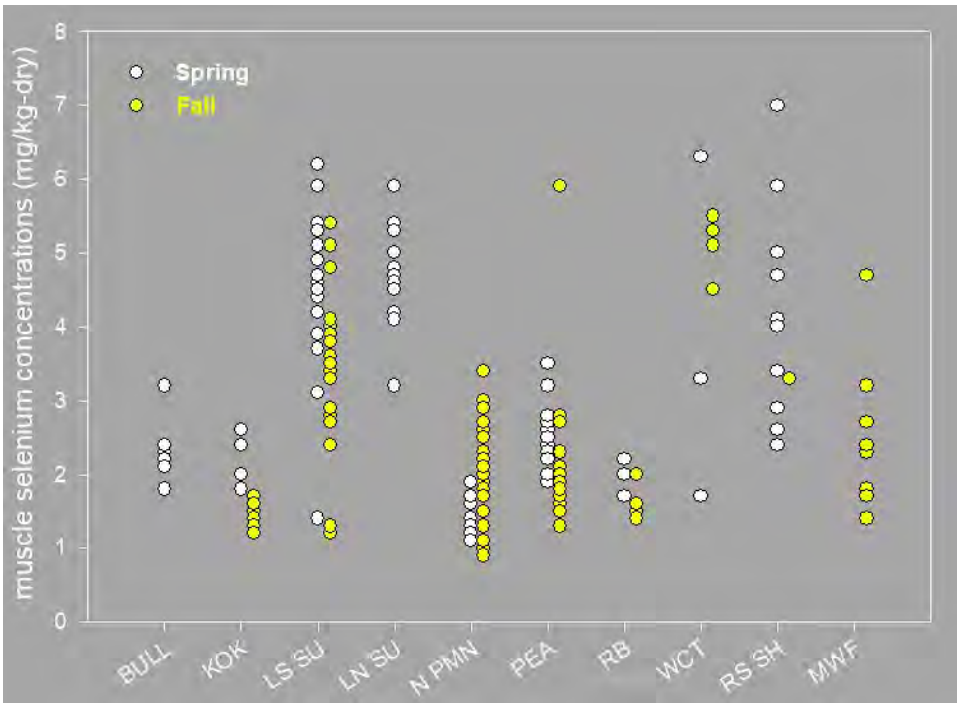


Figure 2-15. Montana Fish Wildlife and Parks 2018 muscle selenium concentrations found in BULL (bull trout), KOK (kokanee), LS SU (largescale sucker), LN SU (longnose sucker), N PMN (northern pikeminnow), PEA (peamouth chub), RB (rainbow trout), WCT (westslope cutthroat trout), RS SH (redside shiner), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

2.3.5 Birds

Lake Koocanusa encompasses transboundary migratory routes and the Pacific Flyway. On the Montana portion of the reservoir, the most common shore bird on Lake Koocanusa is spotted sandpiper (*Actitis macularius*). Less common during the summer months along the shore are killdeer (*Charadrius vociferus*) but they are regulars on the mud flats at Rexford in fall and into winter. Other shore birds are limited on Lake Koocanusa because of the steep shoreline, a result of dam management. Presser and Naftz (2020) reports that the common resident birds on Lake Koocanusa are Bald Eagle (*Haliaeetus leucocephalus*) and Osprey (*Pandion haliaetus*), Common Loon (*Gavia immer*), Ring-billed Gull (*Larus delawarensis*), Canada Goose (*Branta canadensis*), Mallard (*Anas platyrhynchos*), Common Goldeneye (*Bucephala clangula*), Common Merganser (*Mergus merganser*), Western Grebes (*Aechmophorus occidentalis*), and swallow species (*Hirundinidae spp.*). They further detail avian species which pass through Lake Koocanusa during migration, including Eared Grebe (*Podiceps nigricollis*), Lesser Yellowleg (*Tringa flavipes*), Wilson's Phalarope (*Phalaporpus tricolor*), and several species of ducks including American Wigeon (*Mareca americana*), Gadwall (*Mareca strepera*), Redhead (*Aythya americana*), Ring-necked Duck (*Aythya collaris*), Green-winged Teal (*Anas carolinensis*), Blue-winged Teal (*Anas discors*), Hooded Merganser (*Lophodytes cucullatus*), and Lesser Scaup (*Aytha affinis*).

3.0 DATA COLLECTION FOR CRITERIA DEVELOPMENT

This section presents details on enhanced Se data collection efforts conducted to inform criteria development. In addition to routine monitoring (see **Section 2.0**), the state, federal, provincial, and industry monitoring entities followed recommendations provided by the SeTSC to the extent feasible. Specifically, coordinated cross-border monitoring protocols were developed, additional parameters collected, and monitoring sites added. **Table 3-1** describes the parameters collected, years of record, responsible monitoring entity, and if the sampling was associated with routine monitoring or added.

Significant advances in coordinated monitoring efforts occurred between 2015-2019, but as a result of the transboundary nature of the reservoir and dual jurisdictions, perfectly matched datasets for target parameters were not possible. Presser and Naftz (2020) detail data collection efforts specific to the biodynamic modeling and utility of data while explanation on sample collection and analysis are described in the Presser and Naftz (2020) data release.

Table 3-1. Monitoring data, years of collection, and monitoring entities.

Data	Sampling (routine or added for criteria development)	USGS	USACE	Teck	ENV	FWP	USFWS
phyiscochemical parameters	routine		2015-2019	2015-2019			
dissolved selenium	routine	2012-2019	2015-2019	2015-2019	2017		
selenium speciation	added	2015	2016				
particulate selenium	added	2015-2019	2017-2019	2017-2019	2017		
sediment selenium	routine			2015-2019			
periphyton tissue selenium	added				2017	2017	
invertebrate tissue selenium	added			2016; 2018-2019		2018-2019	
zooplanktoon selenium	added	2016-2017	2016-2019	2018-2019			
fish muscle tissue selenium	routine			2015-2019		2015-2019	
fish whole body tissue selenium	routine			2015-2019		2015-2019	
fish egg/ovary tissue selenium	routine			2015-2019		2015-2019	
fish food habits	added					2017-2018	
bird egg selenium	added						2016

3.1 DISSOLVED AND PARTICULATE SE

The ratio of Se concentration of suspended particulate matter (SPM) to dissolved Se in the water is the environmental partitioning coefficient (K_d). For the most accurate understanding of the K_d, it is necessary to measure SPM and dissolved Se values across multiple years and multiple seasons. Throughout spring-fall months, large volume water samples were collected at two depths (epilimnion and hypolimnion), centrifuged, and analyzed for Se. Dissolved Se samples, defined as passing through a 0.45 µm filter, were collected at the same time and location as SPM samples (these are considered matched samples). USGS and USACE each collected matched dissolved and SPM samples at the main MT monitoring sites, the International Boundary, Tenmile, and Forebay. Teck and BC-ENV collected dissolved and SPM Se samples on the BC portion of the reservoir (**Figure 3-1**). A more robust matched dataset exists for the Montana portion of the reservoir.

Biogeochemical processes have the potential to influence Se distribution between particulate and dissolved phases. At each matched sampling site, high resolution vertical profile data was collected including, temperature, pH, specific conductance, DO, and fluorescent dissolved organic matter (fDOM). Details on these parameters are located in Presser and Naftz (2020).

A concerted effort was made to analyze SPM samples for Se speciation but due to limited sample mass as a result of low lake productivity, the laboratory was unable to complete Se speciation on all SPM data. Only a small subset of the SPM data includes Se speciation.



Figure 3-1. Location of sites where water quality and (or) SPM samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3. 2 PERIPHYTON

DEQ and FWP conducted a pilot study to determine how Se moves through the food chain at the periphyton level, subsequently transferred to periphyton-associated macroinvertebrates and, if present, periphyton feeding fish. Sampling of shoreline periphyton occurred at Tenmile and Rexford sites in MT

during September 2017. Teck additionally sampled for periphyton on the BC portion of the reservoir. Full details on these sampling methods are found in Lotic (2019).

3.3 ZOOPLANKTON

Zooplankton is routinely sampled by USACE and Teck for density and identification. In addition, zooplankton selenium concentrations were measured to help understand the trophic transfer function from SPM to zooplankton. Zooplankton was sampled concurrently with water and SPM samples in MT and BC. **Figure 3-2** details the transboundary zooplankton sampling sites.

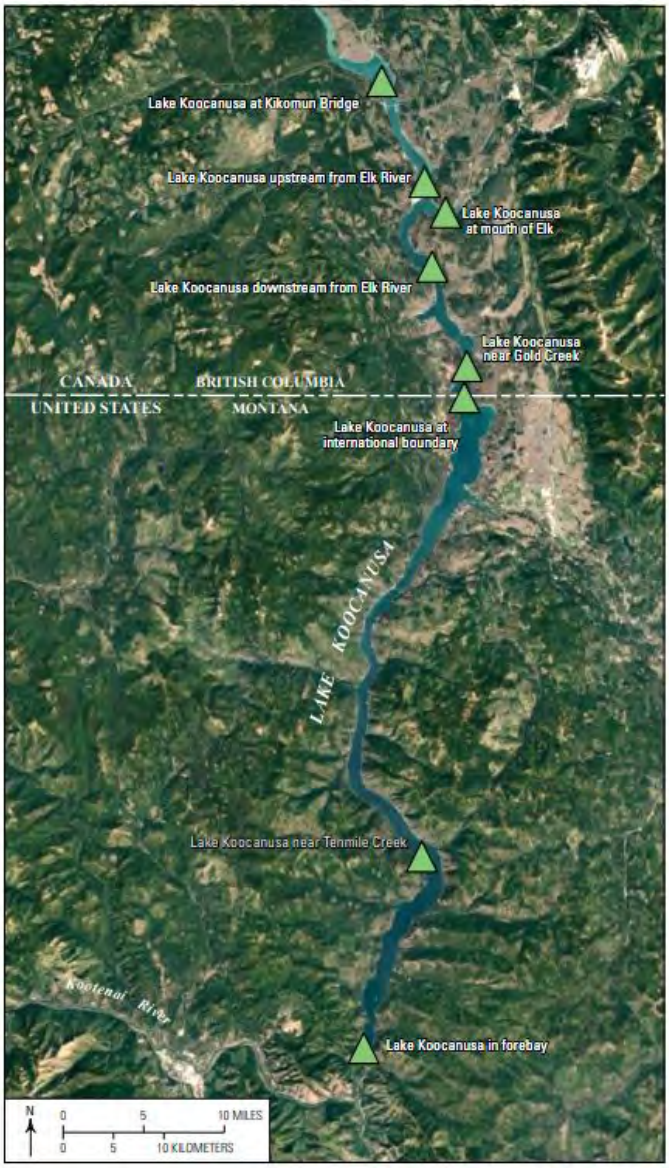


Figure 3-2. Location of sites where zooplankton samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.4 INVERTEBRATES

To better understand the trophic transfer of selenium, FWP collaborated with DEQ to collect and analyze surface and benthic invertebrates in Lake Koocanusa. As part of Teck's monitoring requirements, sampling for invertebrates for selenium analysis continued as routine monitoring on the BC portion of the lake. **Figure 3-3** displays the macroinvertebrate sampling sites in MT and BC.

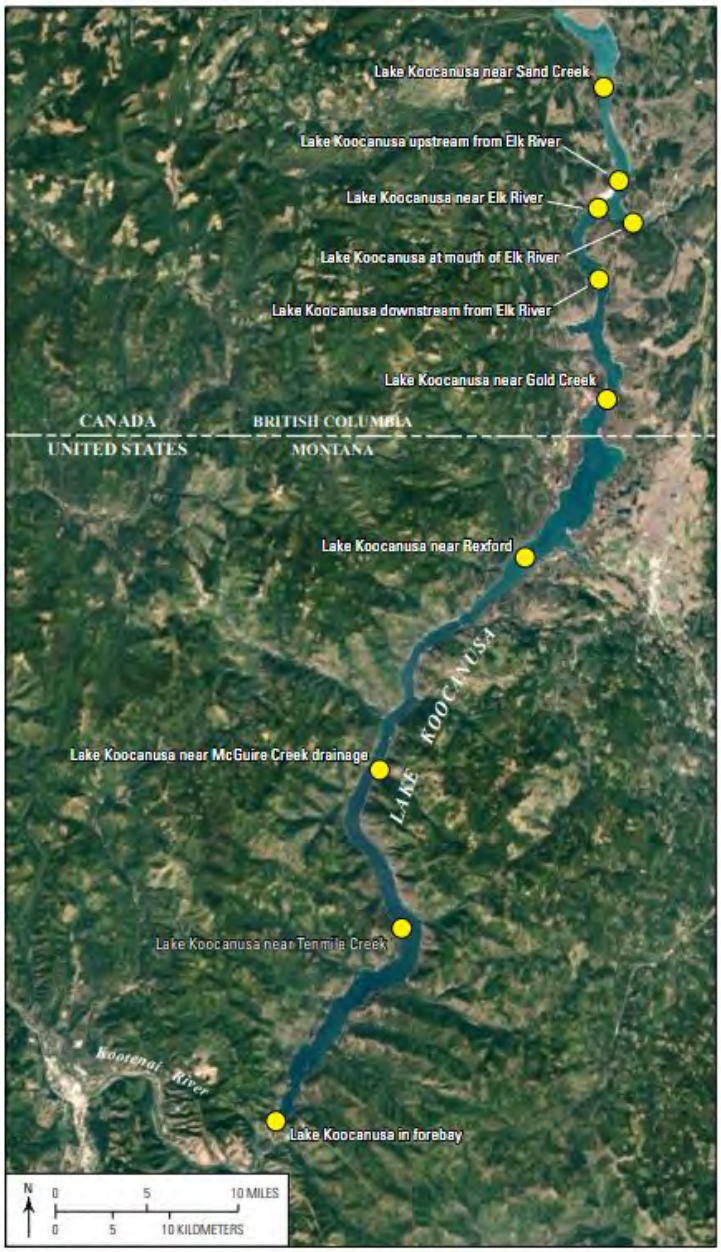


Figure 3-3. Locations of sites where invertebrate samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.5 FISH

FWP and DEQ worked collaboratively to sample fish tissue and continue baseline monitoring of fish tissue Se in Lake Koocanusa and evaluate concentration trends. Since 2008, fish tissues have been collected and analyzed for Se. In 2008, 2013, and 2018 bull trout, longnose sucker, northern pikeminnow, kokanee, peamouth, rainbow trout, and westslope cutthroat trout were targeted by FWP for tissue and opportunistic egg/ovary sampling. The three locations for this effort were; 1) near the mouth of the Elk River in Canada, 2) Rexford, and 3) McGillivray (Tenmile). Egg/ovary samples were taken if ovaries were with eggs, but the stage of development was not noted. In 2018-2019 FWP expanded their Tenmile site further south to the Forebay to give a more complete spatial representation of the lower portion of the reservoir.



Figure 3-4. Fish collection sites for Montana and British Columbia. Explanations of collections within area 1 (South of Elk), area 2 (International Boundary), area 3 (Tenmile), and area 4 (Forebay) are further defined in Presser and Naftz, 2020.

A fish food habit study was conducted by FWP in 2017 to determine any differences from previous food habit information collected from fish in Lake Koocanusa from 1983-1992. Target species for the food habit study were westslope cutthroat trout, rainbow trout, longnose suckers, kokanee, and burbot. Stomach contents were analyzed and no major differences in food habits were determined.

Teck conducted fish sampling and analysis for Se as outlined in their permit requirement. Similarly, Teck sampled whole body, muscle, and fish egg/ovary tissue. Detailed information can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

3.6 BIRDS

USFWS conducted a preliminary risk assessment for avian exposure to Se in Lake Koocanusa. Data were collected in June 2016 for killdeer. Methods and results are described in Skorupa and Nelson (2018). The concentrations found for killdeer were well below the known toxicity for this species. The results of this study suggest birds in the MT portion of Lake Koocanusa are not currently experiencing Se-induced reproductive impairment. The SeTSC determined that fish are the most sensitive endpoint for consideration for modeling, and so fish became the focus for future data collection efforts to inform criteria development. No additional bird studies were conducted on the US portion of the reservoir, although Teck continues to monitor birds, primarily spotted sandpiper in BC, as part of their permit requirement.

4.0 SELENIUM MODELING

This section provides an overview of information on the USGS biodynamic selenium modeling utilized for derivation of a protective water column criterion for Lake Koocanusa. Consistent with the approach used by EPA in developing 304(a) criteria, DEQ partnered with the USGS to employ the mechanistic Ecosystem-Scale Selenium modeling approach for Lake Koocanusa (Presser and Luoma, 2010). The work by Presser and Naftz (2020) tailors the Presser and Luoma (2010) model to the Lake Koocanusa ecosystem. The peer-reviewed report and data release can be found at https://pubs.er.usgs.gov/publication/ofr20201098.

DEQ worked with the SeTSC and the USGS while concurrently following the EPA guidance in Appendix K for criteria derivation.

4.1 ECOSYSTEM-SCALE MODEL OVERVIEW

Presser and Luoma (2010) describe the Ecosystem-Scale Model and its use in understanding bioaccumulation and trophic transfer as essential to managing ecological risks from Se. The modeling process, using key components outlined below, provides a basis for understanding and quantifying dietary uptake and linkages among food webs. **Figure 4-1** illustrates the processes regulating movement of Se through an ecosystem.

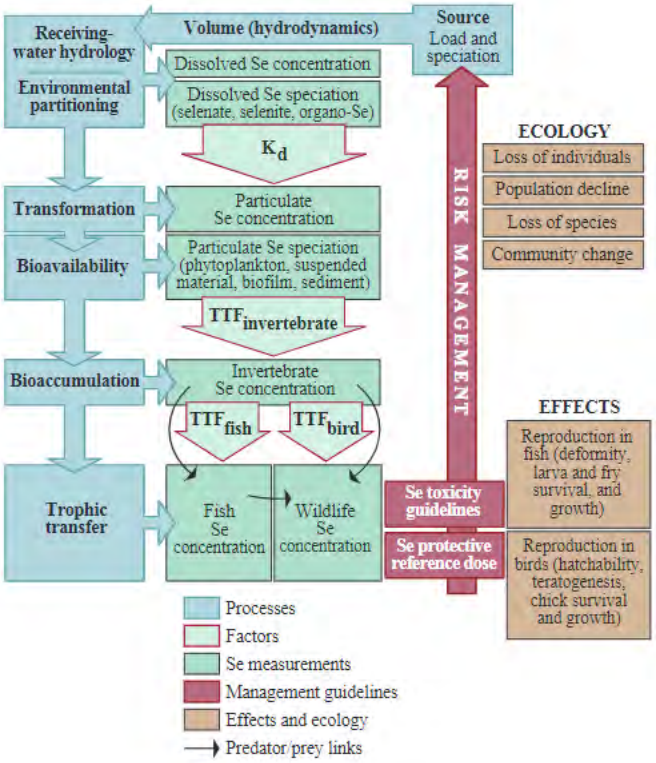


Figure 4-1. Conceptual illustration of the Selenium Ecosystem Scale Model (Presser and Luoma, 2010).

Jenni et al. (2017) first described tailoring the Presser and Luoma (2010) conceptual model to the Lake Koocanusa ecosystem. The key factors of the modeling include a tissue criterion element, a trophic transfer factor (TTF), and a partitioning coefficient (K_d). All of these are required to derive a protective dissolved water column number, as described by the equation below:

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{TTF^{composite} X\ K_d}$$

Where,

C_{target}	= translated site-specific water criterion element (µg/L)
$C_{tissue\ criterion\ element}$	= fish tissue criterion element (mg/kg dw)
$TTF^{composite}$	= product of the species-specific trophic transfer factor (TTF) values in each trophic level of the food web of the target fish species related to the tissue criterion element (no units of measurement)
K_d	= environmental partitioning factor

If the desired tissue criterion element is for an egg-ovary concentration, Jenni et al. (2017) prescribe translating this into a whole body concentration using either EPA guidelines or species-and site-specific data. Each of the key modeling factors incorporated as model inputs are described in more detail below.

4.2 FISH TISSUE CRITERION ELEMENT

It is widely understood that Se toxicity is manifested through chronic dietary exposure to Se. A growing body of research has described egg-laying vertebrates as the most sensitive ecological endpoint for selenium (see **Section 1.4.2**). The SeTSC scientific recommendation was to consider fish as the most

sensitive endpoint in the Lake Koocanusa ecosystem (see **Section 3.7**). Limited toxicity data exists for fish species in Lake Koocanusa, only species in the genus *Onchorhynchus* (rainbow trout and westslope cutthroat trout) and species within *Salvelinus* (bull trout) have known Se toxicity thresholds. **Table 4-1** describes the toxicity thresholds based on 10% effect concentrations (EC10's) described in EPA (2016). Laboratory analysis whereby the effect concentrations results in 10% mortality is referred to as an EC10. This is also referred to as a low effect concentration (LOEC).

Table 4-1. Tested reproductive-effect whole body (WB) concentrations measured directly or converted to WB concentrations from egg-ovary (EO) concentrations (EPA, 2016). Taxon resident in Lake Koocanusa *are Salvelinus*, *O. mykiss*, *Onchorhynchus*.

Taxon*	EO Chronic Value	EO/WB CF	Direct or Calculated WB Repro Chronic Value	Direct Calculation or Basis for EO/WB CF (from Appendix B)
Salvelinus	56.2	1.61	34.9	Dolly Varden EO/M (1.26) x all fish M/WB (1.27)
Esox	34.0	2.39	14.2	Northern pike EO/M (1.88) x all fish M/WB (1.27)
Cyprinodon	27.0	1.20	22.6	Desert pupfish EO/WB
O. mykiss	24.5	2.44	10.0	Rainbow trout EO/M (1.92) x all fish M/WB (1.27)
Rudolph et al. 2008	24.7	1.96	12.6	*Oncorhynchus* EO/WB
Nautilus 2011	27.7	1.96	14.1	*Oncorhynchus* EO/WB
O. clarkii	26.2	NA	13.3	Geometric mean of two studies
Oncorhynchus	25.3	NA	11.6	Geometric mean of *O. mykiss* and *O. clarkii* WB SMCVs
Micropterus	26.3	1.42	18.5	*Micropterus* EO/WB
Salmo	21.0	NA	13.2	Directly calculated EC_{10}
Coyle et al. 1993	26.3	NA	8.6	Directly calculated EC_{10}
Doroshov et al. 1992a	22.6	2.13	10.6	Bluegill sunfish EO/WB
Hermanutz et al. 1992, 1996	14.7	NA	10.6	Directly calculated EC_{10}
Lepomis	20.6	NA	9.9	Geometric mean of three studies
Acipenser	15.6	1.69	9.2	White sturgeon EO/M (1.33) x all fish M/WB (1.27)

EPA (2016) describes Se concentrations in egg or ovaries as the best predictors of Se toxicity but explains the vulnerability of a species is the product of its propensity to accumulate Se from its environment through diet and transfer the Se from its body into the eggs. Therefore, EPA includes guidance in Appendix K to use whole body tissue as a reasonable alternative for modeling site specific criteria derivation. Presser and Naftz (2020) provide details outlining the necessity to model from whole body rather than eggs or ovaries in Lake Koocanusa. Moreover, given the limited toxicity data for the resident species in Lake Koocanusa, the EPA guideline for whole body calculated to 8.5 mg/kg dw was used for the criterion element in modeling (Presser and Naftz, 2020). This value was calculated by EPA using OLS regression based on the known four most-sensitive species in the nation, including the most sensitive species, white sturgeon, which is resident in the downstream Kootenai River. A criterion element of 8.5 mg/kg dw is considered protective of white sturgeon across the US. While Presser and Naftz (2020)

applied the 8.5 mg/kg 304(a) whole body criterion in the modeling, they describe how other values may be applied depending on the level of protection desired and the goals of the modeling.

4.3 TROPHIC TRANSFER FACTORS

Selenium trophic transfer factors (TTFs) describe uptake and efflux of prey and predator species (Presser, 2013). Chapman et al. (2010) discusses the similarity of TTFs found within groups of related species and species with a similar trophic status (level). Chapman further describes an important concept in understanding Se TTFs is that the majority of Se enrichment occurs at the lowest trophic levels through particulates and primary consumers. The implication of this is that secondary and tertiary consumers may not always experience substantially higher Se exposure than lower trophic levels. This differs from contaminants such as mercury (Hg) that consistently bio-magnify at higher trophic levels.

Presser and Naftz (2020) utilized TTFs established from laboratory experiments. Field derived TTFs have greater uncertainty than laboratory derived TTFs and Lake Koocanusa had limited data; therefore, USGS determined it most appropriate to model with laboratory derived TTFs as described in Presser and Luoma (2010). The following TTFs were applied; 2.8 (aquatic insects), 1.5 (zooplankton), and 1.1 (fish). Presser and Naftz (2020) present two choices for bioavailability (100% and 60%). The 60% bioavailability effectively reduces the TTF's by 60% to match observed data.

4.4 FOOD WEB MODELS

Two primary food web models were presented in Presser and Naftz (2020) and described in Tables 6 and 7 of their report. Included are the invertebrate to fish model (IFM) and trophic fish model (TFM).

The IFM model is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish guideline wb}/TTF_{fish}\ /[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

The TFM modeling is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish tissue guideline wb}/TTF_{fishTL4}/TTF_{fishTL3}[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

Focal fish, previously selected by the SeTSC, were grouped into categorized food webs representative of variations in diet of modeled fish. The categorized food webs described for the IFM model are located in **Table 4-2** and for the TFM model are in **Table 4-3**. These tables present different model scenario options, in which the invertebrate fraction (invert faction) in the above equations are modified based on the percentage consumed. The adjustment to the food web, in turn, modifies the bioaccumulation potential (BAP). The BAP is the combined effect of diet and TTFs. The food web consisting of 100% chironomids (aquatic insects) has the greatest bioaccumulation potential.

Table 4-2. Invertebrate to fish (IFM) model: fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
RBT, WCT, RSS, LNS	100% chironomid	3.08	1.85
PMC, LSS, MWF	50% chironomid 50% zooplankton	2.37	1.42
rainbow trout: Dec-Mar	25% chironomid 75% zooplankton	2.01	1.20
kokanee	100% zooplankton	1.65	0.99

Table 4-3. Trophic level fish model (TFM): fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
BT, burbot (winter, benthic), NPM	100% TL3 fish species (100% insectivores)	100% chironomid or aquatic insect	3.39	2.03
BT, burbot, NPM	100% TL3 species (50% IV; 50% PV)	50% chironomid 50% zooplankton	2.60	1.56
[1)]BT, burbot (summer fish), NPM	100% TL3 species (100% planktivores)	100% zooplankton	1.82	1.09

4.5 PARTITIONING COEFFICIENT (K_D)

The partitioning coefficient (K_d) describes the relationship between Se concentrations in particulate and dissolved phases (Presser, 2010). The term K_d has been used interchangeably with enrichment factor (EF), a term more commonly used by EPA. The K_d is a simple ratio described below which could be expanded to include a more complex enrichment function incorporating saturation kinetics. In this modeling process the equation below was used. The collection of matched particulate Se and dissolved Se is described in **Section 3.1** using the terminology suspended particulate matter (SPM) and particulate interchangeably.

$$K_d = [\text{particulate Se}]/[\text{dissolved Se}]$$

K_d values calculated from 2015-2019 ranged from 424.2 to 7,474.5 L/g. Rather than statistically reducing the K_d dataset down to a single representative value to use in the model equation, Presser and Naftz (2020) present each K_d calculation as an independent scenario (n=87). The result of this is that each model scenario includes 87 predicted dissolved selenium concentrations.

4.6 MODELING CONCLUSIONS

Presser and Naftz (2020) present a report and accompanying data release which provide the data, rationale, food web modeling structure, and interactive spreadsheets for the quantitative derivation of a site-specific selenium guideline for Lake Koocanusa.

Model predictions of protective dissolved selenium concentrations were specific to the EPA national guideline of 8.5 mg/kg whole body criterion, while recognizing that this whole-body concentration could be changed to meet the protection goals of BC-ENV. Modeling choices and assumptions used for the modeled scenarios were guided by the goals described in the report, and previously defined by the SeTSC.

Those goals are summarized here as:

- Consideration of ecologically significant species and those important to stakeholders,
- Protection of ecosystems during maximum dietary exposure (i.e., feeding within a benthic food web),
- Protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding in an ecosystem that functions as a lentic reservoir, and
- Long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all Se loading profiles, and all water years.

The IFM model based on the food web with maximum BAP (maximum dietary exposure) was through a 100% chironomid (aquatic insect) diet and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice. As noted in **Section 4.5**, Presser and Naftz (2020) recommend that model runs be undertaken for each measured K_d, in order to provide the full range of candidate criteria for a specified model scenario.

The TFM model was based on the food web with maximum BAP (TL3: trophic level 3) was through a piscivorous diet of 100% forage fish which had a diet of 100% chironomid (aquatic insect), and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice.

Presser and Naftz (2020) did not provide a final recommended protective water column Se concentration. Rather, as described above, the report provided the foundation from which DEQ and BC-ENV were able to co-develop a protective water column Se standard.

5.0 CRITERIA DEVELOPMENT AND IDENTIFICATION

The scientific expertise of the SeTSC guided the development of Lake Koocanusa's site specific water column Se standard, from the early stages of data collection to the final recommendations for ecosystem-scale model factors. The USGS modeling results described in **Section 4.0** describes the foundation from which a protective water column value would be co-developed between DEQ and BC-ENV. This section presents the scientific recommendations of the SeTSC and analysis by DEQ and BC-

ENV to co-develop a numeric water column standard that prevents impairment of the aquatic life beneficial uses of Lake Koocanusa.

5.1 SeTSC Recommendations

As part of the criteria development process and different protection goals between BC and the US, the LKMRWG Steering Committee (comprised of a BC and DEQ representative) solicited individual SeTSC recommendations on model input parameters (whole body tissue criterion, food web, TTF, and K_d). The SeTSC was additionally requested to provide recommendations on a final protective water column Se standard for Lake Koocanusa. Recommendations were discussed at length throughout a half-day teleconference held August 25, 2020. A subset of the members submitted additional written recommendations (**Appendix A**). The three USGS SeTSC members recused themselves from providing recommendations beyond what is provided in Presser and Naftz (2020) and Jenni and Schmidt (2020).

5.1.1 Fish Tissue Criterion Element

Presser and Naftz (2020) applied the US EPA criterion of 8.5 mg/kg whole body tissue threshold, yet describe that modification of this value may be appropriate depending on the level of protection desired and modeling goals. The derivation of the national whole body fish tissue threshold includes white sturgeon, the species with the known greatest sensitivity to selenium (EPA, 2016). In spite of that, five out of seven participating SeTSC members recommended applying a lower whole body value. Their recommendations ranged from 4.6 – 7.0 mg/kg dw. Primary rationale for selecting a lower whole body value included; 1) consistency with the lower British Columbia guideline which is considered protective of 100% of species at all life stages, 2) uncertainty around potentially sensitive species and species of cultural importance for which no toxicity data exists, 3) providing assurance for sensitive aquatic dependent wildlife which may become resident in the future, 4) based on selenium toxicity expertise and understanding of the reservoir.

5.1.2 Trophic Transfer Factors (TTFs)

Recommendations for TTFs are intertwined with the bioavailability. As described in **Section 4.3** Presser and Naftz (2020) included a 2.8 TTF for aquatic insects and 1.5 for zooplankton. Additionally, Presser and Naftz (2020) present two bioavailability choices, 100% and 60% bioavailability. The 60% bioavailability was recognized to be the option better tailored to Lake Koocanusa. The 60% bioavailability effectively reduces the 2.8 TTF (aquatic insects) to 1.7 and the 1.5 TTF (zooplankton) to 0.9. However, there was general agreement among the SeTSC that the 2.8 and 1.5 TTFs at the 60% bioavailability may still be over predictive. The SeTSC individual members had varying recommendations for approaching TTFs including; 1) applying the 2.8 and 1.5 at 60% as a conservative measure particularly if using a less conservative whole body threshold (8.5 mg/kg dw), 2) use site specific TTFs (recommendations ranged from 1.1 – 1.2 (aquatic insects) and 0.52-0.85 (zooplankton), 3) combine the Presser and Luoma (2010) and EPA (2016) TTF data to produce a more robust dataset from which to derive a TTF based on the central tendency.

5.1.3 Food Web Model

Presser and Naftz (2020) included two models, the IFM and TFM. Within each are categorized food webs representative of variations in diet of modeled fish. Recommendations focused on using a piscivorous food web at 100% aquatic insects. This food web is referred to as TFM with TL3 100% Aquatic Insects (**Section 4.4**). The rationale for TFM with TL3 100% aquatic insects, the most

conservative food web, was for protection for potentially sensitive piscivorous species and species of cultural importance.

5.1.4 Partitioning Coefficient (Kd)

Two of the SeTSC members recommended additional analysis be done to sub-set the K_d dataset to include only K_d values from the epilimnion. The epilimnion showed slightly greater K_d values overall, and the rationale to include only epilimnion K_d data was to include a conservative approach which may be more representative of Se entering the food chain, specifically through primary producers.

Ultimately, a protective water column value must be selected from the distribution of observed K_d values. Recommendations ranged from the 50th percentile (median) to the 90th percentile depending on the model assumptions (model inputs). The recommendations as to which K_d percentile to select was reliant upon the level of conservatism incorporated into other model parameters, particularly the whole body tissue criterion. There was overall agreement that if applying a lower (more conservative) whole body value, then a median K_d would be protective of the beneficial use. However, if a less conservative whole body value was used, such as the 8.5 mg/kg, then a more protective percentile from the distribution would be recommended to ensure adequate protection.

5.1.5 Water Column Concentration Recommendations of the SeTSC

Recommendations on a final Se water column criterion were presented from four of the seven participating SeTSC members. Final values presented ranged from 0.6 μg/L to 1.5 μg/L, with one committee member describing a range for consideration between 0.73 μg/L to 0.8 μg/L. Three of the four SeTSC members proposed criteria recommendations less than 0.9 μg/L with one recommendation of 1.5 μg/L. The 1.5 μg/L recommendation was proposed with the rationale to follow the EPA 304(a) criteria, rather than utilize the work presented in Presser and Naftz (2020).

5.2 DEQ & BC-ENV SUPPLEMENTAL ANALYSIS

As previously stated, the goal of this work was to co-develop a site-specific water column standard for Lake Koocanusa. A challenge of that work has been the differing protection goals between BC-ENV and DEQ. However, the two agencies worked collaboratively, giving consideration to SeTSC recommendations and the protection goals of each agency, to co-develop three additional model scenarios to consider in conjunction with the model assumptions presented in Presser and Naftz (2020). This section describes the collaborative analysis and the DEQ proposed dissolved Se standard. **Table 5-1** describes model inputs (model assumptions) representative of a balance between the SeTSC recommendations and BC-ENV and DEQ agency goals for the water body.

The scenarios described in **Table 5-1** apply a whole body tissue value of 5.6 mg/kg. As this work was a collaborative process between BC-ENV and DEQ, the 5.6 value was incorporated to remain consistent with BC-ENV's more stringent guidelines. This value falls within the range recommended by five out of seven SeTSC members (see **Section 5.1.1**). This value was derived by applying a westslope cutthroat (a resident species within the known most Se sensitive genus) egg-ovary to whole body conversion factor of 1.96 (EPA, 2016) to the BC provincial egg ovary guideline of 11.0 mg/kg dw.

Based upon recommendations by some SeTSC members to include site specific TTFs, BC-ENV and DEQ applied a bioavailability of 45%. This work was guided both by analysis by Thorley (2020) and the

recommendations by two of the seven SeTSC members. While the 45% bioaccumulation incorporates less conservatism, it was found to be more representative of the observed concentrations. It was determined by BC-ENV and DEQ that this 45% bioavailability would only be appropriate if a more conservative whole body tissue value was also applied (5.6 mg/kg). The 45% effectively reduces the aquatic insect TTF from 2.8 to 1.26 and the zooplankton from 1.5 to 0.68. For aquatic insects, this value is very close to site-specific TTFs recommended by two SeTSC members which ranged from 1.1-1.2 and is within the range of site specific zooplankton TTFs recommended that ranged from 0.56-0.85. The live Excel spreadsheets for the three model scenarios presented in **Table 5-1** are available from DEQ upon request (please contact DEQ's Water Quality Standards & Modeling Section).

Table 5-1. Three additional model scenarios developed by BC-ENV and DEQ incorporating SeTSC recommendations.

Scenario	Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
1	5.6	IFM	100% Aquatic Insects		2.8		45%	50th (median)	0.89
2	5.6	TFM	75% Aquatic Insects/ 25% Zooplankton	1.1	2.8	1.5	45%	50th (median)	0.91
3	5.6	TFM	100% Aquatic Insects	1.1	2.8		45%	50th (median)	0.8

With consideration of the SeTSC recommendations and supplemental DEQ and BC-ENV analysis, DEQ determined Scenario 3 from **Table 5-1,** resulting in a dissolved water column numeric standard of 0.8 µg/L, to be protective of the aquatic life beneficial uses of Lake Koocanusa. Scenario 3 ensures all ecosystem food webs are protected, a stated goal of the SeTSC.

Additionally, DEQ considered the SeTSC recommendation to use the 8.5 mg/kg tissue threshold. Through working collaboratively with BC-ENV, the following model assumptions as described in Presser and Naftz (2020) were applied; IFM 100% Aquatic Insects, 60% bioavailability, and DEQ selected a more conservative percentile from the upper quartile of the K_d distribution (75th percentile). This modeling scenario also meets the protection goals defined by the SeTSC and DEQ. This scenario resulted in a water column Se value of 0.8 µg/L protective of the aquatic life beneficial uses of Lake Koocanusa.

Table 5-2. Model inputs DEQ considered following the two SeTSC member recommendations to apply 8.5 mg/kg and following the recommendations to then identify a more conservative K_d percentile.

Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
8.5	TFM	100% Aquatic Insects	1.1	2.8		60%	75th	0.8

6.0 PROPOSED CRITERIA FOR LAKE KOOCANUSA

Proposed Se standards for Lake Koocanusa contain two classes of selenium standards; fish tissue standards, which limit the amount of Se allowed to accumulate in different fish tissues, and a water column standard which was derived from bioaccumulation modeling also intended to limit Se accumulation in fish tissue.

Table 6-1. Proposed water column and fish tissue Se standards for Lake Koocanusa.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

The national fish tissue standards have a hierarchy of importance; the egg/ovary standard is the most takes precedence because those data are the most indicative of selenium toxicological effects on fish at the reproductive stage. However, fish egg/ovary tissue is not always available. Fish muscle or whole body tissue standards can be used in the absence of fish egg/ovary tissue. The fish tissue standards supersede the water column standard only when the lake or river is in steady-state, referring to conditions whereby there are no activities resulting in new, increasing, or changing selenium loads to the lake, and selenium concentrations in fish have stabilized. Lake Koocanusa is not currently in steady state (Presser and Naftz, 2020). Therefore, both the fish tissue standards and water column standards are applicable standards for Lake Koocanusa. The department will determine when Lake Koocanusa reaches steady state after review and analysis has been carried out by the department during triennial review. The proposed water column standards are chronic values. There is no acute selenium standard included since the greatest toxicity risk to aquatic life is from chronic dietary exposure.

6.1 FREQUENCY AND DURATION

The proposed recommendation for return frequency is consistent with EPA's current 304(a) guidance based on the 1985 Guidelines for water column criteria. The proposed return frequency for a water column criteria exceedance of not more than once in three years, on average, is based on EPA's determination of the resiliency of the aquatic ecosystem to recover from a toxin when the impacts are associated exclusively with a water column exposure (Stephan et al., 1985).

The duration component of the criteria describes the exposure time-period and restricts the length of time that the concentration in the receiving water can be continuously above a criterion concentration, in order to protect aquatic life. The proposed durations are consistent with the current 304(a) guidance such that the numerical fish tissue criterion elements are specified as instantaneous. The use of an instantaneous measurement (duration) not to be exceeded (frequency) is because fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish population(s) at a given site. Selenium concentrations in fish tissue are generally expected to only change gradually over time in response to environmental fluctuations. The duration for the chronic water column standard is a 30-day average.

6.2 PROTECTION OF DOWNSTREAM WATERS

Federal regulation at 40 CFR 131.10(b) requires the State to consider and ensure the attainment and maintenance of downstream (intra-and interstate waters) WQS. The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream Endangered Species Act (ESA) listed, white sturgeon.

The Kootenai River is in a B-1 use class, identical to what was outlined for Lake Koocanusa (**Section 1.3.1**). The designated use class for the Kootenai River in Idaho is outlined in Idaho's regulations found at 58.01.02.100 as, "*water quality appropriate for the protection and maintenance of a viable aquatic life community for cold water species*." Idaho defines viable aquatic life as communities that are functioning and intact. Additionally, the Kootenai River Native Fish Conservation Program includes a Tribal Sturgeon Hatchery managed by the Kootenai Tribe of Idaho (KTOI) to prevent extinction, preserve the existing gene pool, and rebuild a healthy age class of the ESA listed endangered white sturgeon.

The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream ESA listed, white sturgeon. DEQ modeled white sturgeon using the bioaccumulation model by Presser and Naftz (2020). Applying the white sturgeon whole body sensitivity of 9.2 mg/kg, the food web of TFM with TL3 100% aquatic insects, a TTF of 2.8, and a bioavailability of 60%, the results showed 0.8 μg/L to be protective at the 90th percentile of the Kd distribution. As white sturgeon are not resident within Lake Koocanusa, DEQ finds this to meet the protection of downstream use including protection of white sturgeon.

6.5 PROTECTION OF FEDERALLY LISTED SPECIES

The bull trout was listed as threatened under the ESA on November 1, 1999 (64 FR 58910). Bull trout are native to Lake Koocanusa and the Kootenai river, representing a geographically distinct and important population within the broader bull trout range. In Montana, the management of fisheries including that of bull trout is executed by FWP. Montana FWP biologists monitor spawning sites (redds) annually as a metric for measuring fish reproduction, recruitment, and fisheries management. The monitoring takes place during the fall as most bull trout spawn between late August and early November. Current trends for bull trout in Lake Koocanusa are common abundance with a stable population. The 2002 FWP Bull Trout Report lists current threats to bull trout in Lake Koocanusa (above Libby Dam) as identified by the Montana Bull Trout Scientific Group as including introduced fish species, rural residential development, forestry, mining, agriculture, water diversions, and illegal harvest.

At present, there are no known selenium toxicity studies for bull trout. There are toxicity tests for the taxonomically similar, dolly varden, both in the genus *Salvelinus*. Research shows dolly varden to be among the most tolerant fish species to Se. **Table 4-1** describes the whole body and egg ovary selenium toxicity thresholds for dolly varden being 34.9 (whole body) and 56.4 (egg ovary). DEQ modeled bull trout using the bioaccumulation modeling by Presser and Naftz (2020) by substituting the whole body guideline for the *Salvelinus* whole body toxicity threshold of 34.9 to determine that the proposed standards including the site specific water column standard of 0.8 μg/L is protective such that no individual bull trout will be harmed.

7.0 REFERENCES

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Threatened Bull Trout (Salvelinus confluentus). U.S. Fish and Wildlife Service, Pacific Region, Portland, Oregon

APPENDICES

1. Appendix A – Selenium Technical Subcommittee written recommendations

28th August, 2020

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Sean Moore| Director, Watershed Science and Adaptation, Environmental Sustainability and Strategic Policy Direction, BC Ministry of Environment| LKMRC Co-Chair
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Dear LK MRC Co-Chairs and Members,

Please accept this recommendation on behalf of the Confederated Salish and Kootenai Tribes (CSKT) and the Kootenai Tribe of Idaho (KTOI), constituent governments of the transboundary Ktunaxa Nation. You will find herein our scientific justification and rationale, regarding the request to provide written recommendation on the inputs to the model developed by US Geological Survey (USGS), in support of a site-specific selenium criteria for Koocanusa Reservoir.[1]

The Transboundary Kootenai watershed sits entirely within the transboundary Ktunaxa Nation Territory and provides critical habitat for rare and threatened fish species including bull trout, burbot, westslope cutthroat trout, and endangered Kootenai River white sturgeon. Unabated selenium inputs from the Elk Valley mines into Koocanusa Reservoir demonstrate a clear, increasing trend dating back to 1984.[2] Selenium leaching from the Teck Ltd. mines in the Elk Valley of British Columbia is resulting in degradation of water quality and presenting unacceptable impairment and risks to Ktunaxa Territory resources. As noted in our previous

[1] Presser, T.S., Naftz, D.L. Naftz, 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A. and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020-1098, 40 p., https://doi.org/10.3133/ofr20201098.

[2] Unpublished data from 2019 collected by the U.S. Environmental Protection Agency, U.S. Geological Survey and Kootenai Tribe of Idaho for the Kootenai River and tributaries. 2019.

letters, we are specifically concerned about impacts on the water quality, fish and fish habitat, species at risk, impacts to other species and resources that depend on those waters and fish, and traditional cultural values, including human health impacts from consumption of contaminated fish, in the entire transboundary Kootenai watershed.

Based on historical and recent data for water quality and fish tissue, it is imperative that Montana work now to adopt a site-specific selenium criteria for the health and protection of all fish species in Koocanusa Reservoir and downstream in the Kootenai watershed. We recognize that existing data documents increasing selenium in several species of fish in Koocanusa Reservoir, including three species that exceed the 2016 EPA recommended criteria for selenium in fish tissue. Further, Koocanusa Reservoir is currently unprotected, given that Montana did not adopt the national recommended selenium criteria, as revised and released by EPA in 2016.[3] The best available science, including the 2020 USGS model and report, demonstrates that there are historical, on-going, and projected future inputs of selenium into Koocanusa Reservoir, and it is the responsibility of the State of Montana to adopt a selenium criteria that is sufficiently protective to ensure the immediate and long-term protection and restoration of Koocanusa Reservoir, and downstream uses in the Kootenai River, from the ecological impacts of selenium contamination. Given the current impacts and risk to Ktunaxa territory resources, the KTOI and CSKT are in full support of the commitment by the State of Montana to adopt a site-specific selenium criterion by December, 2020, including initiation of the formal rule-making process in September, 2020.

In addition, we support the scientifically defensible and peer-reviewed report and model developed by USGS in support of criteria development, including the approach of the USGS to base the model on a conservative and protective approach. The authors of the model are among the top selenium experts in North America, with decades of experience in the field of selenium toxicology, and the model they have developed is peer-reviewed and capable of generating a defensible, protective criterion for the reservoir, based on the factors that influence selenium in the reservoir.

Given that Koocanusa Reservoir is already degraded due to input of contaminants from mining in the Elk Valley of British Columbia, we support a criterion that manages the reservoir to improve and restore from the already degraded condition. Current levels of selenium contamination caused by Elk River coal mining above and below Libby Dam is with high probability already causing, and threatens to continue, negative physiological effects to organisms dependent on aquatic resources, including birds, and possibly humans. A conservative site-specific criterion is needed to support management that improves and restores the water quality and aquatic life in the reservoir.

[3] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

There is evidence of significant bioaccumulation of selenium already occurring across the Kootenai ecosystem, including the Idaho and BC portions of the Kootenai.[4] This bioaccumulation has been occurring and will continue even at current water column selenium concentrations that are below the current criteria/exceedance limits. Literature provides evidence that body burden concentrations found in Kootenai River white sturgeon, burbot, mountain whitefish, and freshwater mussels are likely already having significant physiological effects. This is a critical concern to the Ktunaxa Nation governments, given the cultural significance of these species, as well as the tremendous effort and resources dedicated to ecosystem restoration.

The selection of a conservative and protective site-specific selenium criterion is necessary to, at minimum; prevent further increases in selenium into the Kootenai ecosystem. Current data is showing increasing concentrations of selenium in larger portions of the reservoir, which in turn will increase selenium concentrations below Libby Dam.[5] This trend will continue until effective mine impact mitigation is implemented at an appropriate scale.

The overall selenium loading into the reservoir from the Elk River needs to be stabilized and reduced in order to prevent near-future partitioning and release of selenium into the reservoir and also the downstream Kootenai River.

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L. Therefore, based on the specific framework of the USGS model W6, Model run #2, the CSKT and KTOI are specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Based on the attached background, modeling recommendations and rationale, the KTOI and CSKT recommends using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates.

In summary, we are recommending a conservative site-specific criterion for selenium in Koocanusa Reservoir, based on the following uncertainties;

1. Koocanusa Reservoir currently demonstrates system degradation and impairment. This is demonstrated by the following:
    a. Fish tissue concentrations (muscle, whole body, and/or egg ovaries) at times exceed USEPA and B.C. recommend thresholds.

[4] https://governmentofbc.maps.arcgis.com/apps/webappviewer/index.html?id=0ecd608e27ec45cd923bdcfeefba00a7

[5] Presser, TS, and DL Naftz. 2020. Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, USA, and British Columbia, Canada: US Geological Survey Open-File Report 2020-1098, 40 p. https://doi.org/10.3133/ofr20201098.

b. The reservoir has increasing pollutant loads, as demonstrated by B.C. long-term monitoring station on the Elk River at HWY 93.
c. The reservoir has an increasing mass of selenium over an increasing reservoir area (Presser and Naftz, Figure 17).
d. The reservoir has declining burbot populations.
e. Fish populations demonstrate gonadal disfunction and dysfunctional selenium dietary bioaccumulation.

2. Water quality monitoring data indicate the Koocanusa Reservoir is a dynamic system and it is possible that current monitoring efforts have not defined nor captured critical time periods or critical portions of the reservoir.
3. A delay or lag in uptake of selenium into the food web, from the water column, is highly likely and at a magnitude that presents a significant risk. The outcome is increasing and perpetuated bioaccumulation of selenium in benthos and fish above elevated levels.
4. To return to a restored condition, MT DEQ must avoid normalizing current degraded conditions and strive for a condition that is improved from current conditions.
5. On-going revisions to the modeling in the Elk and Fording River, including the Implementation Plan Adjustment to the Elk Valley Water Quality Plan, that increases the observed and modeled future contaminant delivery into Koocanusa Reservoir from the Elk Valley Mines.[6]

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

Sincerely,

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[6] 2019 Implementation Plan Adjustment Annex B - Regional Water Quality Model Modifications
https://www.teck.com/media/Annex-B-Regional-Water-Quality-Model-Modifications.pdf

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August 28, 2020

Dear SeSTC Committee Members and Co-Chairs,

Selenium Technical Sub-Committee members were requested to submit written recommendations to the SeTSC Co-Chairs for the site-specific selenium criteria. Below you will find our recommendations, serving as a representatives of the Kootenai Tribe of Idaho (KTOI) and the Confederated Salish and Kootenai Tribes (CSKT). Please see below for background, recommendations and rationale for the site-specific criteria.

We based on our recommendation on a site-specific criterion that protects burbot (*Lota lota*), the fish species that are most sensitive to selenium bioaccumulation in Koocanusa Reservoir. Burbot have been functionally extirpated from the reservoir and are culturally important to the Ktunaxa Nation community. Burbot populations declined over two decades ago when the ambient reservoir Se concentrations were below what is currently seen today. In published literature, burbot have been shown to be particularly sensitive and susceptible to the bioaccumulation of selenium.[1] Muscatello and Janz observed significant bioaccumulation in burbot (10 ug/g dw WB) at low aqueous (<0.5 µg/L) and benthic invertebrate (0.5-3 µg/g) selenium concentrations.[2] This is reinforced with the general knowledge that the burbot population decline[3] and eventual functional-extirpation in Koocanusa Reservoir coincides with the Elk River Coal Mines operational history and subsequent water pollution caused by those coal mines; and severely complicates the restoration of burbot above Libby Dam.[4]

The burbot population declined when the ambient reservoir Se concentrations were below the aqueous concentrations that are currently seen today. Limited KTOI data is also showing that burbot in the mainstem Kootenai River are accumulating selenium at rates that are known to cause significant negative physiological effects on other fish species. Those effects include reproductive failure, reduced growth, and mortality (KTOI, unpublished data).
Further, mining contaminant inputs into Koocanusa Reservoir present a critical uncertainty in the Kootenai River Ecosystem Restoration program[5], and will continue to act in synergy with

[1] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[2] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[3] Dunnigan, J., J. DeShazer, T. Ostrowski, M. Benner, J. Lampton, L. Garrow, and M. Boyer. 2018. Mitigation for the Construction and Operation of Libby Dam, 1/1/2017 – 12/31/2017 Annual Report, 1995-004-00. 252 pp.
[4] Cope, A. 2018. Upper Kootenay River Burbot Conservation Strategy, Draft Report. 59 pp.
[5] www.http://restoringthekootenai.org

the habitat alterations perpetuating white sturgeon and burbot recruitment failure below Libby Dam.

In addition to burbot, it is critically important that the criterion is based on considerations for protection and restoration of the Kootenai River white sturgeon (*Acipenser transmontanus*) downstream of Libby Dam given their sensitivity to reproductive impacts from selenium toxicity. We note that white sturgeon are the most toxicologically sensitive fish as ranked by the US EPA in its national guidance.[6]

With respect to birds and wildlife, the Kootenai River Basin was once one of the more ecologically productive inter-montaine ecosystems, supporting resident and migratory bird populations; however, Koocanusa Reservoir currently does not support robust shorebird populations. Shorebirds are particularly vulnerable to selenium toxicity, as they are highly sensitive to selenium exposures.[7] Skorupa et al found reproductive failure in aquatic birds with 3.0 µg/g selenium concentrations in their eggs.[8] Birds have been shown to be particularly sensitive to selenium exposures due to their feeding habits that are linked to the aquatic environment.[9] Stanley et al found that a 7 mg Se/kg dietary exposure in mallard ducks caused a >30% embryo mortality.[10]

Hamilton reviewed approximately 40 different studies investigating selenium toxicity for fish, aquatic birds, phytoplankton, and zooplankton.[11] Several tables within this paper provided a comprehensive compilation of species tested, tissues sampled, selenium concentrations tested for effects, corresponding physiological effects, and study citations. The physiological effects concluded by the individual studies listed throughout the review tables are “Mortality”, “Reduced Growth”, “Reproductive Failure”, “Reduced Weight”, and “Reduced Cell Replication”.

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[6] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

[7] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[8] Skorupa, JP, HM Ohlendorf, and RL Hothem. In press. Interpretive guidelines for selenium-exposed waterbirds. J. Wildlife Management.

[9] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[10] Stanley, TR Jr, GJ Smith, DJ Hoffman, H Heinz, and R Rosscoe. 1996. Effects of boron and selenium on mallard reproduction and duckling growth and survival. Environ Toxicol Chem 15:1124-1132

[11] Hamilton, SJ. 2003. Review of residue-based selenium toxicity thresholds for freshwater fish. Ecotoxicology and Environmental Safety 56:201-210.

For several fish and aquatic bird studies listed, the selenium toxicity levels causing mortality, reduced growth, reproductive failure, and/or reduced weight were whole body tissue and/or egg concentrations as low as 1-4 ppm.

Thorley cites data collected from water and fish tissue (whole body and egg/ovary) Se concentrations for Koocanusa Reservoir.[12] Water concentrations ranged 0.5 -1.5 µg/L, and corresponding fish tissues from several fish species ranged from 1.0 – 6.0 ppm for whole body, and ~2.0 to 80.0 for egg/ovary. Even if the 80.0 µg/g observation is an outlier, results from peamouth chub (*Mylocheilus caurinus*), redside shiner (*Richardsonius balteatus*), and Northern pikeminnow (*Ptychocheilus oregonensis*) were predominantly 10.0 – 40.0 µg/g for egg/ovary samples. These are tissue concentrations at water concentrations of 0.5-1.5 µg/L.

Thorley also presents data collected from zooplankton and benthic macroinvertebrate Se concentrations for sample sites located within Koocanusa Reservoir.[13] Zooplankton selenium concentrations ranged between <1 to 5 µg/g, with some samples upwards of 14 µg/g Se. Benthic macroinvertebrate tissue concentrations ranged between <1 to 12.5 µg/g Se, with the mean Se concentration near 5 µg/g Se.

The EPA whole-body threshold of 8.5 mg/kg dw is based upon the known sensitivity of white sturgeon. This is scientifically defensible and appropriate on the national level. However, the 8.5 mg/kg dw whole-body criterion does not account for other potentially sensitive and susceptible fish species or protection of the most sensitive designated use, which includes tribal harvest treaty rights. Whitefish (*Prosopium williamsoni*) and burbot are culturally important fish species that are consumed by Ktunaxa citizens from all three Ktunaxa Nation governments. A minimum whole-body threshold of 5.6 mg/kg dw should be considered. Using the BC MOE egg/ovary guideline of 22 mg/kg dw, and factoring in the safety/assessment factor of 2, and using the EC10 egg/ovary to whole-body conversion for rainbow trout of 1.9, this leads to a more conservative 5.6 mg/kg dw whole-body recommendation. The KTOI and CSKT recommend using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates. The KTOI and CSKT recommend a conservative site-specific criterion for Koocanusa Reservoir until additional science and data collection demonstrate otherwise.

Current reservoir selenium outflows are approximately 1.0 µg/L (range between 0.8 and 1.2 µg/L, depending upon dam operations, time of year, and hydrologic conditions within the basin). Kootenai River white sturgeon egg selenium concentrations in the mainstem river

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[12] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

[13] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

below Libby Dam range between 3.0 and 6.0 mg/kg dw. Of the five whole-body burbot tissue samples collected by the KTOI, one was above the 8.5 mg/kg dw EPA threshold, and mountain whitefish egg concentrations exceed EPA's 15.1 mg/kg dw threshold, with some of these values almost double the EPA recommended criteria (KTOI 2020; unpublished data). These measurements indicate that, like Koocanusa Reservoir, the Kootenai River requires the development of a site-specific water column selenium criterion. KTOI and CSKT understand that this will likely require a multi-year effort to collect adequate data and develop a site-specific criterion for the Kootenai River, and we encourage DEQ to begin this effort immediately in collaboration with both Tribes. For now, KTOI and CSKT support MT DEQ setting an interim criterion for the Kootenai River that is equal to EPA's national recommended value for water column, fish tissue, and egg/ovaries. In summary, we support the adoption of a conservative site-specific criterion for Koocanusa Reservoir now, to reduce uncertainty and risk in the Kootenai River downstream, and the subsequent initiation of a rigorous, scientific process to develop a site-specific criterion for the Kootenai River.

After evaluating multiple scenarios using a reasonable range of variable values within the USGS models provided to the SeTSC, the KTOI and CSKT recommends using the 'W6. TFM with TL3 100% Aquatic Insects' model. This model is conservative and protective of the most selenium-susceptible trophic levels; and is also considered the most protective, as it incorporates whitefish and burbot.

We recognize the variability of TTF's, conversion factors, and K_d values. Given the uncertainty and wide fluctuations in K_d throughout the reservoir (values ranging between 400 and 7000), a conservative K_d should be used. In order to be protective of the reservoir ecosystem across time and location, the 90th percentile K_d should be used to capture the worst-case scenario. The use of the median K_d value is also supported in literature. The use of the 1.1 TTF is supported by literature and is scientifically defensible. To manage the uncertainty in the water concentration guideline, Jenni, Naftz, and Presser (2017) suggested triangular distributions with a TTF for invertebrates (aquatic insects and zooplankton combined) between 1 and 3.5 with a mode of 1.3, a TTF for fish between 0.6 and 1.6 with a mode of 1.1 and a Kd between 800 and 6,500 with a mode of 3,000.

Model Input Recommendations

With respect to the specific model inputs, we provide the following recommendations and rationale; Given the varying K_d values within the reservoir, and the two recommended TTF values for aquatic insects, we ran six variations of the W6 model that incorporate the different K_d and TTF values. Listed below are the outputs from the six model runs.

1. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.22 µg/L (given the model correction of 100% Se bioavailability) to 0.37 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

2. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.37 µg/L (given the model correction of 100% Se bioavailability) to 0.61 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

3. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.53 µg/L (given the model correction of 100% Se bioavailability) to 0.89 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

4. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.29 µg/L (given the model correction of 100% Se bioavailability) to 0.49 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

5. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.49 µg/L (given the model correction of 100% Se bioavailability) to 0.82 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

6. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.71 µg/L (given the model correction of 100% Se bioavailability) to 1.18 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L.

Based on the specific framework of the USGS model W6, Model run #2 as described above, the CSKT and KTOI is specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Current whole-body fish tissue samples from Northern pikeminnow, peamouth chub, redside shiner, and largescale sucker in Koocanusa Reservoir exceed, and in many individuals sampled, greatly exceed, the EPA whole-body criteria in the current aqueous conditions in the reservoir.[14] This clearly indicates to KTOI and CSKT that to be protective of all fish species in the reservoir, the site-specific criterion should be lower than the current selenium concentrations

[14] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

sampled in the reservoir. Also, as noted in Presser and Naftz, 2020, it is important to determine where Koocanusa Reservoir is in an impairment-restoration cycle so as not to base protection on survivor bias, the maintenance of a currently degraded ecosystem, or normalized toxicity. In a broader context, one of the overall consequences of revised selenium regulations is that their derivation is now dependent on being able to define and understand the status of the ecosystem on which protection is based. And, as described in Presser and Naftz, 2020, the Koocanusa Reservoir system demonstrates traits of a currently degraded system (see Table 1 in the report and subsequent discussions). This further illustrates to CSKT and KTOI that a protective site-specific water column selenium criterion should be lower than existing conditions in the reservoir.

Given that there may be a lag in the biological uptake and detection of selenium across the food web in the reservoir, it is important to adopt a more conservative criterion at this time, to ensure protection under unknown future selenium levels and the increasing contaminant trends. Any selenium concentrations above the background concentrations represent an increase from baseline conditions for the Kootenai Basin and are likely already having, and will perpetuate negative impacts upon the ecosystem. According to Chapman et al[15] in the Selenium Risk Characterization chapter 7, Lentic systems were identified to be at an increased risk of Se-caused adverse effects due to the maximized mobility of selenium into the food web, thereby increasing the chance for elevated exposures.

Continuing downriver into the altered lower-river ecosystem driven by Libby Dam operations, the food web in the mainstem Kootenai River is quite different than the reservoir; therefore the movement of selenium from Koocanusa Reservoir through Libby Dam and into the lower-river is relatively unknown. Water and tissue sampling in the Kootenai River below Libby Dam suggests the current selenium concentrations and loading into the river are already having negative impacts on the ecosystem.

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

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[15] Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP. 2009. Ecological assessment of selenium in the aquatic environment: Summary of a SETAC Pellston Workshop. Pensacola FL (USA): Society of Environmental Toxicology and Chemistry (SETAC).

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August 29, 2020

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RE: KNC Selenium Technical Sub-Committee recommendations on Presser and Naftz (2020) selenium bioaccumulation model for Koocanusa Reservoir

In response to the request from the co-chairs of the Selenium Technical Sub-Committee (SeTSC) of the Koocanusa Reservoir Monitoring and Research Working Group, the Ktunaxa Nation Council (KNC) technical representatives of the SeTSC are pleased to offer our recommendations for consideration in setting the water quality benchmark (also "criterion" or "objective") for the protection of designated uses in the Koocanusa Reservoir. Specifically, we are providing our recommendation on the requested topics, including model inputs, quantitative approaches to using the USGS model outputs (Presser and Naftz 2020), and the proposed range of water column concentrations that would be protective of designated uses.

We would highlight that the following technical recommendations were developed based on our current understanding of the Koocanusa Reservoir and the data and information available (e.g., Beaman 2020; DeForest 2020; Lotic Environmental 2018; Presser and Naftz 2020; Thorley 2020). KNC technical representatives are providing recommendations at this time to honour the Se TSC co-chair request in recognition of the timelines required by Montana Department of Environmental Quality (DEQ) and their process for rule-setting. We also must acknowledge that KNC is committed to working government to government with the Province of British Columbia in developing a water quality objective for Koocanusa Reservoir. The technical recommendations made in this memo will be provided to KNC's Lands and Resources Council in September 2020, after which KNC's formal recommendations for the water quality objective for Koocanusa Reservoir will be submitted.

Model Inputs

Target Tissue Concentration

The ecosystem-scale model ("USGS model") used by Presser and Naftz (2020) estimates the concentration of selenium in trophic levels within a food web beginning with particulate material (i.e., sediment, phytoplankton, and detritus) in the water column through primary consumers (e.g., benthic invertebrates and zooplankton) and into higher trophic levels including planktivorous and piscivorous fish. The objective of the USGS model is to derive a range of selenium concentrations in the water column that are predictive of whole-body concentrations in fish that meet the protection goal of preventing adverse effects on fish reproduction. Various tissue-based whole-body selenium guidelines have been proposed to meet this objective, including the USEPA whole-body guideline of 8.5 µg/g dry weight (dw).

KNC technical representatives, along with some other members of the SeTSC, have recommended the BCMOE (2014) tissue-based guideline of 11 µg/g dw in egg/ovary tissue be used to provide assurances that the most sensitive species at the most sensitive life-stage would be protected during indefinite exposure. To facilitate the use of the BCMOE (2014) tissue-based guideline in the USGS model, we have converted the egg/ovary tissue concentration to a whole-body tissue concentration. The BCMOE (2014) guideline is based on an egg hatchability/viability test with rainbow trout. USEPA (2016) provides an egg/ovary:whole-body conversion factor of 1.96 for rainbow trout. Applying the conversion factor results in a whole-body tissue guideline of 5.6 µg/g dw, which can be used directly in the USGS model as the target tissue concentration.

In previous communications, KNC technical representatives requested the evaluation of a tissue-based guideline applied to the diet of fish and other wildlife (i.e., birds and mammals). The threshold of 4 µg/g dw (BCMOE 2014) was recommended as a protective threshold in prey fish (e.g., redside shiner, peamouth chub). However, based on an evaluation of the data available in the reservoir, the trophic transfer factor (TTF) between prey fish (e.g., peamouth chub) and piscivorous fish (e.g., northern pikeminnow) is roughly 1, indicating that the 4 µg/g dw threshold may be lower than the site-specific data warrants.

When setting water quality objectives, it is important to understand whether the protection goal sought (i.e., fish reproduction) would be protective of other designated uses (e.g., wildlife, recreation, human health). The target tissue concentration can be compared to whole-body tissue-based guidelines for other designated uses, including wildlife consumers (e.g., birds) and human consumers. With respect to avian receptors, Ohlendorf and Heinz (2011) suggest diets greater than 5 µg/g dw could reduce egg hatchability in sensitive avian receptors, including the mallard duck (EC_{10} of 4.9 µg/g dw; Joe Skorupa, USFWS, SeTSC member; pers. comm.). J. Skorupa (pers. comm.) also stated that an

appropriate threshold for protection, considering whole-body concentrations, would likely be in the 4.5 to 6 µg/g dw range (pers. comm) when considering an egg/ovary:whole-body conversion factor for burbot (i.e., 2.3) and using either the BCMOE (2014) egg/ovary threshold of 11 µg/g dw or USEPA (2016) egg/ovary threshold of 15.1 µg/g dw, respectively.

Recognizing the importance of food security and the continuity of Ktunaxa practice including hunting, fishing, trapping, camping, ceremonial practices, and the transmission of knowledge and identity to future generations, we also evaluated whether tissue concentrations would be protective of Ktunaxa citizens at their preferred consumption rates. BCMOE (2014) provides human health screening values for low (i.e., 30 g/day) to high (i.e., 220 g/day) fish consumption. By using the approach in BCMOE (2014), we calculated a whole-body threshold that we consider to be protective of cultural practices such as harvesting and consuming fish at preferred rates. By applying the most current estimate of Ktunaxa preferred consumption rates of 245 g/day, and selecting mountain whitefish as a focal species due to our understanding of cultural practices, we estimate that a muscle concentration of 1.6 µg/g wet weight (ww) or 6.8 µg/g dw would be an appropriate screening value. Using the muscle:whole-body conversion factor for mountain whitefish of 1.27 (USEPA 2016), an appropriate whole-body tissue threshold of 5.3 µg/g dw would achieve the BCMOE (2014) screening values and protect Ktunaxa cultural practices at currently understood preferred rates. We note that the Ktunaxa preferred consumption rate is our current best-estimate based on diet surveys and likely underestimates the importance of fish in Ktunaxa diet resulting from the exclusion of anadromous salmon from Ktunaxa Territory (ʔamakʔis Ktunaxa).

Given the estimates of ecological and human health thresholds for whole-body selenium concentrations noted above, we offer the following recommendation.

> **Recommendation 1:** We recommend that the water quality objective for Koocanusa Reservoir protect ʔa·kxaṁʔis q̓api qapsin (All Living Things) and that the Kootenay ecosystem (including the reservoir) be managed in such a way that Ktunaxa rights, title, and practices are protected. Accordingly, a target tissue concentration of 5.3 ug/g dw has been identified as protective of cultural use and a target tissue concentration of 5.6 µg/g dw using the BCMOE tissue-based guideline for the protection of ecological receptors. Therefore, it is our recommendation that 5.3 µg/g dw be used as the target tissue concentration in the USGS model, as it is protective of all uses.

Food Web Model

To assist in the development of a range of selenium concentrations in the water column that are predictive of whole-body concentrations in a variety of fish species, Presser and Naftz (2020) developed a series of food web models accounting for variations in the source (e.g., aquatic insects, zooplankton, fish) and proportions of food sources in the diet of model species. From our perspective, the *TFM w TL3 100% AqIns* model, which reflects the food web of burbot, bull trout, and northern pikeminnow is considered appropriately conservative and protective of the culturally important burbot, which has shown significant population declines (Hardy and Paragamian 2013). This food web assumes a piscivorous feeder with a diet consisting of prey fish that feed on aquatic insects (e.g., juvenile rainbow trout, westslope cutthroat trout, redside shiner, and longnose sucker).

Given the protection goal of protecting all fish species in the reservoir, we offer the following recommendation.

> **Recommendation 2:** We recommend the use of *TFM w TL3 100% AqIns* to represent the food web in the USGS model.

Trophic Transfer Factors (TTFs)

Presser and Naftz (2020) rely on literature derived TTFs in the mechanistic model as presented in Presser and Luoma (2010). The TTFs used, include:

- $TTF_{Particulate\ to\ Insects}$: 2.8
- $TTF_{Particulate\ to\ Zooplankton}$: 1.5
- $TTF_{Prey\ to\ Fish}$: 1.1

To account for any site-specific differences in the TTFs resulting from differences in community structure (e.g., proportion of rotifers, Presser and Naftz 2020; or, proportion of sediment, detritus, and algae in the particulate material, Beaman 2020), Presser and Naftz (2020) applied a bioavailability factor of 60%. As an example, in a food web that includes a focal species feeding on 100% insects, the bioaccumulation factor from particulate material to fish would be 2.8 multiplied by 1.1 or 3.08. To account for site-specific bioavailability, the bioaccumulation factor of 3.08 would be adjusted downwards to 1.85, assuming 60% bioavailability. The bioavailability adjustment was derived through a validation exercise (Presser and Naftz 2020).

Members of the SeTSC and supporting consultants have proposed alternative TTFs. Beaman (2020) proposed to expand the original Presser and Luoma (2010) TTF dataset to include additional studies as presented in (USEPA 2016). Both Thorley (2020) and DeForest (2020) derived site-specific TTFs for aquatic insects/benthic invertebrates of 1.2

(Figure 1). Thorley (2020) and DeForest (2020) also derived site-specific TTFs for zooplankton. Thorley (2020) accounted for seasonality in the zooplankton selenium concentrations (Figure 2), while DeForest (2020) presented statistical estimates of the TTF based on pooled data (i.e., arithmetic mean of 0.53). When considering seasonality, the upper estimate of mean TTFs was 0.85, which corresponds to September. Conceptually, this relationship is supported with data from Woods (1982) where it is demonstrated that primary productivity in the reservoir typically peaks in August; it would therefore be expected, that peaks in primary consumers would follow soon after.

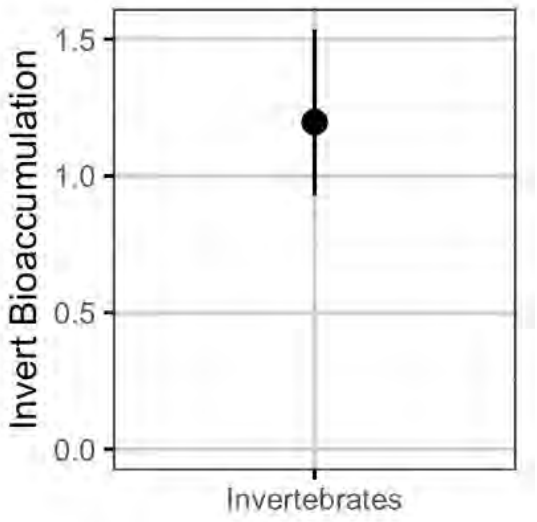


Figure 1. Estimated particulate to invertebrate selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

Presser and Naftz (2020) and Beaman (2020) acknowledged that there are sparse data available on aquatic/benthic insects and zooplankton, which results in greater uncertainty in site-specific TTFs. Secondly, an important assumption of lab- or field-derived bioaccumulation factors is that system is in steady-state with respect to exposure concentrations. Koocanusa Reservoir is not in steady state, driven largely by the dynamic nature of the operation of the Libby Dam and increasing selenium loads from the Elk River (Presser and Naftz 2020). Accordingly, Presser and Naftz (2020) and Beaman (2020) propose the use of literature-based TTFs. Despite relying on literature-based TTFs, Presser and Naftz (2020) apply a bioavailability factor of 60% to account for site-specific conditions via a validation exercise. Thorley (2020) estimated bioavailability factors of 43% and 57% for invertebrates and zooplankton, respectively, using data collected from the reservoir. KNC technical representatives also determined that the literature-based TTFs were likely too high when considering the concentrations observed in the invertebrate and zooplankton data.

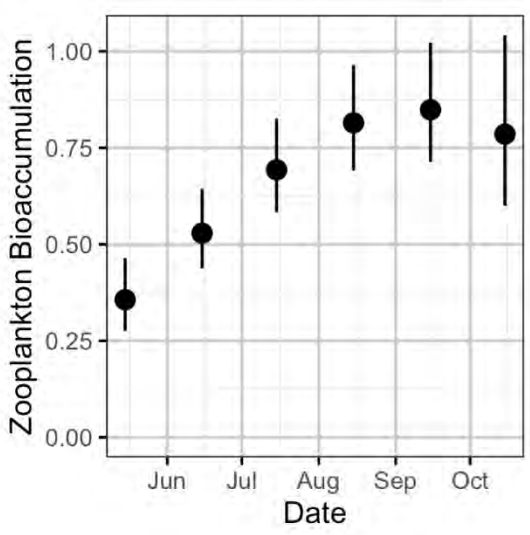


Figure 2. Estimated particulate to zooplankton selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

We consider both approaches to estimating TTFs as reasonable and defensible methods and acknowledge the uncertainties in these estimates due to the non-steady state of the system. The implications of a temporal lag in selenium inputs and biological uptake, as well as with respect to selenium retention within the reservoir, are currently unknown. Given that we do have some information on site-specific bioaccumulation within the system from the previous 5 to 10 years, we recommend relying on the measured site-specific TTFs. It must be acknowledged that the adjusted TTFs from Presser and Naftz (2020) and the site-specific TTFs developed by Thorley (2020) are relatively similar (Table 1).

> **Recommendation 3:** We recommend using the site-specific TTFs of 1.2 for invertebrates and 0.85 for zooplankton.

Kd Results

The USGS model determines an aqueous selenium concentration that would be considered ecologically protective for each of the measured Kd estimates (n = 87) for each food web model (e.g., *TFM w TL3 100% AqIns*). The Kd estimates ranged from 424 to 7,475 with a median Kd of 4,547 (Presser and Naftz 2020). The measured Kds were typically higher in the epilimnion of the reservoir compared to the hypolimnion (Figure 3; Thorley 2020). Given the slight differences in Kd estimates between the epilimnion and hypolimnion, and the greater degree of primary productivity in the epilimnion, the Kd estimates from the epilimnetic zone should be included for any quantitative approach to application of the

Table 1. Summary of trophic transfer factors and bioaccumulation factors evaluated for use in the USGS model.

	TTFs - Presser and Naftz (2020)	TTFs - Site-Specific (Thorley 2020)	Estimated Bioavailability Factors (Thorley 2020)	
Trophic Transfer Step				
TTF_{invert}	2.8	1.2	2.8	--
$TTF_{zooplankton}$	1.5	0.85	--	1.5
TTF_{fish}	1.1	1.1	1.1	1.1
Bioavailability Adjustment	0.6	--	0.43	0.57
Bioaccumulation Factor				
BAFinvert-fish	1.85	1.3	1.3	--
BAFzooplankton-fish	0.99	0.94	--	0.94

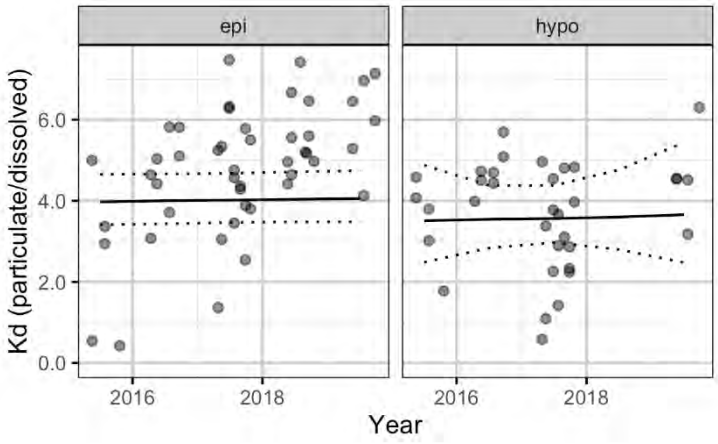


Figure 3. Estimated and observed ratio of particulate to water selenium (Kd = ratio x 1,000) in the epilimnion and hypolimnion with 95% confidence intervals (from Thorley 2020).

model results (see below). This would allow for a more conservative approach to quantifying a Kd for deriving the ecological benchmark considering the uncertainties in the degree of selenium assimilation in the water column of the hypolimnion and also considering that estimated Kd at the sediment water interface in the reservoir are more similar to the epilimnion than the hypolimnion (ranging between 2,641 and 5,812; median: 4,775; Table 2).

Recommendation #4: Kd estimates from the epilimnion (n = 50) should be carried forward into the quantitative assessment of model results below.

Table 2. Predicted Kd estimates in detritus/sediment of the reservoir proper using benthic tissue samples (collected with ponar; predominantly chironomids; Table T19 - Presser and Naftz 2020). Predicted particulate Se was calculated using the site-specific TTF for invertebrates (1.2; Thorley 2020) and associated water quality (mean concentrations by date and depth) in the reservoir (Presser and Naftz 2020).

Site Name	Sample ID	Invertebrate Se (µg/g dw)	Predicted Particulate Se (µg/g dw)	Associated Water Quality (µg/L)	Estimated Kd
Tenmile/Forebay	C3418	5.07	4.23	1.6	2,641
Tenmile/Forebay	C3421	8.37	6.98	1.2	5,812
Rexford	C345-BI-D1	4.50	3.75	1.0	3,750
Rexford	C345-BI-D2	5.73	4.78	1.0	4,775
Tenmile/Forebay	C3426-BI-D	6.43	5.36	0.93	5,761

Quantitative Approaches to Application

It is expected that some primary consumers and higher trophic level receptors (i.e., fish) would reflect reduced variability in selenium tissue burden compared to primary producers under varying selenium concentrations. Biokinetic modeling presented by DeForest *et al.* (2015) suggests that zooplankton would likely exhibit similar, but only slightly reduced variability, while benthic invertebrates and fish would have moderate and high reductions in variability, respectively when considering continuous exposure over varying Kd measurements. However, these higher trophic organisms would also be slower to respond to trends over time. Accordingly, it is reasonable to assume that fish are integrators of the varying selenium concentrations and that some measure of central tendency, with an appropriate level of conservatism to account for changes over time is reasonable.

Analysis of the Distribution

Beaman (2020) recommended a percentile, such as the median be used, to select the Kd for guideline derivation. Thorley (2020) estimated the expected Kd for the epilimnion of Koocanusa Reservoir at roughly 4,000 while the expected Kd in the hypolimnion is 3,500. The upper 95% upper confidence interval in the epilimnion is roughly 4,800 (Figure 3). The median Kd using all measurements is 4,547 while the median Kd in the epilimnetic measurements is 5,017.

> **Recommendation #5:** We recommend the range of Kd estimates for use in the model is between 4,547, the median of pooled Kd estimates (n = 87), and 5,017, the median of epilimnetic Kd estimates (n = 50).

Protective Concentrations

The USGS model was populated with the recommendations above to estimate the range of aqueous concentrations considered to be protective of all uses. It is estimated that the range of protective concentrations in Koocanusa Reservoir is 0.73 to 0.80 µg/L; model results are presented in Table 3. These results align well with the estimated 95% lower confidence interval of the mean dissolved selenium concentration (i.e., roughly 0.9 µg/L) associated with a whole-body concentration in burbot of 5.6 µg/L (Thorley 2020).

Table 3. Estimated range of ecologically protective concentrations using the recommendations for model inputs and Kd selection presented in this memorandum and the USGS model (Presser and Naftz 2020).

[1.] Kd scenario that most closely matches the pooled median of 4,574 and epilimnetic median of 5,071.

Tissue Target Concentration	Aquatic Insect TTF	Fish TTF	Bioavailability Factor	Kd[1]	Benchmark (µg/L)
5.3	1.2	1.1	1.0	5,000	0.73
5.3	1.2	1.1	1.0	4,579	0.80

Considerations for Future Activities/Monitoring

- We recommend that a mass balance analysis be conducted to quantify inputs from the Elk River (and other sources), exports (via Libby Dam) and the pool of selenium in the reservoir and the rate at which it is increasing over time.
- We recommend that fish tissue monitoring be conducted to get an accurate measure of ripe egg selenium concentrations (pre-spawn) to validate conversion factors.
- We recommend efforts focus on the estimation of assimilation efficiency factors (Kds) in periphyton (e.g., through the deployment of artificial substrates, seeded with periphyton)
- We recommend collection of additional benthic invertebrate samples for tissue analysis from the vicinity of the artificial substrates as well in the reservoir proper.
- We recommend collection of additional zooplankton samples in various seasons throughout the year at multiple locations throughout the reservoir.

Closure

The recommendations put forth in this letter provide a range of reasonable and defensible model parameters considering the data and information collected to date. Using the recommendations for model parameters in the USGS model, we estimate a range of ecologically protective benchmarks between 0.73 and 0.80 µg/L. This range is

corroborated by the work conducted by Poisson Consulting, which estimated a criterion for burbot, a high selenium accumulator, of roughly 0.9 µg/L (Thorley 2020). It is important to note that this range of estimates is lower than the current concentrations (i.e., 1 µg/L; Thorley 2020) in the reservoir. Accordingly, these analyses suggest that there is a need to stabilize and reduce loadings of selenium into Koocanusa Reservoir in order to meet protection goals as we do not have confidence that increasing selenium concentrations above these levels would be protective of aquatic life or Ktunaxa cultural practices.

We appreciate the opportunity to provide feedback and look forward to further engagement on this process.

Sincerely,

Jesse Sinclair
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LGL Limited

Heather McMahon
Project Biologist
Ktunaxa Nation Council

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Disclaimer: *Please note the comments and recommendations contained in this document are strictly for Montana's and British Columbia's consideration. The views expressed in these comments and recommendations are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Regarding Montana's submission, the comments do not constitute approval or disapproval decisions under CWA Section 303(c). Neither are these comments a determination by the EPA Administrator under CWA Section 303(c)(4)(B) that revised or new standards are necessary to meet the requirements of the Act. These comments and recommendations do not impose any binding requirements, determine the obligations of the regulated community, change or substitute for any statutory provision or regulation requirement, represent, change or substitute for any Agency policy or guidance, or control in any case of conflict between this discussion and statute, regulation, policy or guidance.*

The Selenium Technical Subcommittee (SeTSC), was established by the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), at the direction of the Steering Committee and is comprised of selenium experts from both the US and Canada and supported by the Montana Department of Environmental Quality and BC Ministry of Environment.

The overall objectives for the Se TSC are to develop selenium criteria/objectives for Lake Koocanusa that are protective of the uses of lake including, but not limited to, aquatic life, human health, recreation, wildlife, and agriculture, with the specific goal of answering the questions:

- Are the current Canadian (selenium target of 2 µg/L, as set out in the BC Water Quality Guideline) or Montana (WQS = 5 ug/L), protective of the uses in Lake Koocanusa in their respective jurisdictions?
- If not, what is an appropriate target value for selenium in Lake Koocanusa that can be adopted and implemented by Montana and British Columbia?

The SeTSC has been meeting regularly for four years through conference calls and in-person workshops with the primary goal of evaluating available data facilitating the development of a site-specific selenium criterion for Lake Koocanusa. More specifically, the process has involved the following tasks:

- Collection and analysis of existing Lake Koocanusa information to identify gaps in scientific understanding of the lake chemistry and ecology that are relevant to the stated research objectives.
- Prioritize monitoring and research activities and tasks
- Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- Definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa.

Seminal products developed during this process include the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019). These products provided critical information enhancing the SeTSC

understanding of the available data and the mechanistic model proposed for use in development of a site-specific water quality threshold protective of the designated uses of Lake Koocanusa.

The SeTSC in consideration of jurisdictional requirements, and with input from stakeholders, developed a set of constraints and considerations critical to the modeling efforts (*Proposed Workplan for Developing a Site-Specific Selenium Water-Column Criterion for Lake Koocanusa*; "the workplan") and resulting outputs including:

- The site-specific criterion will meet the regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.
- The site-specific criterion will also consider ecologically significant species and those important to stakeholders
- The definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir
- The site-specific criterion will provide long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all selenium loading profiles, and all water years (precipitation/runoff scenarios).
- The Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- site-specific criterion also will protect downstream uses including protection of the endangered Kootenai River white sturgeon

Using the requirements and constraints set forth above, four main alternative levels of protection were proposed for model runs for Lake Koocanusa:

- Two scenarios based on the BCMoE egg-ovary selenium guideline of 11.0 mg/kg dw that consider individuals and populations of fish species explicitly.
- Two scenarios based on the USEPA national 304(a) egg-ovary selenium criterion of 15.1 mg/kg dw that consider individuals and populations of fish species explicitly.

An additional scenario was proposed by stakeholders as a potential no effect threshold (BCMoE tissue guideline of 4.0 mg/kg dw) protective of sensitive wildlife receptors. This value is like a "no-effect" threshold of 5.5 mg/kg dw proposed to EPA by USFWS in 2005 (J. Skorupa personal communication).

SeTSC members were charged with providing comments on the USGS modeling report "*Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada*" Open-File Report 2020–1098.

Committee members were asked to comment on:

- Model Inputs (used to confirm or run model scenarios):

- Tissue threshold (use of whole body 8.5 ug/g, 4 ug/g, or other?)
- Model(s) to run, i.e. Invertebrate to fish model and/or Trophic level fish model and species-specific model (Table 10.)
- Food Web / diet fraction(s), e.g. %aquatic insect / %zooplankton: 100/0, 75/25, 50/50
- TTFs (invert to fish): generic option (1.1) or other?
- Bioavailability (100%, 60% or other percentage)
- Kd data set (Specific sub-sets?)

The following comments reflect my personal scientific views on the USGS model assumptions and outputs from the report. The focus of these comments is based on regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

1. Model Inputs - Tissue threshold

The USGS selected the USEPA whole-body (wb) selenium criterion element 8.5 mg/kg dw, a value translated from the egg-ovary criterion of 15.1 mg/kg dw. In EPA, 2016, Species-specific whole-body values were calculated directly from whole body tissue [Se] concentrations measured in reproductive toxicity studies, or more commonly by applying an egg-ovary (EO) to whole-body (WB) conversion factor (CF) based on taxonomic relatedness. The lowest WB concentration in the available data is from the white sturgeon (9.2 mg/kg dw), the most sensitive species in the EPA national toxicity dataset. The criterion (8.5 mg/kg dw) is based on an OLS regression-based projection using an n = 15 taxa. The EPA used a generic (median of all fish [1.78]) conversion factor (CF) to convert the egg-ovary criterion to a whole-body threshold. EPA derived CFs for matched pairs of field-collected egg or ovary and whole body samples and used medians based on the available species-specific distributions of whole body and reproductive tissue data. Concern has been expressed over the EPA's use of the median, as it may not be appropriate to a specific site (i.e., Lake Koocanusa). There is a paucity of field data of sufficient quality to calculate field-based EO-WB conversion factors for resident species. Ideally, laboratory data directly measure reproductive tissues (mature eggs) and whole-body measurements would be more readily available. There are two studies available bluegill (Hermanutz, 1993, 1996) and brown trout (Formation 2009) with directly measured tissues resulting in EO-WB conversion factors of 1.38 and 1.59 respectively, providing support for the EO-WB CF of 1.78 used in the national freshwater selenium criterion EPA, 2016)

National criteria are limited to predicting sensitivity to a given contaminant based on the range of toxicity data available, and the national data set may be modified by applying the Recalculation Procedure (40CFR131.11(b)ii) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while including tested surrogates for untested resident species. As discussed previously (Beaman presentation to SeTSC, October 2019), the fish assemblage in Lake Koocanusa is well comprehensively represented by the EPA selenium toxicity database (EPA, 2016) augmented by other data (DeForest 2012, Teck, draft redside shiner toxicity report 2020). This database provides precise quantitative reproductive toxicity values for 5/13 resident species, as well as qualitative species or genus surrogate level tissue values for an additional 4 species (mountain whitefish, largescale and longnose sucker, and redside shiner), leaving 4/13 species unrepresented in the site-specific toxicity database. Early in the process, the SeTSC agreed that all fish species without data would be deemed equally sensitive to the white sturgeon, the most sensitive species in EPA's toxicity dataset. Therefore, the

white sturgeon whole body value of 9.2 mg/kg dw would be applied to the burbot, northern pikeminnow, peamouth chub, and yellow perch. When these 4 values are added to the censored site database (removing non-resident species), the resulting criterion is 9.2 mg/kg since the 5 most sensitive taxa are all equally sensitive. This provides valuable information demonstrating that the EPA tissue threshold of 8.5 mg/kg dw is likely sufficiently protective of the assemblage of fish species in Lake Koocanusa, including those with no toxicity data.

Recommendation: use the EPA whole body criterion element of 8.5 mg/kg dw, as it provides a protective goal for the species assemblage in Lake Koocanusa.

2. Bioavailability of selenium in Lake Koocanusa & Bioaccumulation Potential of Resident Fish Species
Selenium bioavailability at the base of the food web is impacted by the form of inorganic selenium (inorganic selenate and selenite) and its interaction with different types of particles in the aquatic environment. The relationship between the [Se] in suspended particulate matter (SPM) and the [Se] in the invertebrate (TTF) is a function of the type of particulate that the invertebrate encounters (sediment, detritus, phytoplankton) and the assimilation efficiency (AE) of the organism based on the form of selenium encountered adsorbed on (elemental Se or inorganic Se) or contained in (organic Se) the particulate.

Because selenium speciation data for SPM was not available for Lake Koocanusa, USGS addressed this uncertainty by using AEs of various species from other studies (e.g., Presser and Luoma 2010a) and using different types of particulate matter to account for the site- or species-specific bioavailability of foods likely to be consumed by invertebrates.

Two SPM bioavailability factors (100 percent and 60 percent) are used within each food-web scenario to quantify the efficiency of assimilation of SPM by invertebrates. USGS cited AEs varying from 55 to 86 percent among various invertebrate species, with smaller differences among living food types such as different species of algae. In my review of EPA 2016, I note that the saltwater copepods have AEs of ~50 – 55% whereas other invertebrates, particularly saltwater mollusks have median AEs ranging 61 – 96% (EPA 2016 Appendix B). Conversely, laboratory studies using freshwater species (including surrogates for resident macroinvertebrates:

Species	Median AE	Range AE	Taxonomic surrogacy
Water flea (D. magna)	40.6%	24.9 -57.9%	zooplankton
Blackworm (L. variegatus)	16.5%	9 – 24%	chironomid (benthic detritivore)
Mayfly	39%	38 – 40%	resident aquatic insect

Even available data for mollusks, a taxon known to have high selenium AE, are notably lower in FW (asiatic clam – 55%; zebra mussel – 26% [18-46%]) indicate that lower AE may be more appropriate.

Given the paucity of resident invertebrate data, USGS used [Se] ranges to examine the impact of bioavailability assumptions on model validation. For macroinvertebrates collected in Montana waters, the AE = 100% resulted in significant overpredictions (2X upper limit of 18.8 for predicted vs 9.1 for observed, 2018), whereas the AE = 60% resulted in more comparable results (observed [Se], 0.4–9.1 µg/g dw vs predicted 0.7–11.3 µg/g dw). Similar observations were made for validation comparisons of macroinvertebrates collected in the lake south of the Elk River, as well as zooplankton collected in US and Canadian waters. The table shows a comparison of water values calculated using the TFM or IFM model using assumed bioavailability of 60% or 100%

Table 1. Influence of Bioavailability of water column values in IFM and TFM models

Centile	TFM (100%)	IFM (100%)	TFM (60%)	IFM (60%)
median	0.55	0.61	**0.92**	**1.01**
40th centile	0.52	0.58	**0.87**	**0.96**
30th centile	0.49	0.54	**0.82**	**0.90**
20th centile	0.45	0.50	**0.75**	**0.83**
10th centile	0.40	0.44	**0.66**	**0.73**
5th centile	0.38	0.42	**0.63**	**0.70**

Recommendation: use the bioavailability of 60% based on model validation results and literature values (EPA 2016) that support freshwater AEs ≤ 60%.

Bioaccumulation Potential (BAP)
USGS developed the following scenarios for the insectivorous fish model:

- 100-percent aquatic insect (rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker),
- 50-percent aquatic insect and 50-percent zooplankton (peamouth chub, largescale sucker, mountain whitefish),
- 75-percent zooplankton and 25-percent aquatic insect (rainbow trout December–March), and
- 100-percent zooplankton (kokanee).

The following scenarios were used for piscivores. The scenarios for bull trout, burbot (winter and summer), and northern pikeminnow are as follows:

- 100-percent insectivores,
- 50-percent aquatic insect and 50-percent zooplankton, and
- 100-percent planktivores.

Given the paucity of stomach content data available to confirm older food web studies illustrated in Lotic Environmental 2017, the USGS developed insectivorous and piscivorous food webs using conservative assumptions regarding the weighting of dietary components. This is consistent with the principles discussed in the USGS workplan, that "definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir".

Recommendation: although some refinement of food webs would be more consistent with previous food web studies documented in the Lotic Environmental report, the use of "model food webs" are consistent with the modeling principle that assumes maximum dietary exposure.

3. Water -Particulate Partitioning Coefficient (Kd)
Kds were collected by different agencies at different times during the years 2015 -2019. Kds were not spatially and temporally comparable on a year over year basis. Due to the density, spatial and temporal inconsistencies in Kd sampling between 2015 and 2019, it is difficult to partition a subset of Kds for examination of its influence on modeled water concentrations for Lake Koocanusa. Therefore, USGS used the entire Kd datasets allowing for 87 independent scenarios within each (IFM and TFM) model. The full distribution of scenarios can be assessed statistically, with selection of a protective water value

expressed as a percent of scenarios where maximally exposed fish are protected (based on attainment of the whole-body value of ≤ 8.5 mg/kg dw using conservative food web assumptions.

Table 2. Centile Distribution of Kds and Water Values (ug/L) generated by IFM and TFM model scenarios

			Model	
Centile	Kd	Centile	IFM	TFM
median	4547	median	1.01	0.92
60th centile	4788	40th centile	0.96	0.87
70th centile	5090	30th centile	0.90	0.82
80th centile	5569	20th centile	0.83	0.75
90th centile	6311	10th centile	0.73	0.66
95th centile	6611	5th centile	0.70	0.63

This table displays the mean epilimnetic and hypolimnetic Kds by year and location.

Table 3. Annual Average Kd by Site, Sampling Year, and Layer

Location	Layer	Sampling Year				
		2015	2016	2017	2018	2019
South of Elk River	epi			4525	5482	
	hypo			2722		
US/Can Border	epi	3971	4256	3748	5745	6573
	hypo	2956	4625	3662		5012
Tenmile	epi				5222	
Forebay	epi	1446	5150	5458	5480	5518
	hypo	4188	4878	3150		3864

Kds collected in the hypolimnion were typically substantially lower than those collected in the epilimnion, however this is not unexpected. Particulate in the hypoliminion is typically composed of detritus (scenescing phytoplankton from the epilimnion or other particulates). In reducing environments such as the epilimnion, selenium released from detritus is recycled as selenite or organoselenium, but is not typically re-incorporated in particulate, resulting in lower Kds. These Kds should not be discounted, since detritivores that tolerate hypoxic conditions such as chironomids are an important macroinvertebrate food source to benthic invertivores and omnivores. These fish species are then preyed upon by demersal predators like the burbot.

Kds in the epilimnion of the lakes forebay were consistent between 2015 and 2019. Epilimnetic Kds were more variable at the international border and appear to be increasing from 2017 – 2019. This is uncertain due to the presence of an extreme value (Kd = 7139) in a small dataset (n = 3) in 2019.

A more refined species-specific approach applying more toxicological knowledge about the resident fish community could allow for examination of individual Kds using Table 10. Because we know that applying the 8.5 mg/kg dw threshold is overprotective for a number of species, I substituted the actual available toxicity data for resident species and their surrogates using data and information from EPA, 2016, DeForest, 2012, and Teck, personal communication (See attached tables, Table 10 SSC Comparison). For the species with no toxicity data (pikeminnow, yellow perch, burbot, and peamouth),

the whole-body threshold of 8.5 mg/kg dw was used as a default. The default TTF of 2.8 was used for invertebrates, and assumed TTFs were used for each fish species, however a TTF of 1.7 was used for burbot. Then, centiles of the Kd distribution (median, 60th, 70th, 80th, and 90th) were selected to represent a "steady state" exposure at the base of the food web throughout the lake. This provided a species-specific water threshold corresponding to each whole-body value from the toxicity database (n = 12 species). The median, 40th, 30th, 20th, and 10th centile values of the distributions are displayed below.

Table 4. Site Specific Water Thresholds (ug/L) for Fish Species Assemblage in Lake Koocanusa

	Lake Koocanusa Kd				
Centile	**Median**	**60th**	**70th**	**80th**	**90th**
	4547	**4788**	**5090**	**5569**	**6311**
Median	1.32	1.25	1.18	1.08	0.95
40th centile	1.16	1.11	1.04	0.95	0.84
30th centile	1.03	0.97	0.92	0.84	0.74
20th centile	0.83	0.78	0.74	0.67	0.59
10th centile	0.76	0.73	0.68	0.62	0.55

Observations:

- The maximum possible water value that might be considered protective of the fish assemblage at Lake Koocanusa is 1.32 ug/L, lower than the EPA national default lentic concentration of 1.5 ug/L.
- Approximately 50% of the water values are above 0.9 ug/L, including a water value representing a 90th centile of potential exposure based on Kd (0.95 ug/L).
- Based on the overall distribution of Kds in the dataset, the selection of a Kd between the 60th and 80th centile would provide robust temporal and spatial coverage for of the Kd distribution collected from the lake between 2015 and 2019

Sources of uncertainty related to various aspects of the toxicity dataset:

- The default WB EC10s for four species with no toxicity data.
- The median CF (EPA 2016) applied to species vs site specific CFs

The sensitivity of the untested species will likely have the largest impact on the range of protective values for species in Lake Koocanusa, so this could influence the selection of protective water value. If one or more of the untested species are tested and more sensitive, selection of a lower water value from the distribution may be more defensible particularly if this species is highly exposed to a food web with high bioaccumulation potential.

Recommendation: Selection of water values should be based on Kd, (either selection of a protective centile of the range of water threshold outputs in the IFM/TFM models or consideration of a conservative Kd based and considering the sensitivity of the species assemblage.

4. Trophic Transfer Factor (TTF)

Due to the paucity of insect data collected, and the lack of concurrently collected particulate data, USGS selected TTFs derived and used in previous studies (Presser & Luoma 2010).

The mean "all insect" TTF (2.8) that USGS is using to model Lake Koocanusa is composed of:

mayfly, caddisfly, cranefly, stonefly, damselfly, corixid (waterboatmen), and chironomid (midge)
The median "all insect" TTF used in EPA 2016 is composed of:
mayfly (match), Diptera (cranefly surrogate – match?), damselfly (match) dragonfly, waterboatmen (match), and chironomid (midge) - match

Both the P&L 2010 and EPA 2016 datasets have significant (4/7) taxonomic overlap.

The USGS model uses the mean as its central tendency estimate, whereas the EPA 2016 derivation uses the median ratio. The median ratio is not influenced by extreme values. For example, the median TTF for the waterboatmen dataset (n = 29) is 1.48, whereas the mean is 57% higher (2.32). The range of the distribution of 28 TTFs values is 0.139 – 5.62, with one extreme value, 21.0. If this extreme value was excluded, then the mean of the distribution would be 1.65, similar to the median.

Another uncertainty is the [Se] and TTFs associated with terrestrial insects. The Lotic Environmental food web report indicated that insectivorous fish species and lifestages consume a substantial proportion of terrestrial insects. For example, trout consumed between 40-50% of terrestrial insects between April and November (Table A.3.5 from Dalbey, 1996). These insect's exposure to Se is uncertain and is likely a product of local site-specific terrestrial conditions due to most insects small home range. For example, terrestrial insects from riparian habitats near the Elk River likely have higher [Se] than terrestrial insects inhabiting nearshore areas of the forebay. This makes prediction of TTFs for insectivores more complex.

Recommendation: Combine the P&L 2010, and the EPA 2016 TTF datasets to maximize the available TTF data, producing a more robust dataset from which to derive a central tendency estimate. Examine TTF distributions and select the most appropriate central tendency distribution on a species-specific basis. I Use of the median (vs the mean) of the more robust combined datasets avoids conducting additional analyses to determine censorship of extreme values (outliers).
The EPA TTF dataset (EPA 2016) has been provided to the SeTSC co-chairs upon their request.

5. Protection of Downstream Uses:
A key principle of the modeling effort is to ensure that the site-specific criterion adopted for Lake Koocanusa will protect downstream uses including protection of the endangered Kootenai River white sturgeon. This is consistent with the regulatory requirements of U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

The available data for eggs collected from white sturgeon in the Kootenai River from 2015 – 2019 indicate moderate elevation of [Se]. The maximum [Se] observed in the dataset was 5.76 mg/kg dw roughly 63% lower than the white sturgeon EC10 from Linville, 2006 (EPA, 2016).

Table 5. Selenium from white sturgeon eggs sampled in Kootenai River.

	2015	2016	2017	2018	2019
average	4.2	3.3	4.1	3.9	4.2
75th centile	4.7	3.4	4.7	4.2	4.5
95th centile	5.3	3.4	5.6	4.8	5.3

Based on current conditions, selenium concentrations in sturgeon eggs do not show an increasing trend between 2015 and 2019, however water thresholds adopted in Lake Koocanusa should ensure that this

trend does not increase over time. An important data gap is the loading of selenium (both particulate and total, and its fate in the downstream Kootenai River. Continued monitoring of sturgeon should continue to enable early detection of increasing selenium in mature eggs to insure protection of this important species. Future understanding of selenium loading may allow for refinement of the Lake Koocanusa SS water threshold, as well as water quality standards proposed for the Kootenai River by Montana.

Overall Recommendation for adoption of a water column threshold for Lake Koocanusa:
I believe that the SSC water value should strike a balance between protection of the fish assemblage in Lake Koocanusa and downstream uses (protection of white sturgeon) based on current conditions, and the opportunity to refine regulatory thresholds in the future based on future monitoring actions targeted on refining the USGS model.

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MEMORANDUM

To: Lauren Sullivan (MT DEQ) and Sheldon Reddekopp (BC ENV)

From: David DeForest

Subject: Comments on Koocanusa Reservoir Selenium Modeling and Recommendations for Site-specific Selenium Criteria

Date: August 28, 2020

This memorandum provides my comments on the Koocanusa Reservoir selenium model developed by the U.S. Geological Survey (Presser and Naftz 2020) and my recommendations for site-specific selenium criteria. Based on review of the U.S. Geological Survey (USGS) model and other lines of evidence, including empirical monitoring data for the reservoir, it is my opinion that the U.S. Environmental Protection Agency's (USEPA's) surface water quality criterion of 1.5 µg/L for lentic water bodies is protective of fish and the aquatic community in Koocanusa Reservoir. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessment. The following summarizes the lines of evidence that support this conclusion.

1 Selenium - Fate and Effects in Freshwater Aquatic Systems

There is scientific consensus that selenium concentrations in fish tissue, and particularly in fish eggs, are the strongest indicator of potential selenium toxicity to fish (Chapman et al. 2010). Fish primarily bioaccumulate selenium from their diet in the form of organic selenium. Selenium bioaccumulated by females is maternally transferred to ovaries and eggs. Adult fish are insensitive to selenium, but if concentrations are sufficiently high in the eggs, selenium may cause mortality, deformities, or edema in developing larvae as the yolk sac is absorbed (Janz et al. 2010).

Selenium is typically mobilized or released into surface waters, from both natural and anthropogenic sources, as inorganic selenium (typically selenate or selenite) (Maher et al. 2010). Inorganic selenium species are taken up at the base of the food web (e.g., algae), and transformed to organic selenium species. Site-specific surface water characteristics have a substantial influence on selenium speciation and bioaccumulation potential (Stewart et al. 2010). For example, a lentic (standing) water body with high biological

productivity, a long retention time, and strong reducing conditions has a greater selenium bioaccumulation potential then a lotic (flowing) water body with low biological productivity, a short retention time, and oxic conditions. This means that surface water selenium concentrations that may reach a toxic concentration in fish eggs may range by two orders of magnitude or more among sites.

1.1 USEPA Selenium Criteria

Based on selenium's fate and effects in aquatic systems, the USEPA derived ambient water quality criteria for selenium with the following hierarchy (USEPA 2016):

1. Fish egg selenium concentration of 15.1 mg/kg dry weight (dw)
2. Adult fish muscle selenium concentration of 11.3 mg/kg dw or adult fish whole body selenium concentration of 8.5 mg/kg dw
3. Surface water selenium concentration of 1.5 µg/L for lentic waters or 3.1 µg/L for lotic waters

The fish egg (or ripe ovary) selenium criterion of 15.1 mg/kg dw supersedes (is given priority over) the selenium criteria for muscle or whole body tissue and for surface water concentrations.

1.1.1 Fish Egg Selenium Criterion

The USEPA fish egg selenium criterion is based on maternal transfer studies in which parent females were exposed to diet-borne organic selenium in the laboratory or naturally exposed to diet-borne organic selenium in the field. Larval survival and development were assessed in the offspring of exposed parent females and selenium EC10s (10% effect concentrations) calculated based on the egg selenium concentration.

The USEPA's egg selenium criterion is based on genus mean EC10s for eight different genera. White sturgeon (*Acipenser transmontanus*) has the lowest EC10, which is 15.6 mg/kg dw. The EC10s for the remaining seven genera used to derive the USEPA's egg selenium criterion range from 20.6 mg/kg dw for bluegill (*Lepomis macrochirus*) to 56.2 mg/kg dw for Dolly Varden (*Salvelinus malma*). The fish egg selenium criterion of 15.1 mg/kg dw is based on the 5th percentile of the genus sensitivity distribution, which is extrapolated to a concentration less than the white sturgeon EC10 (Figure 1).

1.1.2 Fish Muscle and Whole Body Selenium Criteria

Muscle and whole body selenium criteria were developed using the same approach as for the egg selenium criterion. The USEPA compiled EC10s based on muscle or whole body selenium concentrations in parent females from maternal transfer studies. Direct measures of muscle and whole body selenium concentrations were used to calculate EC10s when reported. When necessary, egg-to-muscle or egg-to-whole body conversion factors were applied to estimate muscle or whole body selenium EC10s. As for the egg selenium criterion, the muscle and the whole body selenium criteria were calculated

based on the 5th percentile genus mean EC10s, and both criteria were extrapolated to concentrations less than the EC10 of white sturgeon (Figures 2 and 3).

1.1.3 Surface Water Selenium Criterion

The USEPA surface water criterion was developed to ensure protection of fish and the aquatic community for sites with high selenium bioaccumulation potential. This means that the surface water criterion may be conservative for sites with moderate to low selenium bioaccumulation potential. Simply put, the surface water selenium criterion was back-calculated from the egg criterion, which is driven by the sensitivity of white sturgeon, based on sites with high selenium bioaccumulation potential.

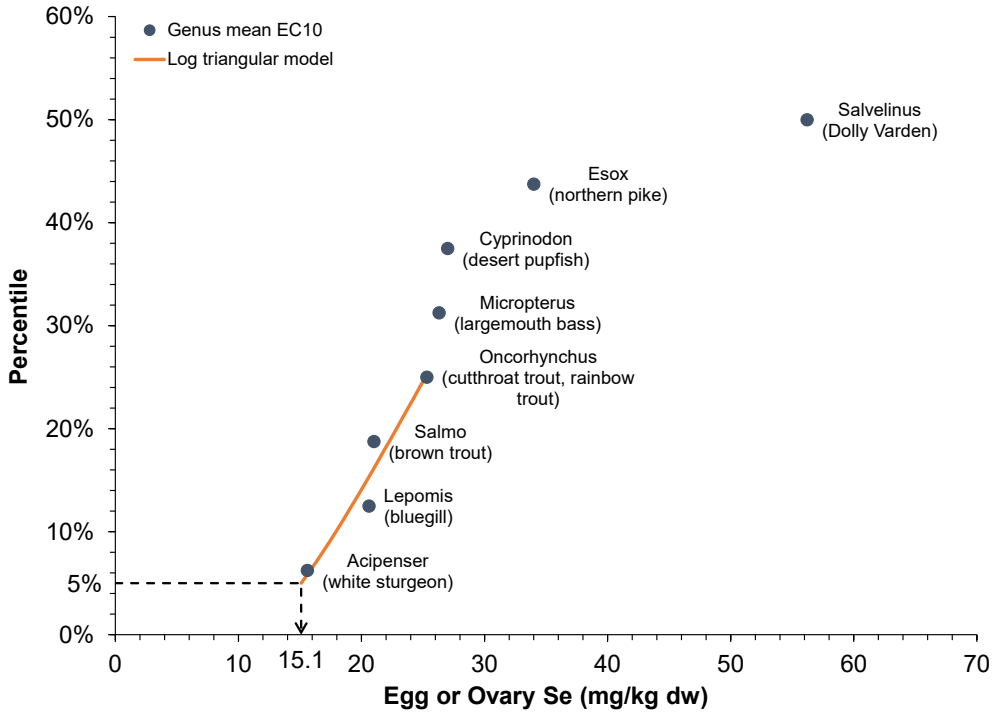


Figure 1. Derivation of the USEPA's egg/ovary selenium criterion of 15.1 mg/kg dw.
Note: Blue symbols are genus mean EC10s with the labels identifying the genus and, in parentheses, the species that comprise each genus mean EC10 (with the exception of two trout species comprising *Oncorhynchus*, all other genus mean EC10s are comprised of a single fish species). The orange curve is the log triangular distribution model that the USEPA traditionally uses to calculate the 5th percentile of the genus sensitivity distribution for criteria development. This model is fit to only the four most sensitive genera. Although the total number of genus mean EC10s is eight, the total sample size is based on an n of 15, which is why the percentiles on the y-axis do not extend to 100%. The sample size of 15 accounts for less sensitive fish genera for which definitive EC10s could not be calculated and less sensitive invertebrates.

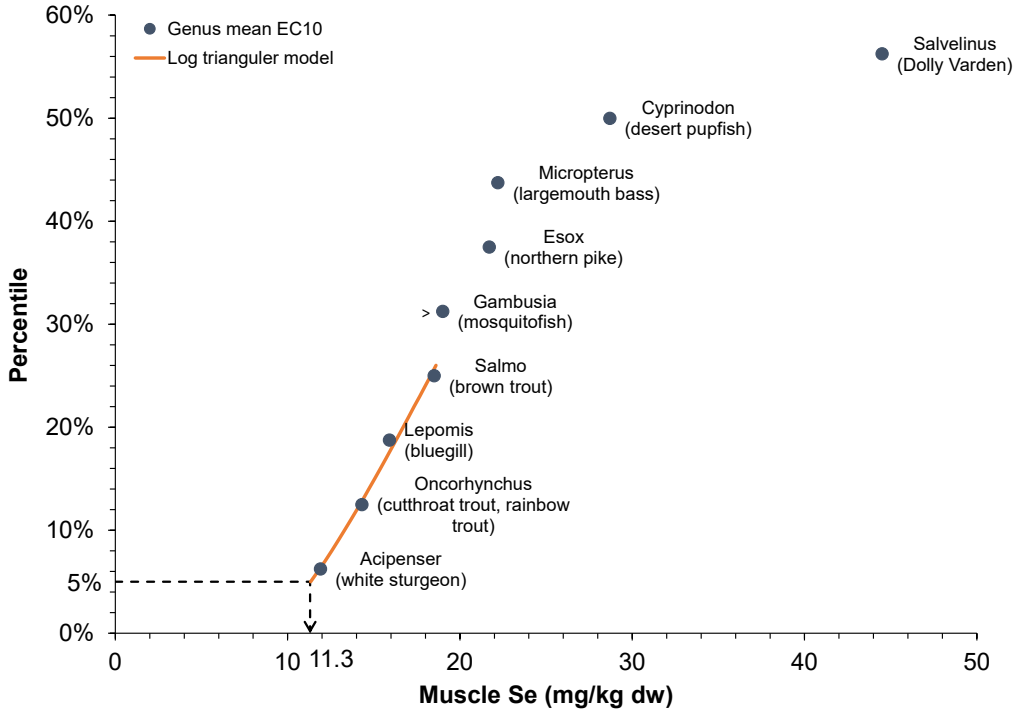


Figure 2. Derivation of the USEPA's muscle selenium criterion of 11.3 mg/kg dw.
Note: See Figure 1 for description.

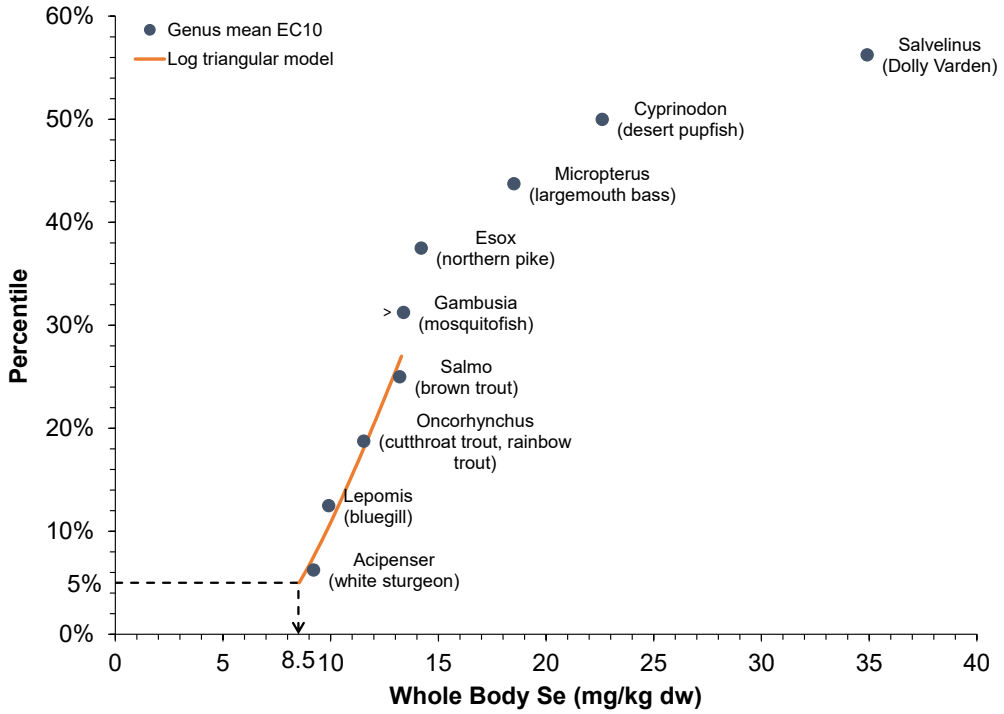


Figure 3. Derivation of the USEPA's whole body selenium criterion of 8.5 mg/kg dw.
Note: See Figure 1 for description.

1.2 BC Environment Selenium Guidelines

1.2.1 Fish Egg Selenium Guideline

BC Environment compiled selenium toxicity data for a variety of fish species, including many of the same species considered by the USEPA in deriving its selenium guideline (BCMOE 2014). As outlined within the guideline, it was determined that toxicity data for the genus *Oncorhynchus* would be a sensitive surrogate for fish in BC. Egg selenium EC10s of 22.05 and 21.97 mg/kg dw for rainbow trout and westslope cutthroat trout were compiled. An uncertainty factor of 2 was applied to each of these, which resulted in a mean of 11 mg/kg dw. The basis for the uncertainty factor of 2 was described as "a value that meets the balance between adequacy and protection, while addressing the inherent uncertainties in published toxicity threshold estimates." However, the basis for determining that it meets this balance was not described. Selenium data for comparison to the guideline should be based on the mean concentration of at least eight samples (eggs or ripe ovary from 8 individual females) (BCMOE 2014).

1.2.2 Fish Muscle and Whole Body Guideline

BC Environment derived a whole body fish selenium guideline of 4 mg/kg dw, which was developed by weighting literature-based evidence and the mean of published effects data for multiple species with an uncertainty factor of 2 (BCMOE 2014). BC Environment similarly derived an interim muscle selenium guideline of 4 mg/kg dw, which was based on low effect concentrations for rainbow trout, brown trout, and bluegill. However, the guideline was defined as interim due to uncertainty and limited primary toxicity data in their review. As for egg selenium, collection of selenium data for comparison to the whole body and muscle guidelines should be based on the mean concentration of at least eight whole body or muscle samples (BCMOE 2014).

1.2.3 Surface Water Selenium Guideline

The BC surface water selenium guideline of 2 µg/L was based on several studies that had lowest observed effect concentrations that converged around a water selenium concentration of 10 µg/L—this concentration was divided by an uncertainty factor of 5 to derive the guideline of 2 µg/L (BCMOE 2014). An alert guideline of 1 µg/L was also derived based on evidence from some studies that concentrations above this could pose a risk to aquatic life (BCMOE 2014). The water selenium concentration measured for comparison to the alert concentration and the guideline should be the mean of five evenly spaced samples over 30 days (BCMOE 2014).

1.3 USEPA Selenium Criteria are Protective of Fish

The USEPA's egg selenium criterion of 15.1 mg/kg dw is 1.4-times greater than the BC egg selenium guideline of 11 mg/kg dw, and the USEPA's muscle and whole body selenium criteria of 11.3 and 8.5 mg/kg dw are 2.8-times and 2.1-times greater, respectively, than BC's muscle and whole body guidelines of 4 mg/kg dw. The USEPA's lentic water criterion of 1.5 µg/L falls between the BC alert concentration of 1 µg/L and

guideline of 2 µg/L. The BC fish tissue guidelines are less than the USEPA's fish tissue criteria, but this difference is driven by the applied uncertainty factor of 2. In my opinion, the uncertainty factor of 2 is overly conservative and not supported by the science. As such, USEPA's fish tissue-based selenium criteria are protective of fish.

As previously noted, USEPA's fish tissue-based selenium criteria are based on the 5th percentile of genus mean EC10s (which are less than the EC10 for the most sensitive species, white sturgeon). This is a conservative approach for selenium criteria development for several reasons:

- First, EC10s cannot literally be considered 10% effect concentrations. This is because EC10s often fall within the statistical "noise" of selenium toxic effect thresholds. Most concentration-response data are inadequate to calculate concentrations associated with extremely low effects (e.g., EC0 or EC1) without having extremely high uncertainty (USEPA 2015). More importantly, very low effect concentrations are often indistinguishable from natural biological variability in data used to develop the concentration-response relationship. Concentration-response data for sensitive coldwater fish species are provided in Figure 4, which show that the USEPA's ovary selenium criterion of 15.1 mg/kg dw falls within the variability of responses at the egg selenium concentrations associated with the "flat" region of the concentration-response curve. As such, the USEPA's egg selenium criterion of 15.1 mg/kg dw and BC egg guideline of 11 mg/kg dw are equally protective.

  Another way to demonstrate this is to plot the concentration-response data for the most sensitive fish species in the USEPA's ambient water quality criteria document (USEPA 2016). Concentration-response models were fit using USEPA's Toxicity Relationship Analysis Program (TRAP) (USEPA 2015), which the USEPA used in developing its selenium criteria. TRAP defines the "control value" as Y0, which is the plateau in the concentration-response relationship before an inflection point indicating an adverse response is detected and then increasing levels of effect are fit by the model. Concentration-response data are normalized for their respective Y0 value so that data from multiple species and tests can be plotted together, as shown in Figure 5. Individual data points are given open symbols to denote those treatments that comprised Y0 (i.e., considered part of the control population) and filled symbols denote those treatments not considered part of the control population. As shown in Figure 5, the USEPA's egg selenium criterion of 15.1 mg/kg dw (i.e., the 5th percentile of genus mean EC10s) falls within the grouping of open symbols, which shows that use of EC10s to derive criteria are within the "noise" of no-effect concentrations.

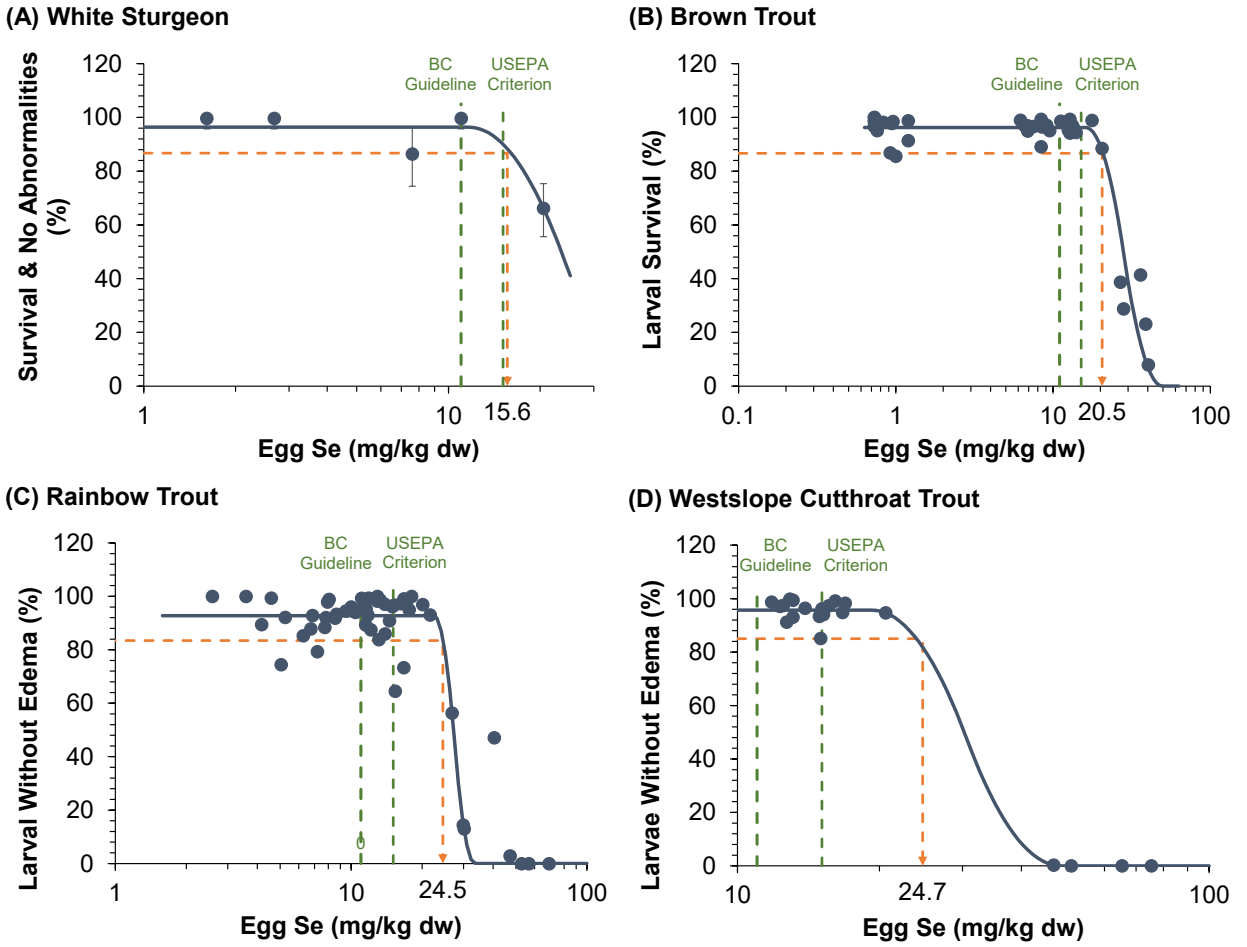


Figure 4. Examples of concentration-response relationships for (A) white sturgeon; (B) brown trout; (C) rainbow trout; and (D) westslope cutthroat trout.
Note: Orange line denotes the EC10, which falls within the variability of the biological response at low egg selenium concentrations. Green dashed lines denote the USEPA's chronic egg/ovary selenium criterion of 15.1 mg/kg dw and the BC chronic egg/ovary selenium guideline of 11 mg/kg dw.

- Second, with the exception of white sturgeon, all egg selenium EC10s compiled in the USEPA's selenium criteria document are ≥20.6 mg/kg dw. The USEPA (2016) egg selenium toxicity data for criteria development, along with data for additional species, are plotted in Figure 6 (sources of data are provided in Table 1). As shown, the first fish species for which egg selenium thresholds could be calculated were fathead minnow and bluegill in the early 1990s. As additional species have been tested, only one (white sturgeon) has been identified as being more sensitive than bluegill. In fact, there tends to be an increasing pattern of less sensitive species being tested with time. This suggests the likelihood of identifying species that are more sensitive than the most sensitive species tested to date is low.

Based on the above, the remainder of my comments focus on evaluations relative to the USEPA's fish selenium criteria.

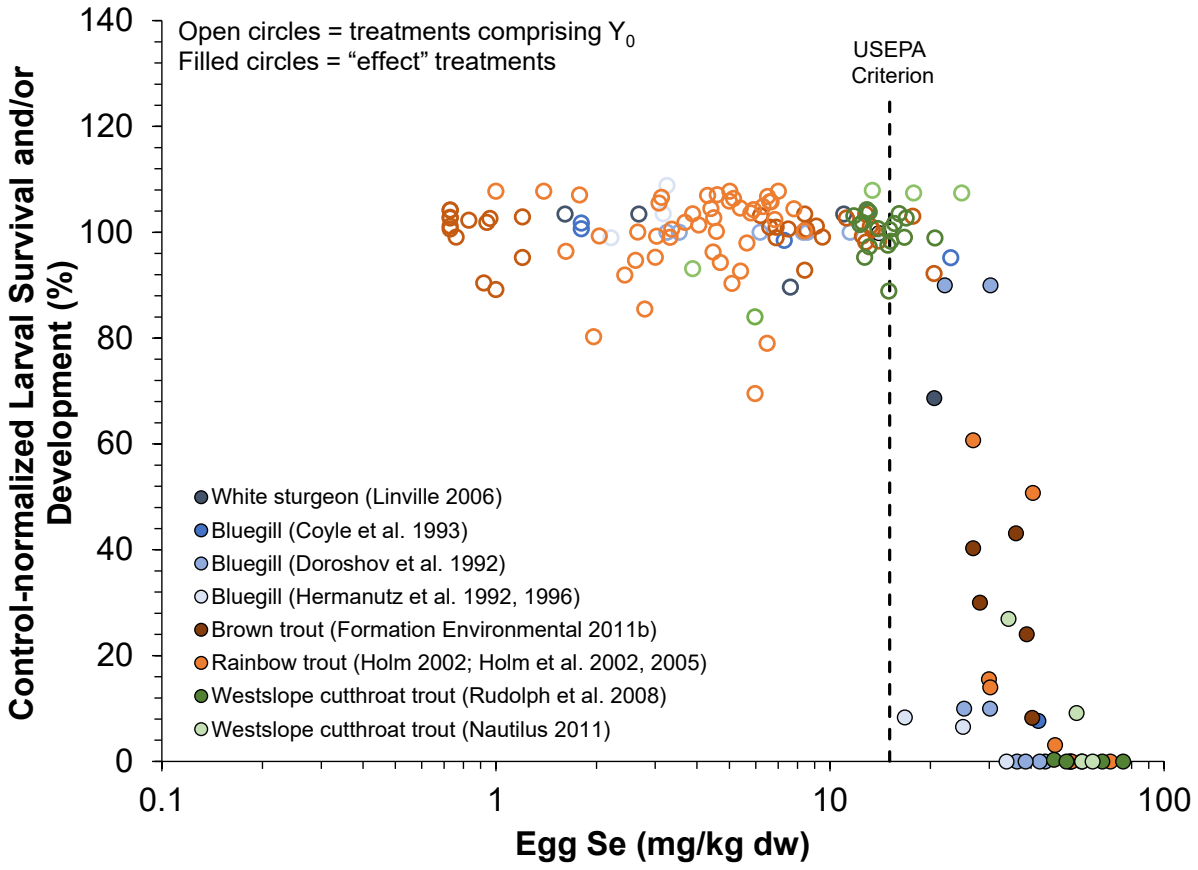


Figure 5. Concentration-response data for the four most sensitive genera in the USEPA's ambient water quality criteria document for selenium (USEPA 2016): (1) *Acipenser* (white sturgeon); (2) *Lepomis* (bluegill); (3) *Salmo* (brown trout); and (4) *Oncorhynchus* (rainbow trout, westslope cutthroat trout).
Note: Open symbols denote treatments that comprised Y0 (i.e., considered part of the control or reference population) and filled symbols denote those treatments that do not comprise Y0.

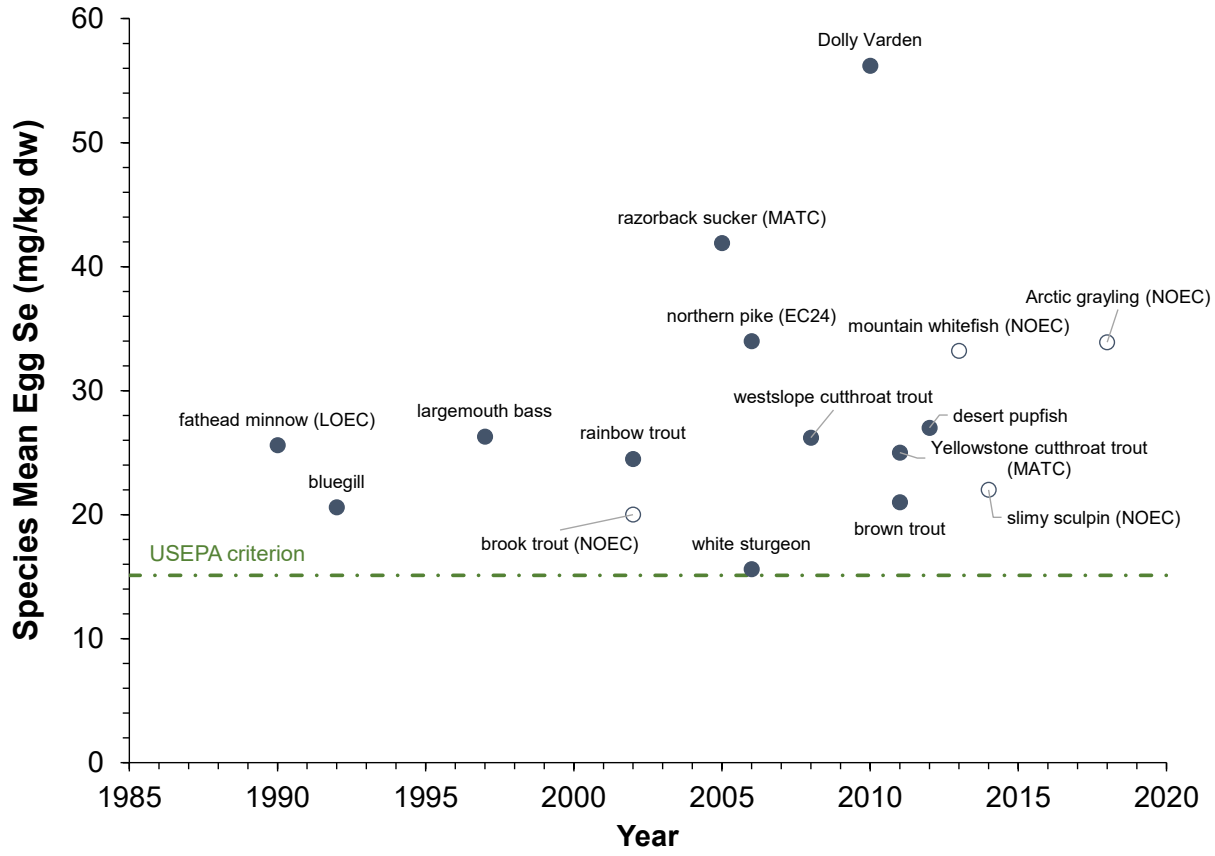


Figure 6. Relationship between fish egg selenium EC10s (and other toxicity thresholds) as a function of the year published or reported.
Note: Open symbols denote no-observed-effect concentrations (NOECs) from tests in which EC10s could not be estimated.

Table 1. Egg/ripe ovary selenium toxicity thresholds for North American freshwater fish.

Species	Statistical Endpoint	Species Mean Egg or Ovary Se (mg/kg dw)	Genus Mean Egg or Ovary Se (mg/kg dw)	Original Study Reference	Source of Toxicity Value Selected[1]
Salvelinus malma (Dolly Varden)	EC10	56.2	56.2[a]	McDonald et al. 2010	USEPA 2016
Salvelinus fontinalis (brook trout)	NOEC	>20		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Xyrauchen texanus (razorback sucker)	MATC	41.9	41.9	Hamilton et al. 2005a,b	DeForest et al. 2012
Esox lucius (northern pike)	EC24	34	34	Muscatello et al. 2006	USEPA 2016
Thymallus arcticus (Arctic grayling)	NOEC	>33.9	>33.9	Windward et al. 2018	Windward et al. 2018
Prosopium williamsoni (mountain whitefish)	NOEC	>33.2	>33.2	Nautilus 2013	Nautilus 2013
Cyprinodon macularius (desert pupfish)	EC10	27	27	Besser et al. 2012	USEPA 2016
Micropterus salmoides (largemouth bass)	EC10	26.3	26.3	Carolina Light and Power 1997	USEPA 2016
Pimephales promelas (fathead minnow)	LOEC	<25.6	<25.6	Schultz and Hermanutz 1990	USEPA 2016
Oncorhynchus clarkii lewisi (westslope cutthroat trout)	EC10	26.2	25.2[b]	Rudolph et al. 2008; Nautilus 2011	USEPA 2016
Oncorhynchus clarkii bouvieri (Yellowstone cutthroat trout)	MATC	25		Formation Environmental 2011a	DeForest et al. 2012
Oncorhynchus mykiss (rainbow trout)	EC10	24.5		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Cottus cognatus (slimy sculpin)	NOEC	>22	>22	Lo et al. 2014	Lo et al. 2014
Salmo trutta (brown trout)	EC10	21	21	Formation Environmental 2011b	USEPA 2016
Lepomis macrochirus (bluegill)	EC10	20.6	20.6	Doroshov et al. 1992; Coyle et al. 1993; Hermanutz et al. 1992, 1996	USEPA 2016
Acipenser transmontanus (white sturgeon)	EC10	15.6	15.6	Linville 2006	USEPA 2016

[1] EC10 values (or alternative statistical endpoints) were not always provided in the original study source, so source of value is provided.
[a] The genus mean value for *Salvelinus* was set equal to the EC10 for Dolly Varden, as no effects were observed in brook trout at the highest concentration tested.
[b] Although the statistical endpoint for Yellowstone cutthroat trout was an MATC, it was geometrically averaged with the EC10 values for westslope cutthroat trout and rainbow trout because values for all three species were similar.
EC10 = 10% effect concentration
EC24 = 24% effect concentration
NOEC = no-observed-effect concentration
LOEC = lowest-observed-effect concentration
MATC = maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC)

2 Empirical Fish Tissue Selenium Data for Koocanusa Reservoir

This section compares empirical fish selenium concentrations to the USEPA's egg, muscle, and whole body selenium criteria. Data for non-Cyprinids and Cyprinids are discussed separately in Sections 2.1 and 2.2, respectively.

2.1 Non-Cyprinids

Out of more than 1,200 individual samples of non-cyprinid fish species in the reservoir, there have been only three measurements (0.2%) that exceeded criteria. These include two ovary selenium measurements (Figure 7) and one muscle selenium measurement (Figure 8). No exceedances of the whole body selenium criterion have been observed (Figure 9). For each of the aforementioned individual selenium criteria exceedances, mean selenium concentrations from other samples of the same species, from the same location and time did not exceed criteria:

- Rainbow trout ovary Se concentration of 19.8 mg/kg dw
  - Mean of 14.0 mg/kg dw for sampling location and time (n = 2) and less than the rainbow trout-specific EC10 of 24.5 mg/kg dw
- Longnose sucker ovary Se concentration of 21 mg/kg dw
  - Mean of 12.8 mg/kg dw for sampling location and time (n = 3)
- Yellow perch muscle Se concentration of 15 mg/kg dw
  - Mean of 4.6 mg/kg dw for sampling location and time (n = 10)

Consequently, empirical monitoring of selenium concentrations in non-cyprinids supports that these species are not adversely impacted by selenium in the reservoir.

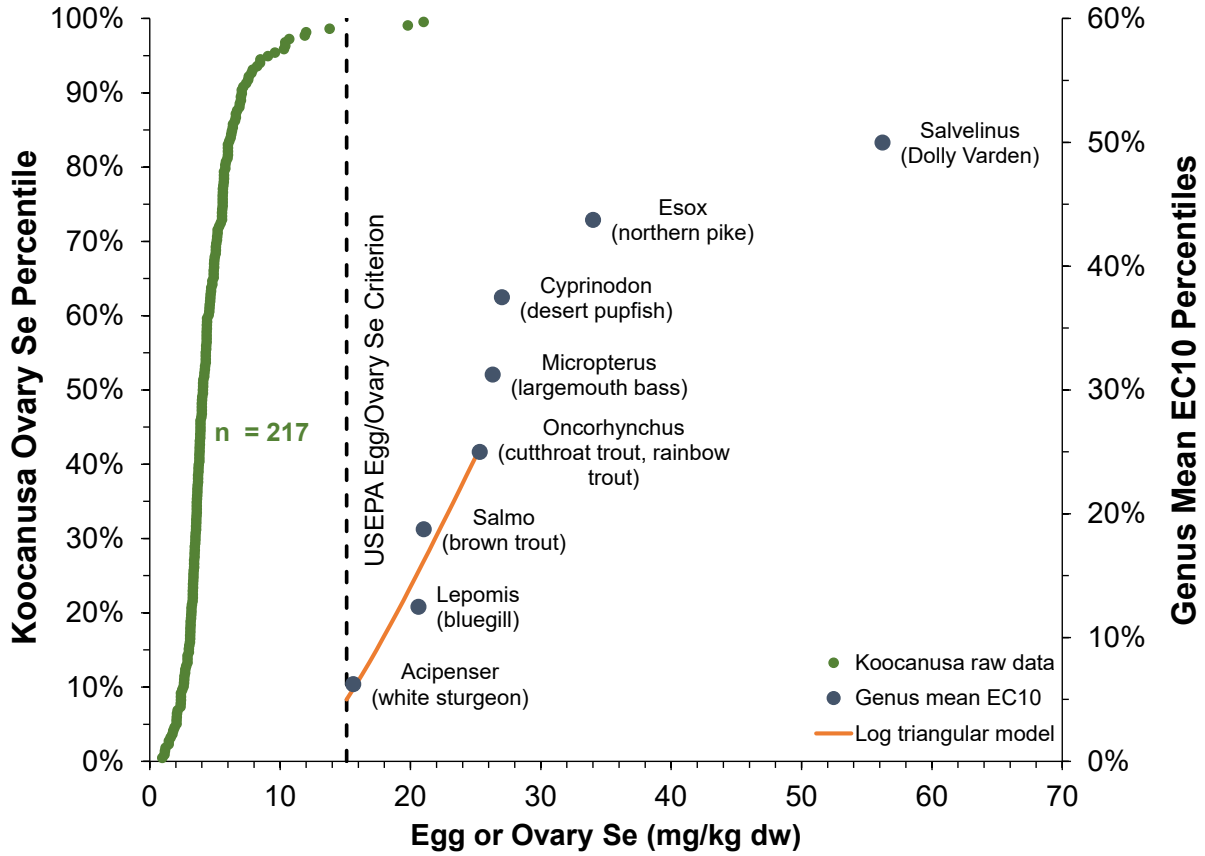


Figure 7. Cumulative distribution of empirical selenium concentrations in fish ovary samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of egg/ovary selenium concentrations used to derive the USEPA egg/ovary selenium criterion.

Note: Ovary selenium data for northern pikeminnow, peamouth chub, and redside shiner are excluded (see Section 2.2 for discussion of these cyprinids).

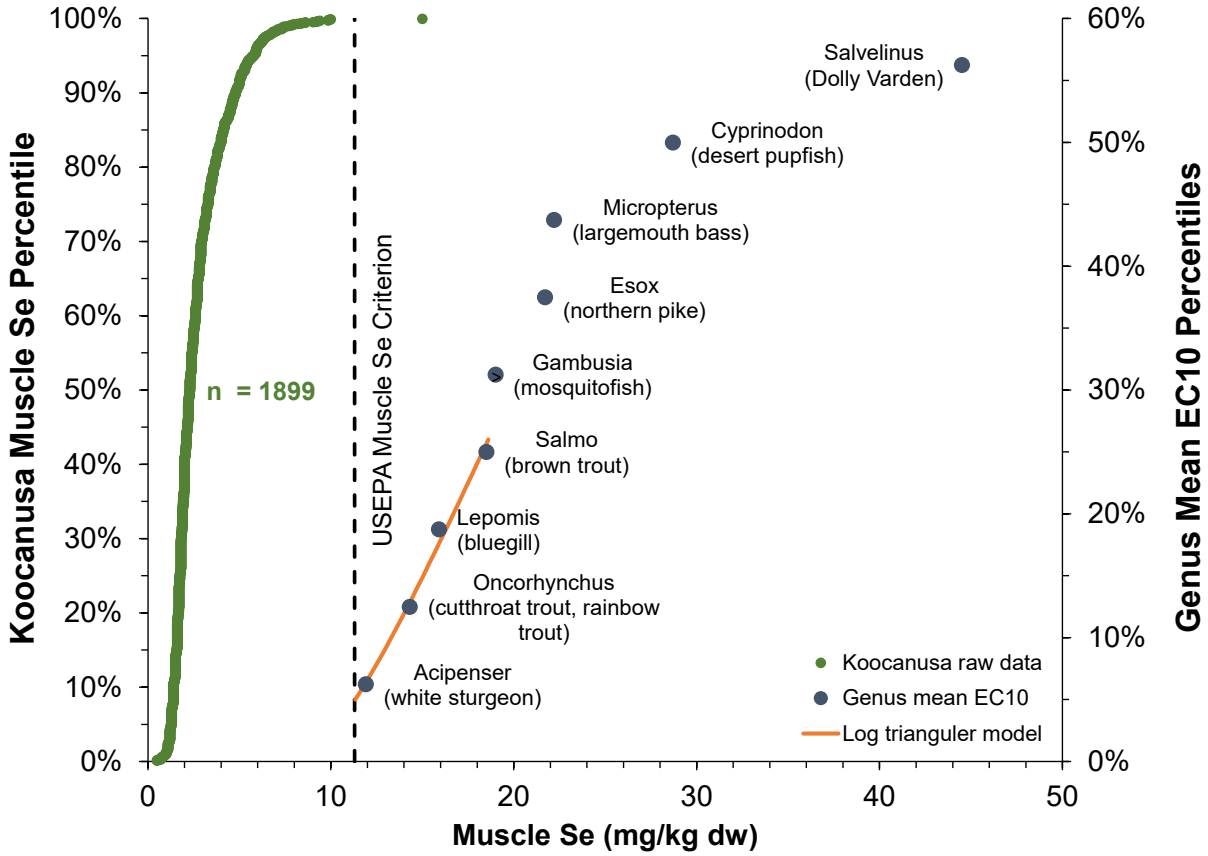


Figure 8. Cumulative distribution of empirical selenium concentrations in fish muscle samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of muscle selenium concentrations used to derive the USEPA muscle selenium criterion.

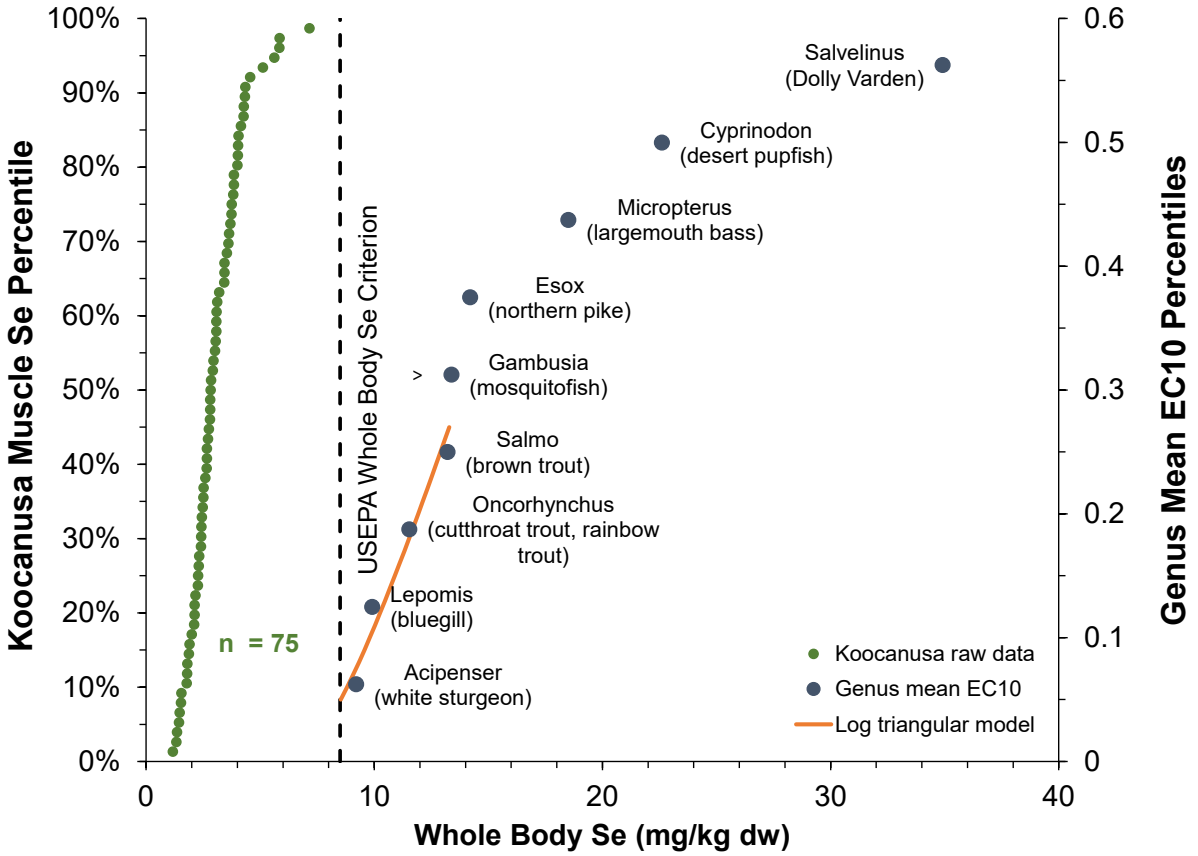


Figure 9. Cumulative distribution of empirical selenium concentrations in whole body fish samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of whole body selenium concentrations used to derive the USEPA whole body selenium criterion.

2.2 Cyprinids

The three cyprinids in Koocanusa Reservoir are northern pikeminnow, peamouth chub, and redside shiner. Muscle and whole body selenium concentrations in these three species have never exceeded USEPA criteria. Ovary selenium concentrations for these species have exceeded the USEPA's egg selenium criterion but, as discussed further below, most of the ovary selenium data were not collected during spawning and concentrations are likely overestimated and should not be used. Further, the USEPA has compiled evidence that cyprinids are not uniquely sensitive to selenium.

2.2.1 Ovary Selenium Concentrations and Maturity

Based on routine monitoring of northern pikeminnow ovary selenium concentrations in Koocanusa Reservoir, it was previously observed that concentrations were higher and more variable in the BC portion of the reservoir, and lower and less variable on the Montana side. Further evaluation of these data revealed that fish collected in BC were

smaller than those collected in Montana, reflective of different sampling techniques. In addition, there was an indication that ovary selenium concentrations and the gonado-somatic index (GSI) were inversely related in northern pikeminnow collected in BC (GSI had not been measured in the northern pikeminnow collected in Montana). The GSI is a measure of ovary maturity, so ideally selenium would be analyzed in ovaries of fish with a GSI that is reflective of the fish's condition at the time of spawning.

These observations led to the northern pikeminnow study conducted in 2019, which included additional sampling at different times during the spawning period to collect fish with a range of sizes and GSI values. This report concluded that ovary selenium concentrations should not be used for assessing selenium risks to northern pikeminnow when the GSI is <5%, as selenium concentrations in the ovaries of these fish overestimate selenium concentrations that are relevant to the time of spawning (EcoTox et al. 2020). The study report was provided to the SeTSC and the study presented to the SeTSC by Dr. Brix in June 2020 (for completeness a copy the report is provided in Attachment 1).

A preliminary evaluation of ovary selenium concentrations and GSI for peamouth chub in Koocanusa Reservoir likewise indicates that there is a minimum GSI below which ovary selenium concentrations should not be used for assessing selenium risks. Based on a study of peamouth chub in the Columbia River (WA, USA), females had a mean GSI of about 8% during the period of spawning (Figure 10; Gray and Dauble 2001). Of the 153 peamouth chub ovary selenium concentrations measured in Koocanusa Reservoir, 29 (19%) exceed the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 11). Of those, the GSI was >8% in just three of the samples. Although this evaluation is not as robust or definitive as the northern pikeminnow study, it similarly highlights that future monitoring of selenium concentrations in peamouth chub should target spawning periods.

Although not a cyprinid, a recent evaluation of mountain whitefish data similarly found that ovary selenium concentrations are inversely related to GSI (Brix et al. 2020). Thus, there is an increasing body of information highlighting the importance of measuring selenium concentrations in ripe ovaries during spawning, as measurement of selenium in immature ovaries may overestimate selenium concentrations at spawning. The mountain whitefish evaluation is included as Attachment 2.

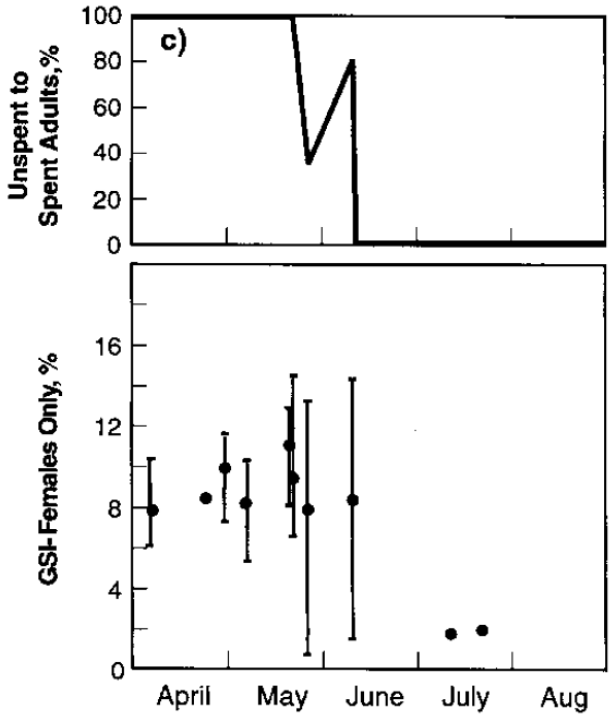


Figure 10. Spawning periods (top panel) and female GSI for peamouth chub in the Columbia River (WA, USA).
Note: Figure 3 in Gray and Dauble (2001).

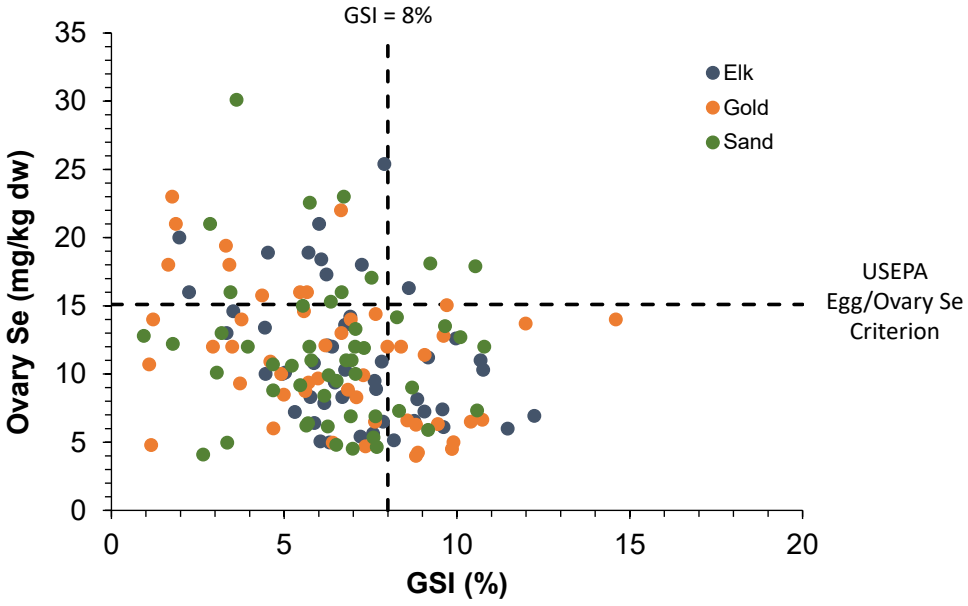


Figure 11. Relationship between ovary selenium concentrations in peamouth chub and GSI.

2.2.2 Conversion Factors for Ripe Ovaries

As noted in Presser and Naftz (2020), egg-ovary tissue can be substituted in the selenium model if the appropriate tissue-to-tissue conversion factors are available. Muscle-to-egg/ovary and whole body-to-egg/ovary conversion factors from USEPA (2016), for example, may be obtained for species of interest. Ideally, site-specific tissue-to-tissue conversion factors may be calculated. If site-specific muscle-to-ovary or whole body-to-ovary conversion factors are derived from Koocanusa Reservoir data, caution must be used to ensure that the ovary data used to calculate the conversion factors are based on ripe ovaries during the fish's spawning period. The following provides an example based on northern pikeminnow.

The northern pikeminnow study resulted in the conclusion that ovary selenium concentrations should not be used to assess selenium risk if the GSI is <5%. Based on this, I derived a site-specific northern pikeminnow muscle-to-ovary conversion factor from those muscle and ovary pairs from fish with a GSI >5% (Figure 12). Following USEPA (2016) methods, the conversion factor is calculated as the median, which is 2.5 for this dataset. This median muscle-to-ovary conversion factor of 2.5 was then applied to northern pikeminnow muscle data to estimate selenium concentrations in ripe ovaries representative of spawning conditions. Reservoir-wide annual mean estimates of selenium concentrations in ripe ovaries range from 3.3 to 7.7 mg/kg dw (Figure 13). Mean estimated selenium concentrations in ripe ovaries as a function of sampling location and sampling time range from 3.0 to 13.0 mg/kg dw, which are all still lower than the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 14). This northern pikeminnow example demonstrates that evaluation of selenium concentrations in ripe ovaries has an important influence in evaluating potential selenium-related impacts.

2.2.3 Cyprinid Sensitivity to Selenium

In developing its ambient water quality criteria for selenium, the USEPA conducted a review of cyprinid sensitivity based on a review of field and laboratory data (Appendix E in USEPA [2016]). Based on field studies of regions in the United States with elevated selenium concentrations, there was no evidence of selenium-related impacts and the USEPA concluded that "native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species." Based on these conclusions, the USEPA's egg selenium criterion, driven by the sensitivity of white sturgeon, is conservative for cyprinids. Current studies on the sensitivity of redside shiner to selenium, and planned studies on the sensitivity of northern pikeminnow to selenium,[1] will provide additional information on the sensitivity of cyprinids.

[1] The 2019 northern pikeminnow study was intended to also test its sensitivity to selenium, but an insufficient number of ripe females were captured from which eggs could be manually expressed.

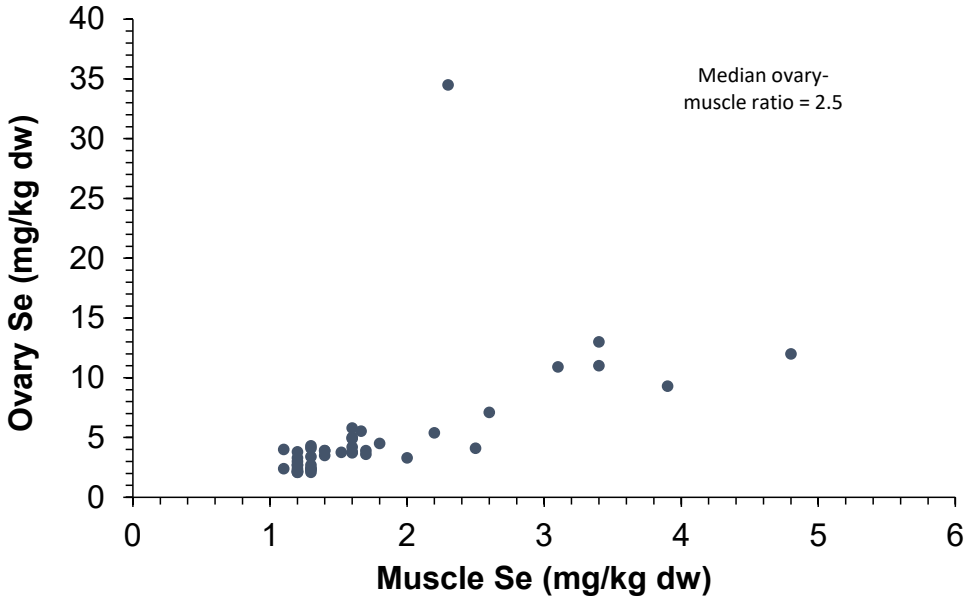


Figure 12. Site-specific relationship between ovary and muscle selenium in northern pikeminnow with a GSI >5%.
Note: The median ovary-muscle ratio is 2.5 with and without the apparent high outlier.

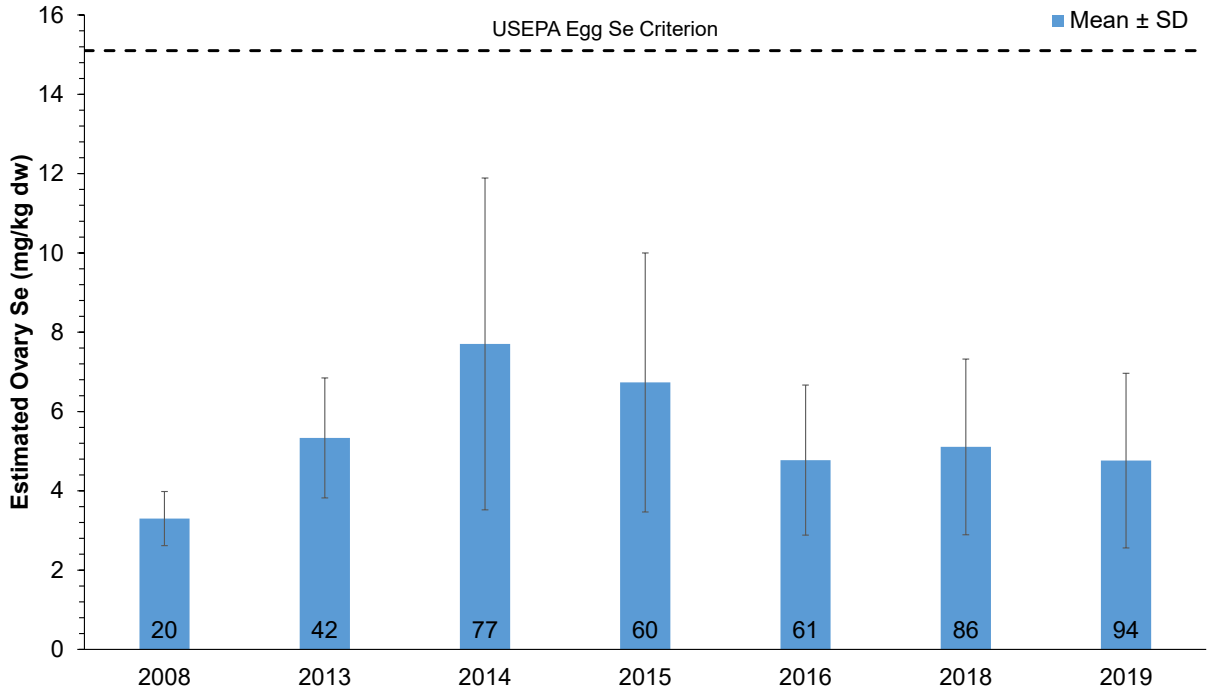


Figure 13. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow based on all samples in Koocanusa Reservoir by year.
Note: Median ovary-muscle ratio is 2.5. Numbers within base of column denote sample sizes.

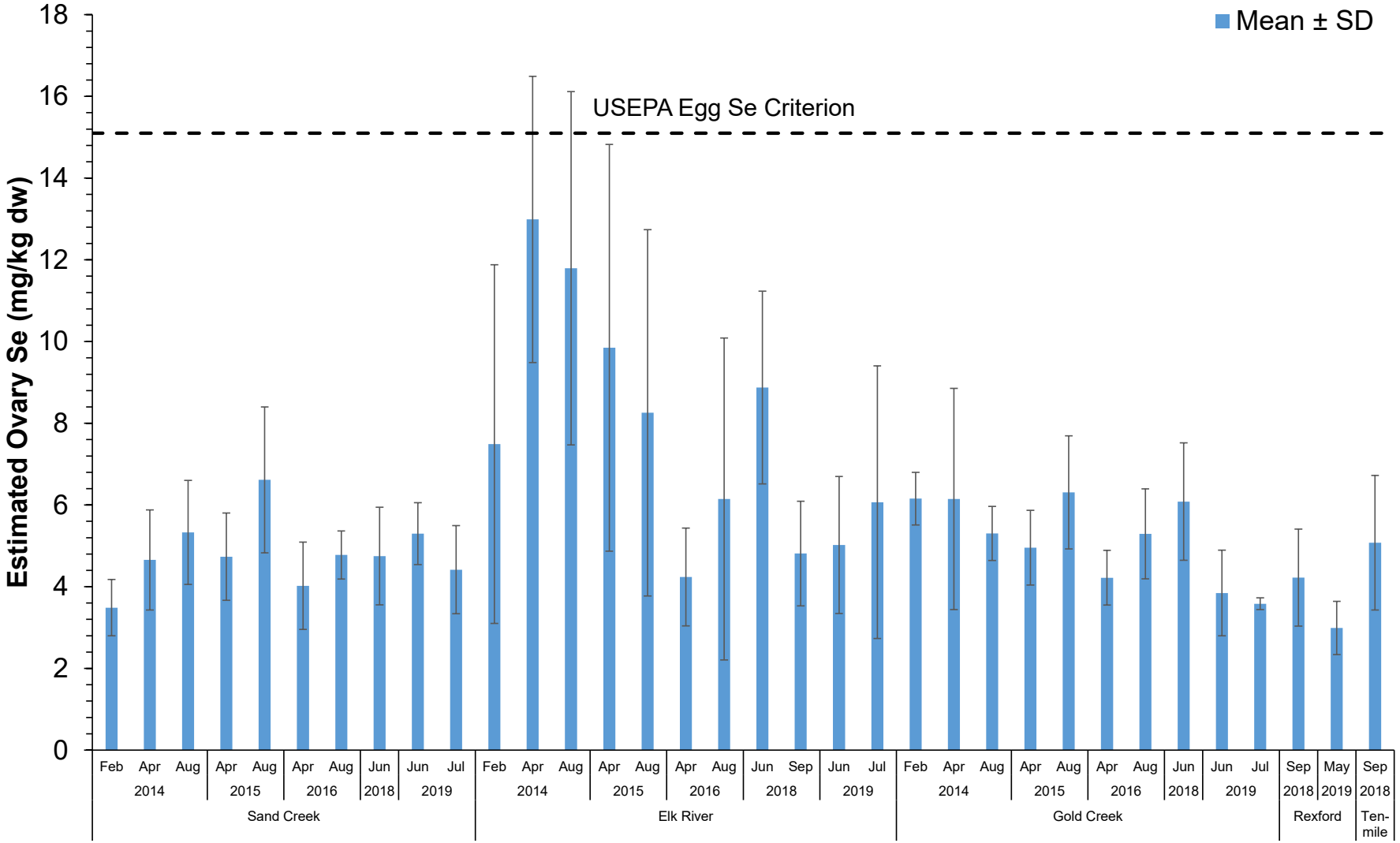


Figure 14. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow by location and sampling time within Koocanusa Reservoir.
Note: Median ovary-muscle ratio is 2.5.

3 Review and Validation of Koocanusa Reservoir Selenium Bioaccumulation Models

Section 3.1 first discusses the USGS selenium model (Presser and Naftz 2020) and modifications to model inputs based on consideration of site-specific data for invertebrates are provided in Section 3.2. Validation of model outputs based on site-specific fish selenium data is then provided in Section 3.3.**USGS Model**

The USGS selenium model was developed to support derivation of a site-specific water selenium criterion for Koocanusa Reservoir (Presser and Naftz 2020). This model was based on the framework for Koocanusa Reservoir previously presented in Jenni et al. (2017), which was developed following the ecosystem-scale selenium modeling methodology described in Presser and Luoma (2010). This model uses k_d values to describe partitioning of selenium from water to particulates at the base of the food web and trophic transfer factors (TTFs) to describe partitioning of selenium between food web components (e.g., from particulates to invertebrates and from invertebrates to fish). For example:

$$C_{fish} = C_{water} \times k_d \times TTF_{invert} \times TTF_{fish} \times 0.001 \quad (1)$$

Where: C_{water} = Se concentration in water (µg/L)
C_{fish} = Se concentration in fish tissue (mg/kg dw)
k_d = ratio of Se concentration in particulates and water (L/kg dw)
TTF_{invert} = ratio of Se concentration in invertebrates and particulates
TTF_{fish} = ratio of Se concentration in fish and invertebrates
0.001 = conversion factor to convert from µg to mg

Based on k_d and TTF data or assumptions, and a target fish selenium concentration of interest (e.g., a criterion), Equation 1 can be rearranged to solve for the water selenium concentration predicted to result in the target fish selenium concentration:

$$C_{water} = \frac{C_{fish}}{k_d + TTF_{invert} + TTF_{fish}} \quad (2)$$

Equations 1 and 2 can be modified to include additional trophic level steps, such as a prey fish consumed by piscivorous fish. The equations can also be modified to include relative dietary fractions of invertebrates, such as 50% benthic invertebrates and 50% zooplankton. The following summarizes model inputs used in Presser and Naftz (2020):

- **k_d:** Selenium k_d values were calculated from 87 paired particulate and surface water selenium concentrations[2] measured in large volume suspended sediment (LVSS) samples. These samples were collected at the epilimnion and hypolimnion

[2] At the time of writing, these complete data have not been made available by the USGS and were requested on August 18, 2020.

from four locations in the reservoir: (1) south of Elk River; (2) the international boundary; (3) Tenmile (hypolimnion only); and (4) the forebay.

- **TTF_{invert}:** TTFs of 2.8 and 1.5 were assumed for benthic invertebrates and zooplankton, respectively. These TTFs were derived and reported in Presser and Luoma (2010) based on data from other studies and are not specific to Koocanusa Reservoir. Presser and Naftz (2020) incorporated a bioavailability fraction of 0.6 (60%), which effectively reduce the benthic invertebrate TTF from 2.8 to 1.7 and the zooplankton TTF from 1.5 to 0.9.

- **TTF_{fish}:** A whole body selenium TTF of 1.1 was assumed in the presentation of the model in the report, which is the mean TTF from 25 fish species as reported in Presser and Luoma (2010). It was noted, however, that species-specific TTFs could be considered and examples were provided.

- **C_{fish}:** The USEPA's whole body selenium guideline of 8.5 mg/kg dw was used in the report, but it was noted that other fish tissue selenium concentrations of interest could be considered.

For presentation and discussion of the model herein, water selenium concentrations are back-calculated from the whole body criterion of 8.5 mg/kg dw based on a k_d of 5,000 L/kg dw and benthic invertebrate, zooplankton, and fish TTFs of 2.8, 1.5, and 1.1, respectively (with and without the bioavailability factor of 0.6 for invertebrates). Water concentrations were back-calculated for four different food chain scenarios to bracket the range of exposure conditions: (1) 100% benthic invertebrates; (2) 100% zooplankton; (3) 50% benthic invertebrates and 50% zooplankton; and (4) 100% fish. These example calculations are similar to those provided in Table 10 of Presser and Naftz (2020). Presser and Naftz (2020) did not recommend selection of a single representative k_d for the reservoir, but a k_d of 5,000 L/kg dw was selected because this value was used in their Table 10 and it is also approximately the median k_d for the reservoir and thus a reasonable estimate of central tendency.

Based on the above model input assumptions and food chain scenarios, the water selenium concentrations projected to result in the whole body selenium criterion of 8.5 mg/kg dw range from 0.5 µg/L (100% fish diet) to 1.0 µg/L (100% fish diet) with a bioavailability factor of 100% (first set of green-highlighted rows in Table 2). If the bioavailability factor of 0.6 is assumed, the range in back-calculated selenium concentrations increases to 0.84 to 1.7 µg/L (second set of green-highlighted rows in Table 2).

Table 2. Examples of water selenium concentrations projected to result in whole body selenium criterion of 8.5 mg/kg dw based on different k_d, TTF, and food chain scenarios.

Scenario	Diet	TTF			SPM Bioavail-ability Fraction	k_d (L/kg dw)	WB Criterion (mg/kg dw)	Water Se (µg/L)	Mean Water Se (µg/L)
		Insects	Zoo-plankton	Fish					
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1	100% Insects	2.8	1.5	3.1*	1	5000	8.5	**0.55**	**0.70**
	100% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**1.0**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**0.72**	
	100% Fish	2.8	1.5	3.1*	1	5000	8.5	**0.50**	
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	100% Insects	2.8	1.5	3.1*	0.6	5000	8.5	**0.92**	**1.2**
	100% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.7**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.2**	
	100% Fish	2.8	1.5	3.1*	0.6	5000	8.5	**0.84**	
Alternative Assumptions									
Alternative Assumptions: Median k_d from USGS (2020) and median site-specific TTFs for inverts	100% Insects	1.1	0.52	1.2*	1	4547	8.5	**1.5**	**2.1**
	100% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**3.3**	
	50% Insects / 50% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**2.1**	
	100% Fish	1.1	0.52	1.2*	1	4547	8.5	**1.4**	
Alternative Assumptions: 75th percentile k_d from USGS (2020) and 75th percentile site-specific TTFs for inverts	100% Insects	1.2	0.65	1.3*	1	5268	8.5	**1.2**	**1.5**
	100% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**2.3**	
	50% Insects / 50% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**1.6**	
	100% Fish	1.2	0.65	1.3*	1	5268	8.5	**1.1**	

SPM = suspended particulate matter
TTF = trophic transfer factor
WB = whole body
*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

3.2 Alternative Inputs for Selenium Model

As discussed above, TTFs for benthic invertebrates and zooplankton in the USGS model are based on data compiled in Presser and Luoma (2010) and are not specific to Koocanusa Reservoir. As a point of comparison to those invertebrate TTFs, I developed zooplankton and benthic invertebrate TTFs based on site-specific data.

Site-specific selenium TTFs for zooplankton were developed by co-locating zooplankton and surface water selenium samples. In this paired dataset, zooplankton selenium concentrations ranged from 0.34 to 4.21 mg/kg dw and particulate selenium concentrations ranged from 2.0 to 6.6 mg/kg dw (Figure 15A). With one exception, resulting zooplankton TTFs were less than 1 and the median TTF was 0.52 (Figure 15B). The one exception was a TTF of 1.9, was calculated from the lowest particulate selenium concentration in the paired dataset. This observation is consistent with patterns in other studies, in which TTFs are greater at low selenium exposure concentrations (DeForest et al. 2007). **The median TTF of 0.52 based on site-specific data is less than the mean zooplankton TTF of 1.5 in the USGS model, as well as the TTF of 0.9 in the USGS model if the 60% bioavailability factor is applied.** As such and consistent with comments from other SeTSC members on August 25, 2020, TTFs for zooplankton as proposed by the USGS are overly conservative.

For benthic invertebrates, co-located selenium concentrations in particulates are not available. As such, a two-step approach was used to estimate site-specific selenium TTFs for benthic invertebrates. First, benthic invertebrate and surface water selenium samples were co-located. Benthic invertebrate selenium concentrations in these co-located samples range from 0.38 to 9.1 mg/kg dw and surface water selenium concentrations range from 0.15 to 2.4 µg/L (Figure 16A). These co-located data were used to develop bioaccumulation factors (BAFs), which are calculated as the benthic invertebrate selenium concentration divided by the surface water selenium concentration (multiplied by 1,000 to convert to units of L/kg dw). As observed in other selenium BAF datasets (DeForest et al. 2007), there is an inverse relationship between BAFs and water selenium concentration (Figure 16B). This relationship is described by a linear regression model in log-log scale (Figure 16C).

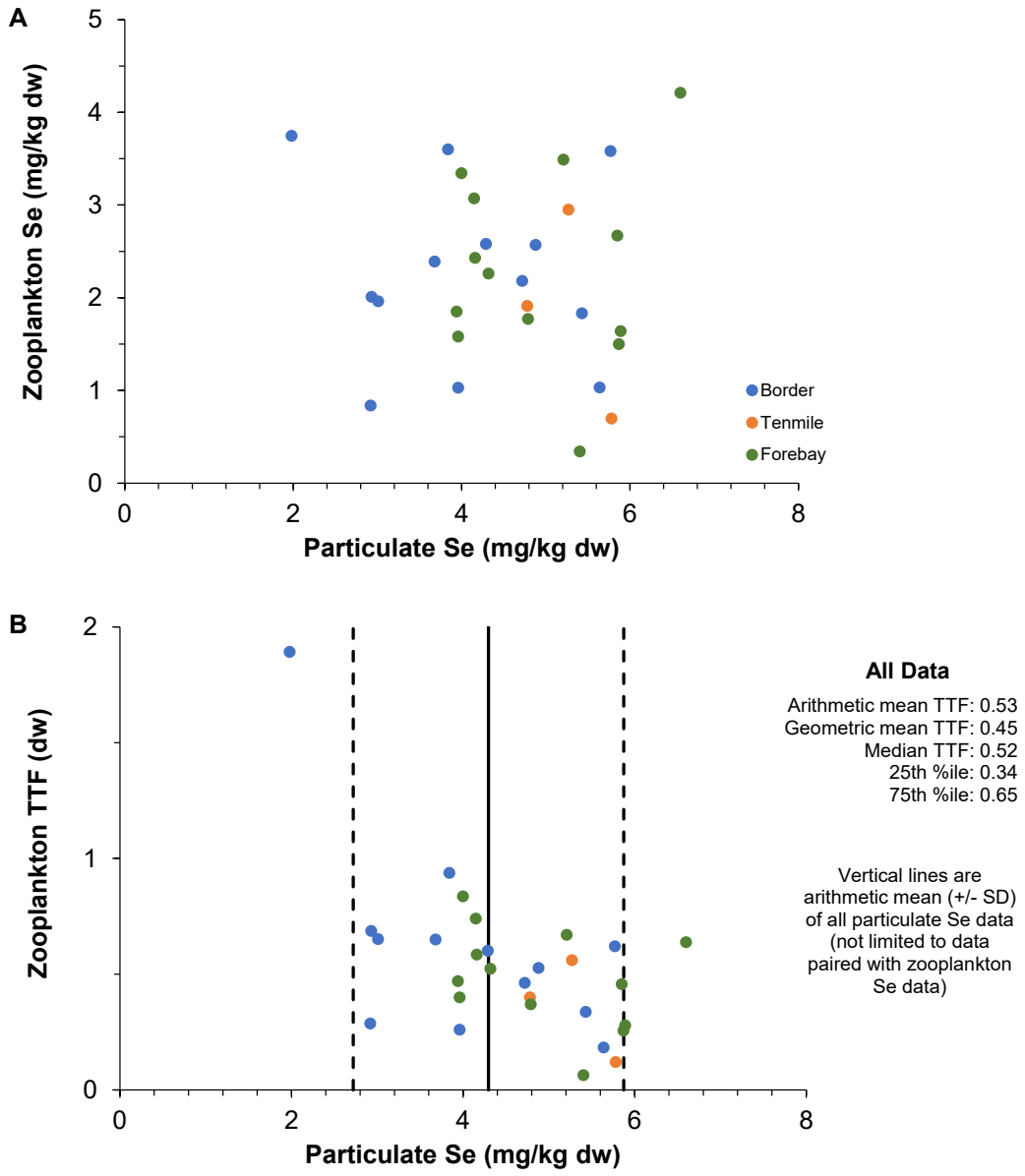


Figure 15. (A) Relationship between selenium concentrations in co-located zooplankton and particulate samples; and (B) relationship between zooplankton TTFs and particulate selenium concentrations.

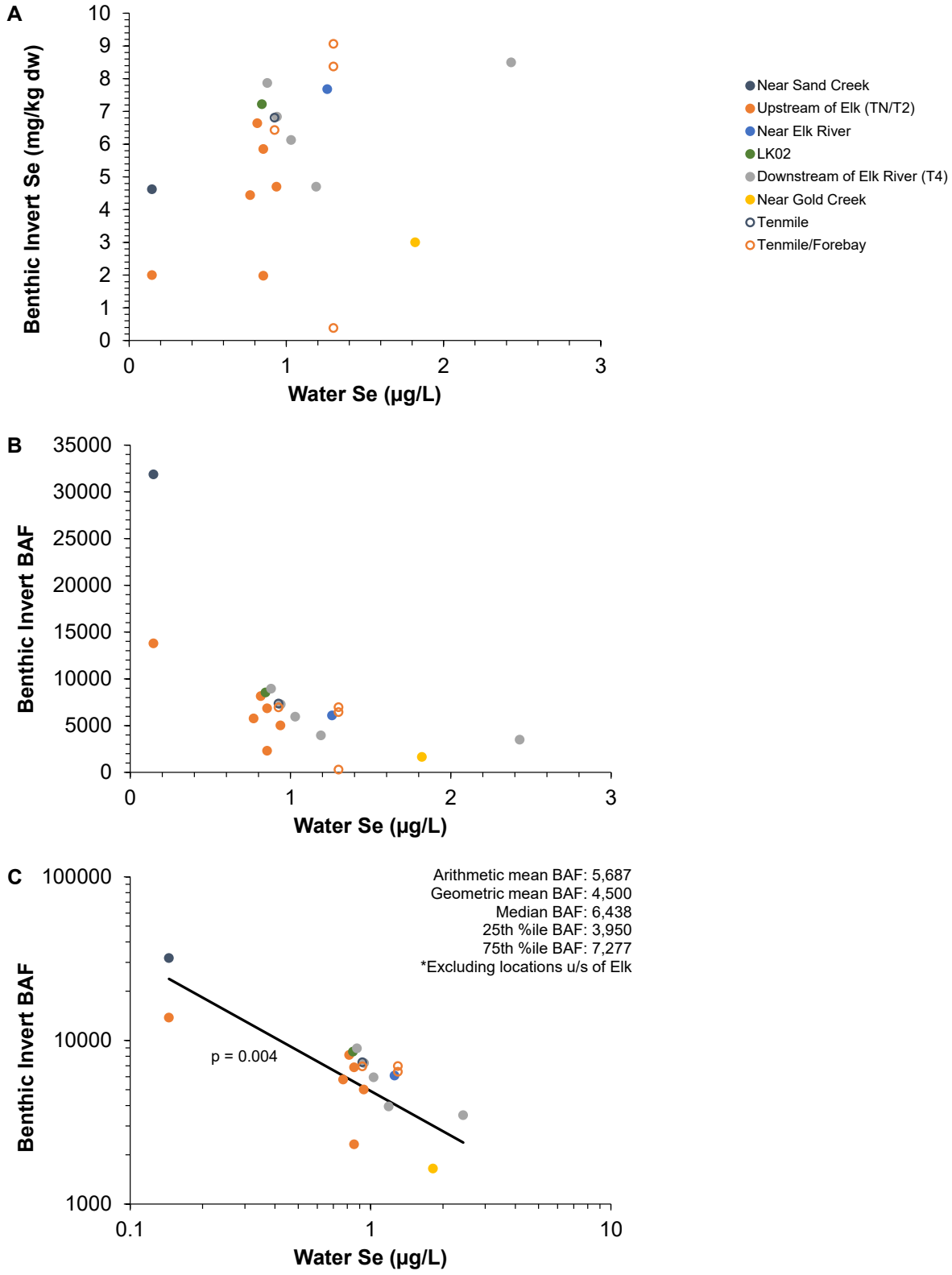


Figure 16. Relationships between (A) co-located benthic invertebrate and water selenium concentrations; and benthic invertebrate BAFs and water selenium concentrations in (B) normal scale and (C) log-normal scale.

The second step was to apply the benthic invertebrate BAF model from Figure 16C to surface water selenium concentrations used to develop the selenium k_d values. Thus, for each water selenium concentration in the k_d dataset, there is a co-located measure of particulate selenium and a co-located estimate of benthic invertebrate selenium (Figure 17A). Benthic invertebrate TTFs were then calculated based on the paired measured particulate selenium concentrations and estimated benthic invertebrate selenium concentrations. Based on the inverse relationship between benthic invertebrate BAFs and surface water, there is also an inverse relationship between estimated benthic invertebrate TTFs and particulate selenium concentrations (Figure 17B). To avoid undue influence of high TTFs at low selenium exposure concentrations and low TTFs at high selenium exposure concentrations, the benthic invertebrate TTF summary statistics were calculated over the range of the mean measured selenium concentrations plus and minus one standard deviation (Figure 17B). The median TTF within this particulate selenium range is 1.1. **The estimated TTF from site-specific data is less than the benthic invertebrate TTF of 2.8 in the USGS model, as well as the TTF of 1.7 in the USGS model if the 60% bioavailability factor is applied.**

Two additional model scenarios were then evaluated using the above-mentioned site-specific data. One model scenario considered the 50th percentile k_d and the 50th percentile TTFs of 1.1 and 0.52 for benthic invertebrates and zooplankton, respectively. The second scenario was more conservative by considering the 75th percentile k_d and 75th percentile TTFs of 1.2 and 0.65 for benthic invertebrates and zooplankton, respectively. Based on the 50th percentile assumption, the water selenium concentrations predicted to result in a whole body selenium criterion of 8.5 mg/kg dw range from 1.4 µg/L (100% fish diet) to 3.3 µg/L (100% zooplankton diet) (first set of blue-highlighted rows in Table 2). Based on the 75th percentile assumption, water selenium concentrations predicted to result in the whole body selenium criterion of 8.5 mg/kg dw range from 1.1 µg/L (100% fish diet) to 2.3 µg/L (100% zooplankton diet) (second set of blue-highlighted rows in Table 2).

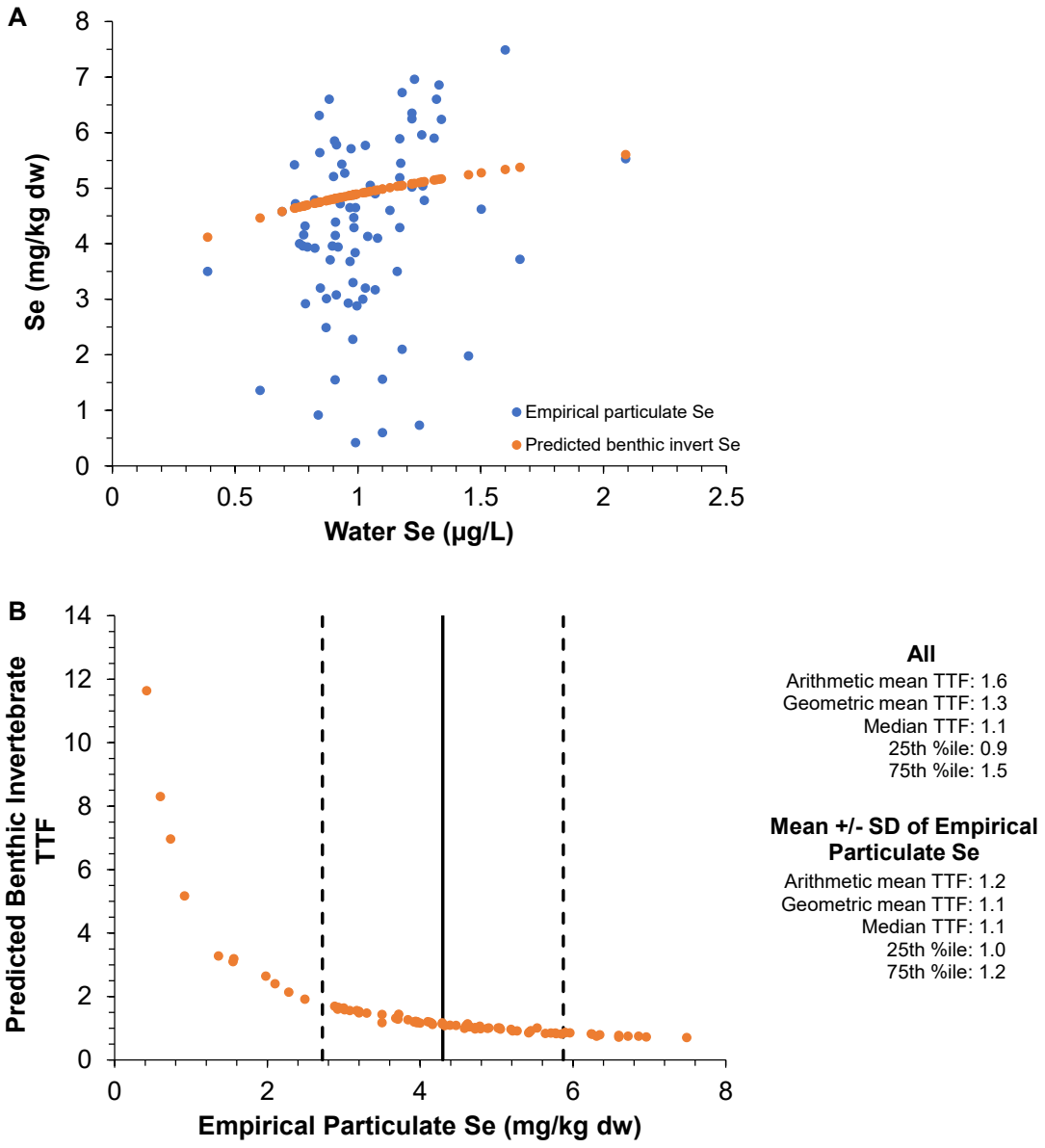


Figure 17. Relationships between (A) empirical particulate Se concentrations and predicted benthic invertebrate Se concentrations; and (B) between predicted benthic invertebrate TTFs and particulate Se concentrations.

3.3 Validation of Models

Empirical fish tissue selenium data for Koocanusa Reservoir were not considered in the development or validation of the selenium model described in Presser and Naftz (2020). It appears that empirical fish selenium data were not considered because it was assumed that the reservoir was currently degraded from the cumulative effects of various stressors and that, by inference, the suggestion appears to be that selenium concentrations in fish in the reservoir are not representative of what would be expected in an unstressed

system. A reservoir is of course a managed system and issues related to drawdown and flow-regime changes, even in the absence of any other stressors, can have a substantial influence on habitat and aquatic communities. In my opinion, consideration of empirical fish selenium data is a critical piece of evidence for evaluating selenium bioaccumulation in the reservoir. In addition, fish selenium data in the reservoir will presumably be important for determining compliance with selenium criteria in the future.

I conducted a validation of the USGS model and alternative input assumptions based on the invertebrate TTF evaluation described in Section 3.2. The first step of the validation process was to first identify paired fish tissue and water selenium concentrations in the reservoir. Fish selenium concentrations were then predicted from water selenium concentrations based on various k_d and TTF assumptions. The selection of TTFs was based on the dietary assumption for the fish species being evaluated in the validation. For example, peamouth chub are assumed to have a diet comprised of 50% benthic invertebrates and 50% zooplankton, so for this fish species the benthic invertebrate and zooplankton TTFs were each given 50% weight.

Validation of four different modeling scenarios (consistent with those described above) was evaluated:

1. USGS model with suspended particulate matter bioavailability fraction of 100%. A k_d of 5,000 L/kg was used for consistency with the model examples provided in Table 10 of Presser and Naftz (2020) (Table 3).

2. USGS model with suspended particulate matter bioavailability fraction reduced to 60% (Table 3).

3. Alternative model inputs based on the 50th percentile k_d from the USGS model and the 50th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

4. A more conservative alternative model with inputs based on the 75th percentile k_d from the USGS model and the 75th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

Table 3. Model scenarios included in the validation.

Scenario	k_d (L/kg dw)	SPM Bioavail-ability Fraction	TTF		
			Insects	Zoo-plankton	Fish
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1.0	5000	1	2.8	1.5	3.1*
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	5000	0.6	2.8	1.5	3.1*
50th %ile site-specific k_d and invert TTFs	4547	1	1.1	0.52	1.2*
75th %ile site-specific k_d and invert TTFs	5268	1	1.2	0.65	1.3*

*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

Predicted fish selenium concentrations were then compared to observed fish selenium concentrations. Comparisons were made using a standard selenium bioconcentration and trophic transfer figure, similar to that provided in Figure 18. This figure format visually shows the transfer of selenium from surface water to particulates, invertebrates, and fish as a function of assumed k_d values and TTFs.

Validation evaluations presented here are based on selenium concentrations in fish muscle and whole body tissue. The validation is focused on these two tissue types because of uncertainties in the ovary selenium data due to the timing of ovary sampling relative to spawning periods and ovary maturity. Further discussion of ovary selenium concentrations and maturity is provided in Sections 2 and 4.

Over the validation scenarios evaluated, measured muscle and whole body selenium concentrations were consistently over-predicted by the USGS model regardless of model assumptions (Figure 19). Based on the USGS model with the 60% bioavailability assumption, predicted fish selenium concentrations were over-predicted, on average, by a factor of 5.0 (Table 4). In most of the scenarios evaluated, this resulted in muscle and whole body selenium concentrations that were predicted to exceed the muscle selenium criterion of 11.3 mg/kg dw and whole body selenium concentration of 8.5 mg/kg dw, while measured fish selenium concentrations were generally less than 50% of criteria concentrations. Even when considering site-specific k_d summary statistics and site-specific invertebrate TTFs the USGS model predicts muscle and whole body selenium concentrations that, on average, are a factor of 2.9 greater than observed (Table 4).

In my opinion, the consistent over-prediction of selenium concentrations in fish tissue is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.

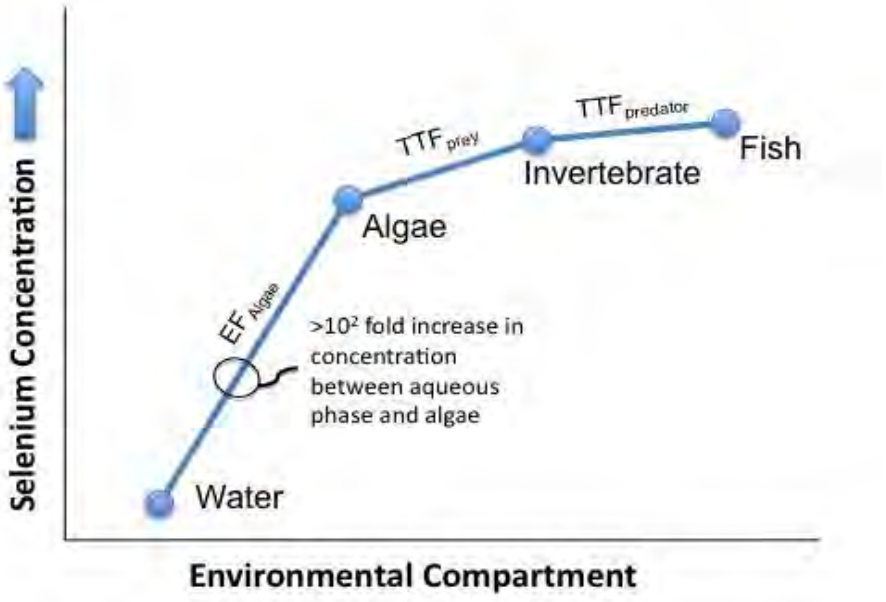


Figure 18. Example of multi-step selenium bioconcentration and trophic transfer plots in a fish food chain.
Source: Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Bioaccumulation and trophic transfer of selenium. In: Ecological assessment of selenium in the aquatic environment. Pensacola, FL:SETAC Press, 93-139.

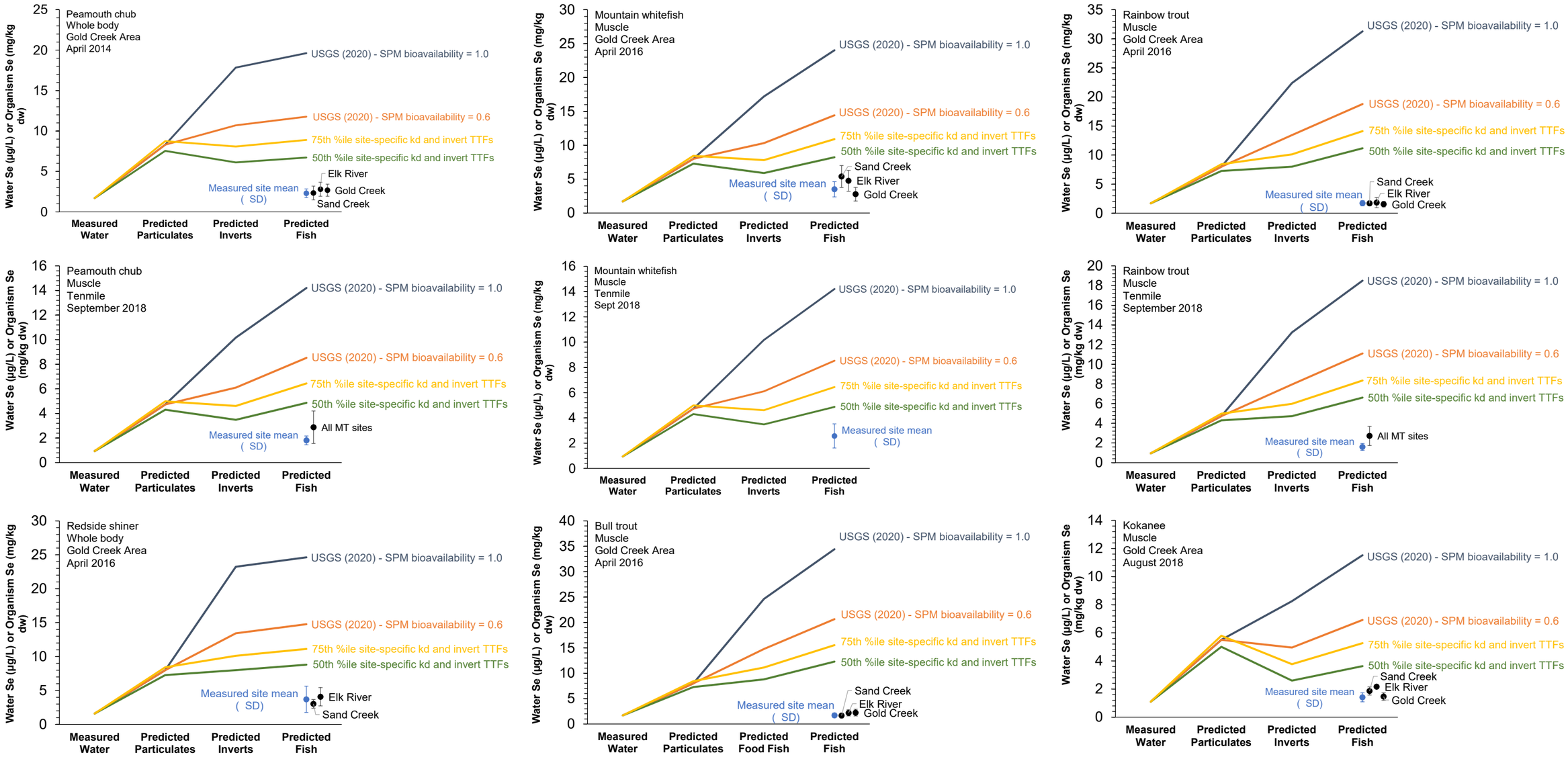


Figure 19. Comparisons of predicted and observed whole body selenium concentrations in Koocanusa Reservoir fish.
Note: See text boxes within each panel for the species, tissue type, sample location, and sampling data.

Table 4. Examples of observed and predicted fish selenium concentrations in Koocanusa Reservoir based on different modeling scenarios.

Site	Scenario	Water Se (µg/L)	kd (L/kg dw)	Predicted Particulate Se (mg/kg dw)	Bioavailability Fraction	TTF			Food Chain Se (mg/kg dw)			Fraction Diet			TTF Target Fish	Predicted Fish Se (mg/kg dw)	Mean Measured Fish Se (mg/kg dw)	SD Measured Fish Se (mg/kg dw)	Predicted-to-Observed Ratio
						Insects	Zoo-plankton	Fish	Insects	Zoo-plankton	Insect-ivorous Fish	Insects	Zoo-plankton	Fish					
Peamouth Chub - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	1	2.8	1.5	1.1	23.2	12.5	25.6	0.5	0.5	0	1.1	19.6	2.3	0.54	8.5
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	0.6	2.8	1.5	1.1	13.9	7.5	15.3	0.5	0.5	0	1.1	11.8	2.3	0.54	5.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.7	4547	7.5	1	1.1	0.52	1.1	8.3	3.9	9.1	0.5	0.5	0	1.1	6.7	2.3	0.54	2.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.7	5268	8.7	1	1.2	0.65	1.1	10.5	5.7	11.5	0.5	0.5	0	1.1	8.9	2.3	0.54	3.9
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	1	2.8	1.5	1.1	3.6	2.0	4.0	0.5	0.5	0	1.1	3.1	1.4	0.30	2.2
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	0.6	2.8	1.5	1.1	2.2	1.2	2.4	0.5	0.5	0	1.1	1.8	1.4	0.30	1.3
North of Elk (Kikomun Area)	50th %ile site-specific k_d & invert TTFs	0.26	4547	1.2	1	1.1	0.52	1.1	1.3	0.6	1.4	0.5	0.5	0	1.1	1.1	1.4	0.30	0.8
North of Elk (Kikomun Area)	75th %ile site-specific k_d & invert TTFs	0.26	5268	1.4	1	1.2	0.65	1.1	3.8	0.6	4.2	0.5	0.5	0	1.1	2.5	1.4	0.30	1.8
Peamouth Chub - Muscle																			
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	1.8	0.36	7.8
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	1.8	0.36	4.7
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	1.8	0.36	2.7
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	1.8	0.36	3.6
Mountain Whitefish – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0.5	0.5	0	1.4	24.0	3.5	1.1	6.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0.5	0.5	0	1.4	14.4	3.5	1.1	4.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0.5	0.5	0	1.4	8.2	3.5	1.1	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0.5	0.5	0	1.4	10.9	3.5	1.1	3.1
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	2.6	0.96	5.5
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	2.6	0.96	3.3
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	2.6	0.96	1.9
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	2.6	0.96	2.5
Rainbow Trout – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.4	31.3	3.5	1.12	8.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.4	18.8	3.5	1.12	5.4
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.4	11.2	3.5	1.12	3.2
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.4	14.1	3.5	1.12	4.0
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	1	0	0	1.4	18.5	1.6	0.35	11.6
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	1	0	0	1.4	11.1	1.6	0.35	6.9
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	1	0	0	1.4	6.6	1.6	0.35	4.1
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	1	0	0	1.4	8.4	1.6	0.35	5.2
Bull Trout - Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0	0	1	1.4	34.4	2.5	0.91	13.8
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0	0	1	1.4	20.7	2.5	0.91	8.3
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0	0	1	1.4	12.3	2.5	0.91	4.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0	0	1	1.4	15.5	2.5	0.91	6.2
Redside Shiner - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.1	24.6	3.7	1.9	6.7
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.1	14.8	3.7	1.9	4.0
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.1	8.8	3.7	1.9	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.1	11.1	3.7	1.9	3.0
Kokanee – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	1	2.8	1.5	1.1	15.4	8.3	16.9	0	1	0	1.4	11.5	1.4	0.32	8.2
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	0.6	2.8	1.5	1.1	9.2	5.0	10.2	0	1	0	1.4	6.9	1.4	0.32	4.9
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.1	4547	5.0	1	1.1	0.52	1.1	5.5	2.6	6.1	0	1	0	1.4	3.6	1.4	0.32	2.6
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.1	5268	5.8	1	1.2	0.65	1.1	7.0	3.8	7.6	0	1	0	1.4	5.3	1.4	0.32	3.7

4 Summary and Recommendations

- The USEPA's fish tissue selenium criteria are conservative and protective of fish in Koocanusa Reservoir. These criteria are based on the 5th percentile of genus mean EC10s, which is extrapolated to a concentration that is less than the white sturgeon EC10. There is less uncertainty in the egg selenium criterion (which supersedes the muscle and whole body selenium criteria) due to differences in muscle-to-egg and whole body-to-egg relationships among species, but conservatism in the criteria calculation method ensures that the muscle and whole body selenium criteria are still protective.
- With the exception of cyprinids (which are discussed in the next bullet), there have been just three exceedances of a fish tissue criterion based on over 2,800 measurements in individual fish within Koocanusa Reservoir. These include a rainbow trout and a longnose sucker ovary sample (see Figure 7), and a yellow perch muscle sample (see Figure 8). No whole body selenium concentrations have exceeded the USEPA's whole body selenium criterion (Figure 9). Based on evaluations of ovary selenium concentrations and ovary maturity discussed for cyprinids (as well as mountain whitefish), there is uncertainty in some of the ovary selenium data for other fish species, specifically the potential to over-estimate exposure due to immature ovaries. Despite this uncertainty, most ovary selenium concentration fall well below the USEPA's egg selenium criterion so this uncertainty is unimportant for most of the cases.
- Three cyprinids—northern pikeminnow, peamouth chub, and redside shiner—have had the highest ovary selenium concentrations in Koocanusa Reservoir and these concentration have exceeded the USEPA's egg selenium criterion. As shown in the northern pikeminnow study, however, the elevated ovary selenium concentrations are associated with immature ovaries as samples were not collected at time of spawning. A similar pattern appears to be observed in peamouth chub, while recent studies with redside shiner will soon provide more information on selenium bioaccumulation in redside shiner eggs and effects. These data on cyprinids in Koocanusa Reservoir, coupled with the USEPA's conclusion that cyprinids are not uniquely sensitivity based on evaluations of data from sites in the U.S. with high selenium concentrations, indicates it is unlikely that cyprinids in the reservoir are uniquely sensitive to selenium, and in fact may be relatively insensitive.
- Based on more than 2,800 empirical selenium concentrations in non-cyprinids that exceeded criteria in just three samples, along with an increasing level of understanding of selenium concentrations in cyprinids, there are currently no data to indicate that fish in Koocanusa Reservoir are at risk from selenium under current conditions. Surface water selenium concentrations in the reservoir over the period of fish selenium monitoring have ranged between 1 and 2 µg/L in the BC

portion of the reservoir downstream from the Elk River. Based on consideration of both empirical fish and surface water selenium concentrations, **it is my opinion that the USEPA's lentic criterion of 1.5 µg/L is protective of fish in Koocanusa Reservoir**. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessments consistent with the USEPA criterion.

- Additional support that the USEPA's lentic criterion of 1.5 µg/L is provided by the evaluation of alternative model inputs and the model validation relative to empirical fish selenium data. As shown in Figures 19 and in Table 4, even the least conservative model scenario evaluated (use of the 50th percentile k_d and site-specific invertebrate TTFs) consistently over-predicted measured fish selenium concentrations. This conservative model translated the whole body fish selenium criterion of 8.5 mg/kg dw to surface water selenium concentrations of:
    - 1.4 µg/L for piscivorous fish (100% fish diet),
    - 1.5 µg/L for fish with a 100% insect diet,
    - 2.1 µg/L for fish with a 50% insect and 50% zooplankton diet, and
    - 3.3 µg/L for fish with a 100% zooplankton diet.

  In my opinion, the consistent over-prediction of selenium concentrations in fish tissue by the USGS model is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.
- The USEPA's lentic criterion of 1.5 µg/L would also be protective of white sturgeon in the Kootenai River downstream of the Libby Dam. Current surface water monitoring data for the forebay and in the river downstream of the dam indicate that the selenium concentrations are similar. Egg selenium concentrations in white sturgeon collected from the Kootenai River from 2015 to 2019 have ranged from 3.0 to 5.7 mg/kg dw with an average of 4.1 mg/kg dw (n = 38). Thus, even the maximum egg selenium concentration is just 38% of the USEPA's egg selenium criterion of 15.1 mg/kg dw.

5 CLOSURE

Thank you for the opportunity to provide these comments. Please do not hesitate to contact me (DavidD@windwardenv.com; 206-812-5426) if you have any questions or if there is additional information I can provide.

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ATTACHMENT 1

Evaluation of Selenium Concentrations in Ovary of Northern Pikeminnow (*Ptychocheilus oregonensis*)

FINAL REPORT

EVALUATION OF SELENIUM CONCENTRATIONS IN OVARY OF NORTHERN PIKEMINNOW (*Ptychocheilus oregonensis*)

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Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Elk River, BC

Appendix B. Northern Pikeminnow Ovary Selenium, Muscle Selenium, and GSI Data for the Koocanusa Reservoir: 2008-2019

Appendix C. Northern Pikeminnow Metal Tissue Data: 2019

1. INTRODUCTION

Ongoing monitoring of Koocanusa Reservoir indicates ovary Se concentrations from some northern pikeminnow (NPM; *Ptychocheilus oregonensis*) collected from the Canadian side of the Reservoir were above both the 11 mg kg^{-1} dry weight (dw) egg Se guideline established by the British Columbia Ministry of the Environment and Climate Change Strategy (BC ENV, 2014) and the 15.1 mg kg^{-1} dw egg Se criteria established by the U.S. Environmental Protection Agency (USEPA, 2016). However, the sensitivity of NPM to Se is currently unknown and so the ecological risk posed by observed egg Se concentrations is uncertain. Further, historical ovary Se concentrations were collected from unripe fish (i.e., not in spawning condition) and the influence of gonadal maturation stage on egg Se concentrations is uncertain. The following presents results from a study to characterize the influence of gonadal maturation stage, fish size, and fish sampling location on ovary Se concentrations in NPM collected from Koocanusa Reservoir.

Efforts to also conduct a toxicity test evaluating the effects of maternally transferred Se on NPM embryo-larval development were unsuccessful in 2019 due to the inability to collect a sufficient number of female fish in spawning condition. As such, this test is not discussed further in this report.

2. BACKGROUND

Northern pikeminnow are distributed throughout the Pacific drainages as far north as the Nass River drainage in BC, Canada to the Columbia River drainage in the U.S. They are most common along sandy, cobble, gravel, boulder or bedrock shorelines during summer and deeper waters during winter (Scott and Crossman 1973, Coker et al. 2001). Northern pikeminnow are late spring-summer spawners, typically spawning when water reaches 14-18 °C with males generally present in larger congregations on breeding grounds over gravel and cobble shallows (Gadomski et al., 2001). Females may have multiple spawning bouts with more than one male throughout the season. Eggs hatch after 8-10 days at 15-17 °C (Coker et al., 2001; Gadomski et al., 2001; Scott and Crossman, 1973).

Koocanusa Reservoir was formed by Libby Dam, located 30 km northeast of Libby, Montana at river mile 221.9 of the Kootenai River[1]. The reservoir is 145 km long, of which 68 km are in BC, Canada. The predominant source of water to the reservoir is the Kootenay River, of which the Elk River is a tributary. Northern pikeminnow are resident to Koocanusa Reservoir and have been sampled for ovary and muscle Se in BC, Canada and Montana (MT), U.S. over the last 11 years.

1. The Kootenay River is referred to as the 'Kootenai River' in the U.S.

Monitoring data indicate NPM ovary Se concentrations on the MT side of the reservoir have low variability within and across sampling years compared to fish collected from the BC side of the reservoir (Figure 1). Some fish collected on the BC side of the reservoir are above both the BC ENV guideline and the USEPA fish egg Se criteria of 11 and 15.1 mg kg^{-1} dw, respectively. These data also indicate ovary Se concentrations in fish collected from the BC side of Koocanusa Reservoir appear to be significantly ($p<0.05$) higher than those collected from the U.S. side.

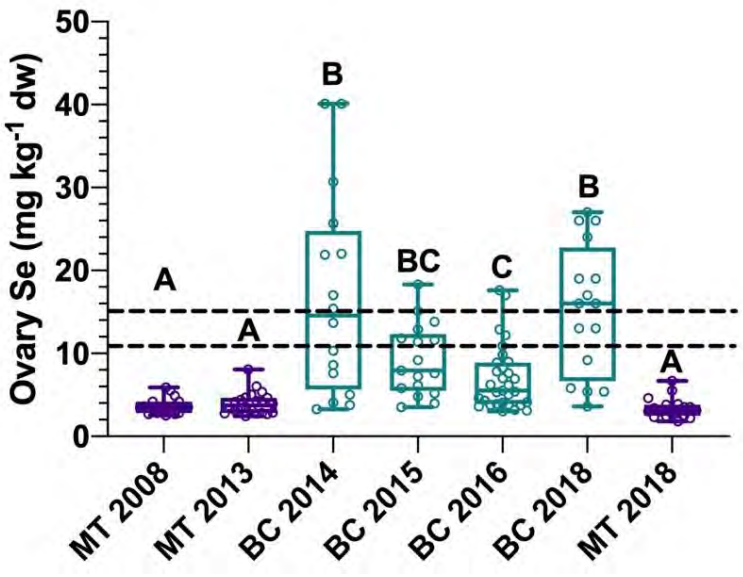


Figure 1. Ovary Se concentrations on the Montana (MT) and British Columbia (BC) sides of Koocanusa Reservoir (2008-2018). Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines. Different letters indicate significant differences ($p<0.05$).

There are several potential biases in the data collected to date that complicate the interpretation of differences in NPM ovary Se data. First, NPM typically reach spawning condition when they have a gonado-somatic index (GSI) of 8-12% (Gray and Dauble, 2001; Petersen and Ward, 1999). While GSI data are not available for fish caught in MT, only a single female on the BC side of the reservoir has been collected with a GSI in this range. The impact of collecting unripe ovaries on observed ovary Se concentrations is unknown, but much of the variability in ovary Se concentrations in the existing BC data is associated with a GSI <2%. Further 55% of ovaries collected from fish with a GSI <2% are above the BC ENV egg Se guideline of 11 mg kg^{-1} dw, while only 7% of ovaries collected from fish with a GSI >2% are above this guideline (Figure 2).

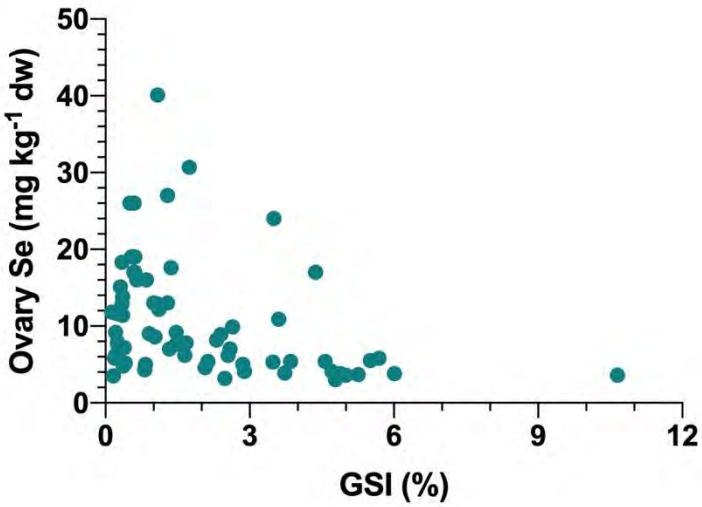


Figure 2. Relationship between ovary Se and GSI for northern pikeminnow collected on the BC side of Koocanusa Reservoir.

Second, there is a significant relationship between fish size and ovary Se concentrations (Figure 3A), and fish collected on the BC side of the reservoir tend to be smaller than those collected in MT (see Figure 3B). Collection of smaller fish on the Canadian side of the reservoir may be the result of sampling bias as fish collection has been restricted to angling, while on the MT side of the reservoir fish are collected using gill nets.

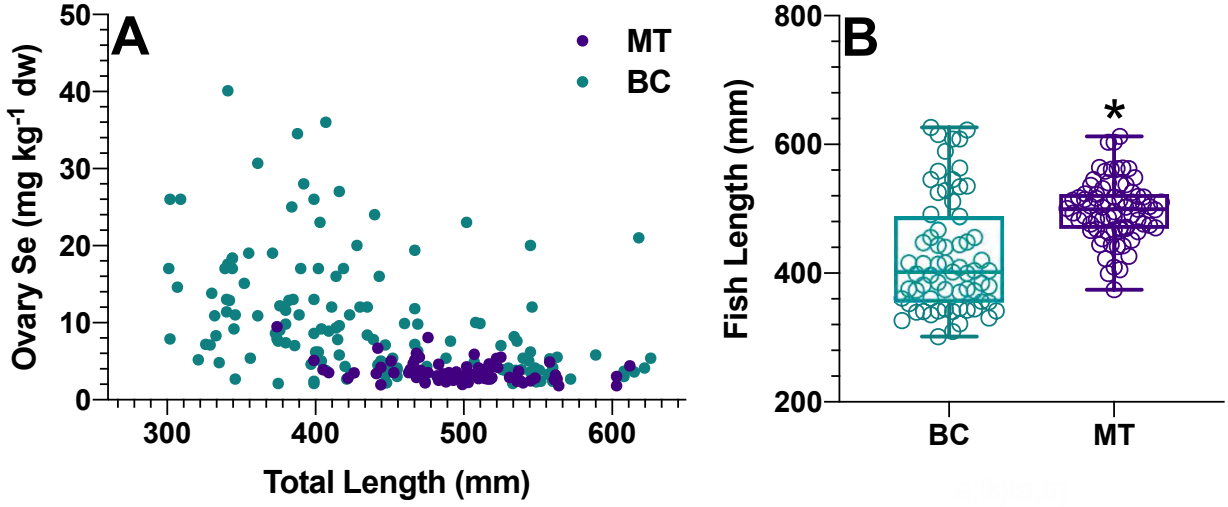


Figure 3. Relationship between ovary Se and fish length (A) and fish length distributions (B) for northern pikeminnow collected from Koocanusa Reservoir. Box plots represent mean, quartiles, maximum and minimum values. * = significant difference in the MT fish length compared to the BC fish lengths ($p<0.001$).

Overall, these observations suggest data collected to date on NPM ovary Se concentrations may be biased. However, this conclusion is uncertain due to the lack of ovary Se data in ripe fish, along with associated size and GSI data. Regardless of potential biases in historical ovary

Se sampling, the sensitivity of NPM to maternally transferred Se is not known. The original objectives of this study were to address both of these uncertainties.

3. OBJECTIVES AND STUDY DESIGN OVERVIEW

This study originally had two objectives:

1) To determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations; and
2) To evaluate the relationship between ovary Se concentrations and ovary development, fish size, and sampling location.

As discussed above, the inability to collect a sufficient number of female fish in spawning condition resulted in the first objective not being achieved. However, the extended effort to collect female fish in spawning condition lead to the collection of a large number (n=79 on the BC side of the reservoir) of samples for ovary Se analysis in support of the second objective. To achieve the second objective, the study had the following key elements:

1) Prior to NPM reaching spawning condition, unripe ovaries/eggs and muscle were collected and GSI measured in sexually mature females (30-60+ cm) to provide information on the relationships between ovary Se, GSI, fish size, and sampling location. As described earlier, historical data indicated fish size and sampling might influence egg Se concentrations, though these potential relationships may be confounded by other variables. The home range of NPM within the reservoir is unknown and so the extent to which ovary Se may reflect exposure to local Se sources (e.g., the Elk River) is also unknown. The developmental stage of a subset of ovaries were also characterized using histological techniques.
2) Attempts were made to collect a gradient of egg Se concentrations from ripe fish by collecting adult NPM of varying size (30-60+ cm) from several locations in Koocanusa Reservoir. This was ultimately unsuccessful but led to the collection of an increased number of ovary samples for Se analysis.

4. FIELD SAMPLING

Details of the field sampling strategy and methods employed are provided in the NPM Study Plan (EcoTox et al., 2019) and summarized here.

4.1 Sampling Strategy

There were originally two phases to the NPM field sampling program. In Phase 1 (beginning June 14, 2019), female NPM were collected from the BC side of the reservoir prior to reaching spawning condition to further characterize the effects of GSI, fish size, and sampling location on ovary Se concentrations as well as monitor spawning condition of the fish. Phase 2 sampling was

intended for once NPM reached spawning condition, and would involve collecting both male and female fish for the Se toxicity study. As only a few ripe fish were collected, Phase 2 sampling was never realized.

Although not explicitly part of this study, there was also an additional NPM sampling effort on the Montana side of the reservoir. In this effort (May 15, 2019), 15 female NPM were collected from Rexford in collaboration with personnel from Montana DEQ. This effort was made to ensure GSI data were collected and they represent the only fish from the Montana side of the reservoir for which GSI data are available.

Mature NPM were collected from various locations in Koocanusa Reservoir (BC side) using multiple sampling methods, consistent with scientific fish collection permit conditions and detailed in the NPM Study Plan (EcoTox et al., 2019). Six locations in Koocanusa Reservoir were initially identified in the study plan, but ultimately 10 locations were sampled in an attempt to collect additional females in spawning condition for the Se toxicity study (Figure 4). Sampling in these areas focused on inlets based on the assumption that NPM would congregate in these areas prior to moving upstream to spawn.

4.2 Sampling Methods

Northern pikeminnow were captured using multiple methods subject to and consistent with fish collection permit conditions. Short-set gill nets (starting with a maximum set time of 20 minutes) were used to reduce fish mortalities (Buchanan et al., 2002). Gill netting was anticipated to be the most efficient capture method and both cotton and monofilament 3-5” mesh nets were deployed. Short set times were used to avoid stress to both NPM and by-catch, particularly as species of concern, bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Onchorhynchus clarki lewisi*) are present in the reservoir. Three foot-diameter hoop nets were deployed and left to fish overnight (i.e., approximately 24 h). Cod pot traps were an additional capture method used, but not originally considered in the study plan. They function similarly to a minnow trap but on a larger scale (65” long x 40” wide, with 4” opening). These traps sit on the bottom substrate similar to the hoop net but sample a smaller area. Cod traps are quicker to deploy and pull; but are more difficult to transport as the metal frame cannot be collapsed. Similar to hoop nets, these traps were deployed and left to fish overnight (approximately 24 h). Angling was conducted from sampling boats. Angling was mainly employed between gill net sets as it has much lower catch per unit effort (CPUE) and often targets smaller individuals. Angling was also employed to scout the lower Elk River below the Elk River bridge at Kootenay Hwy 93. All fish captured were identified to species, enumerated, and all non-target individuals were released alive at the point of capture.

Northern pikeminnow sampled during Phase 1 were sacrificed by a decisive blow to the head. Fish processing and handling for tissue sampling was consistent with provincial guidelines (BC ENV, 2016).

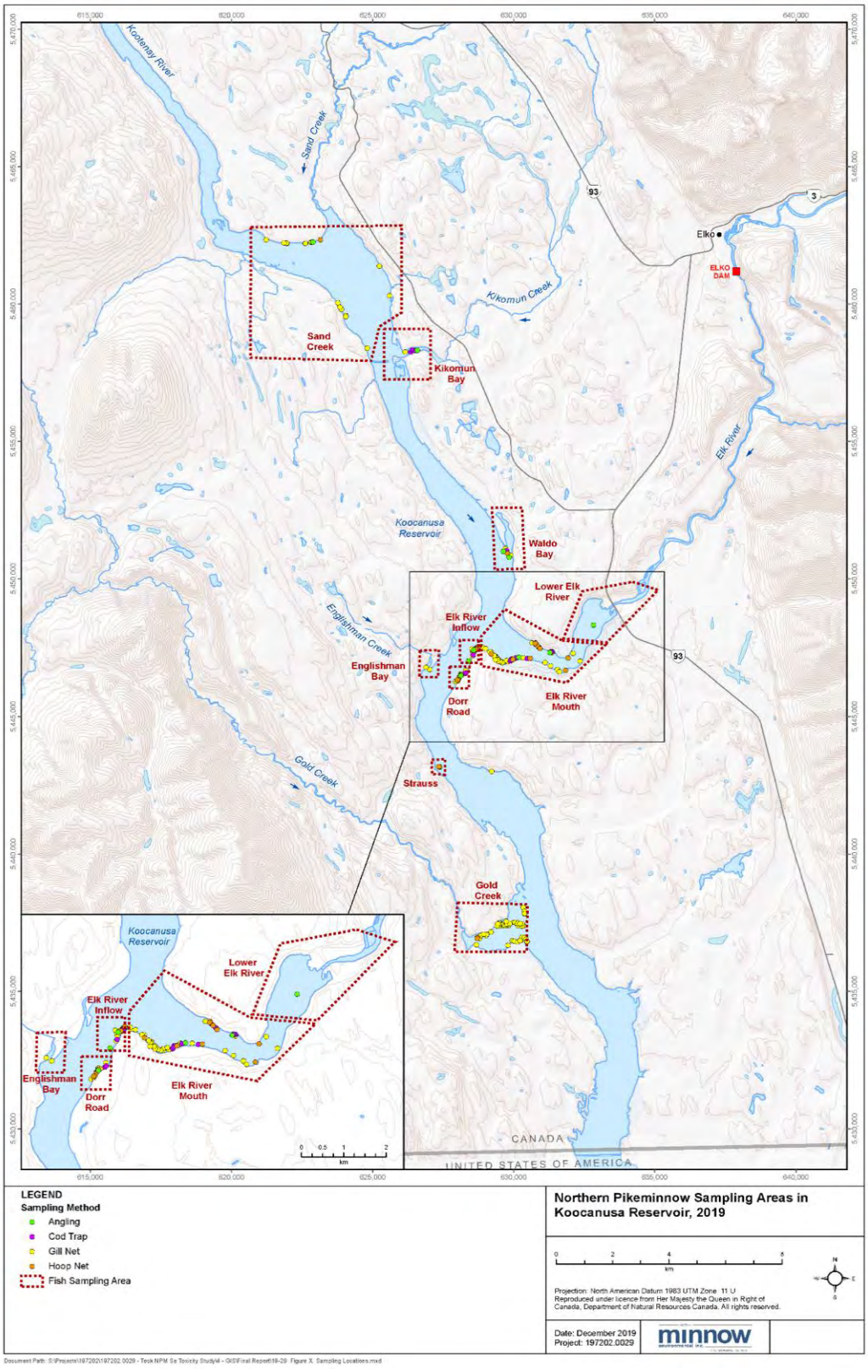


Figure 4. Northern Pikeminnow Sampling Areas on the Canadian Side of Koocanusa Reservoir.

Fish were kept on ice in coolers and transported to a dedicated field laboratory for processing as soon as possible following capture (i.e., within hours). Fork and total lengths and body weights were measured. Each fish was opened and the sex and/or sexual maturity recorded. Whole gonads and livers were removed from each fish and weighed to the nearest milligram using an analytical balance to allow for calculation of gonado-somatic indices. Whole ovaries were collected from each female and placed in separately labelled, polyethylene (Whirl-Pak®) bags. A skinless, boneless muscle fillet sample was also collected from each fish to provide supplemental data on muscle Se concentrations. Following these measures, age structures (i.e., otoliths) were removed from each fish. Each age structure was wrapped separately in waxed paper and placed inside a labelled envelope and archived for analysis. Internal or external deformities, erosions (fin and gill), lesions, or tumours (DELT) observed during processing (Sanders et al., 1999) and parasites were recorded. Tissue samples (ovaries, muscle, and age structures) were stored frozen pending shipment to the respective laboratory for analysis.

Mature female NPM retained for gonad and muscle collection were weighed and measured for total and fork length. Obvious external deformities, erosions (fin and gill), lesions, and tumours (i.e., DELT survey) and parasites observed during processing were recorded.

Ovary and muscle samples were all sent to Saskatchewan Research Council (SRC) in Saskatoon, SK for chemical analysis.

4.3 Permits

A permit for fish collection was obtained from the BC Ministry of the Environment and Climate Change Strategy (BC ENV *Application to Collect Fish for a Scientific Purpose*) and an additional permit was obtained for transport of eggs to the University of Saskatchewan (UoS) facility in Saskatoon, SK from Fisheries and Oceans Canada (License #119412), BC ENV (License #119412) and the Government of Saskatchewan (SK Import #2019-16).

5. LABORATORY METHODS

5.1 Ovarian Histology

All methods for histology preparation followed the UoS Toxicology Centre's draft standard operating procedure for histology (Appendix A). Field-collected NPM were dissected at the field laboratory and gonads excised, weighed and then immediately preserved in 10% buffered formalin. After 24 hours samples were transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming

table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

As, to the best of our knowledge, no previous studies have characterized NPM gonads histologically, oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish (OECD, 2009). Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

5.2 Analytical Chemistry

Ovary and muscle samples collected in the field were submitted for chemical analysis at SRC in Saskatoon, SK. In addition to Se, ovaries, eggs, and muscle were analyzed for 24 other elements (listed in Table 5 of the Study Plan). Results for these other elements are provided in Appendix C but are not discussed further in this report. Samples were analyzed using high resolution inductively coupled plasma mass spectrometry (HR-ICPMS). The detection limit for Se was 0.01 $\mu g\ g^{-1}$ dw. Moisture content was measured by freeze drying and results were reported on a dry weight basis along with moisture content to allow conversion to wet-weight values.

Standard quality control procedures for sample analysis were included as detailed in the Study Plan (EcoTox et al., 2019).

6. RESULTS

6.1 Fish Sampling

Four different methods were employed to capture NPM during the study: hoop nets, cod pot traps, gill nets and angling. The fish sampling was separated into 2 phases. The goal of Phase 1 was to sample approximately 36 sexually mature females of varying sizes and ranges of gonadosomatic index (GSI) values, with half being from sites directly influenced by the Elk River. This phase also allowed tracking of spawning condition within the population. When ripe fish were initially collected, Phase 2 sampling was initiated to focus on collecting fish for fertilization and assessment of larval deformities as a function of egg Se concentrations.

Different sampling methods had varying degrees of success in catching mature females and CPUE changed through the sampling period (Table 1). Monofilament gill nets were more successful than cotton mesh gill nets so after approximately two weeks remaining gill net sampling only used 3” and 4” monofilament nets. Overall, gill nets were the most successful capture method for mature females over the longest sampling period. Though gill nets had high

incidence of bycatch, survival rates were high (4 mortalities over 92 hours of effort in 9 sample areas) due to short set times. Elk Mouth, Elk Inflow and Gold Creek sites were sampled with greatest effort over the longest periods of time in response to capture success rates. Gold Creek and Elk Mouth, both at locations of tributary inflow, had the highest CPUE through the last weeks of June and tended to decrease through July. Elk Inflow area, where the Elk tributary inflow opens into the reservoir, had peak CPUE through mid-July then drastically declined moving into the last two weeks of July (Table 2).

Table 1. Female northern pikeminnow CPUE and total effort (hours) by gear type and sampling area.

CPUE	Dorr Rd.	Elk Inflow	Elk Mouth	Lower Elk	Sand Cr.	Gold Cr.	Englishman Bay	Strauss	Waldo Bay	Kikomun Bay	Total Gear CPUE	Total Gear Effort
Gill Net	5.24	60.00	55.34	-	7.78	136.61	0.00	8.11	0.00	-	273.08	
Gill Effort	7.68	18.00	19.47	-	10.85	31.45	1.70	1.50	1.20	-		91.85
Hoop Net	20.20	0.04	1.47	-	0.17	0.04	-	0.04	0.34	-	22.30	
Hoop Effort	596.25	67.68	492.08	-	46.43	93.50	-	95.18	117.37	-		1508.50
Cod Trap	0.43	0.19	0.25	-	-	-	-	-	0.00	0.04	0.91	
Cod Effort	238.43	237.76	420.62	-	-	-	-	-	24.00	99.42		1020.23
Angling	0.25	4.00	0.00	0.00	0.67	-	-	-	0.00	0.00	4.92	
Angling Effort	4.00	9.83	0.07	6.00	1.50	-	-	-	0.66	1.00		23.06
Total CPUE	26.12	64.23	57.06	0.00	8.62	136.65	0.00	8.15	0.34	0.04	**301.21**	
Total Effort	846.36	333.27	932.24	6.00	58.78	124.95	1.70	96.86	143.23	100.42		**2643.64**

Table 2. Gill net CPUE at most successful sample areas through the sampling period.

CPUE	Elk Inflow	Elk Mouth	Gold Creek
June	18.00	35.20	62.71
July 1-7	Not sampled	15.00	57.40
July 8-14	128.96	5.14	Not sampled
July 15-21	9.50	0.00	8.05
July 22-26	0.00	0.00	8.44

When sampling commenced many mature females had higher GSI than anticipated and there was difficulty capturing low GSI/large individuals and high GSI/small individuals. More fish were processed in an effort to capture the desired range of GSI and size. As the field season progressed and few ripe fish were captured, more fish were processed than originally anticipated with a total of 79 fish processed by end of the study (Table 3). This allowed inclusion of a greater GSI and size range as well as a range of egg development for histology analysis (15 fish). The high GSI/small size categories were eventually captured at Elk influenced sites but not at other sites (Table 3 and Figure 5).

Table 3. Fish GSI and total length for Rexford, Elk River and all other sites combined.

GSI/SIZE	300-400 mm	401-500 mm	501-600+ mm
Rexford, MT (n=15)			
<2%	2	6	0
2-4%	0	0	7
4-7%	0	0	0
7+%	0	0	0
Elk River, BC (n=49)			
<2%	3	6	1
2-4%	3	3	2
4-7%	2	3	3
7+%	3	11	9
Other Sites, BC (n=30)			
<2%	9	6	1
2-4%	0	2	1
4-7%	1	2	1
7+%	0	3	4

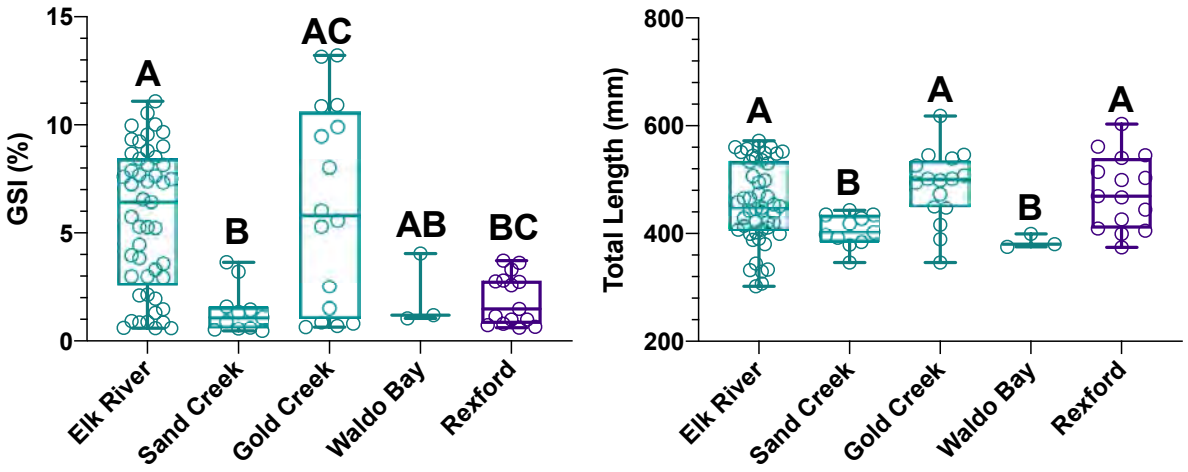


Figure 5. Northern pikeminnow GSI and total length by location for samples collected in 2019. Different letters indicate statistically significant differences ($p<0.05$) as determined by ANOVA.

6.2 Ovarian Histology

Between July 8 and 19, 2019, ovaries from 15 NPM were collected for histological analysis of maturation stages of oocytes across fish of different sizes and reproductive development (weight: 250 – 1800 g; fork length: 33.2 – 61.8 cm) and GSI (range: 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (Figure 6 and Table 4-1 in Appendix A). While there was no obvious relationship between the size of fish and GSI, there was a clear correlation between GSI and ovarian maturation stage (Figure 7) with fish having GSI >5% all being at oocyte maturation stage 3, with one exception. Similarly, there was a significant linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($r^2 = 0.81$), revealing that ovaries of mature fish with a GSI > 5% consisted of over 50% LVOs (Figure 6).

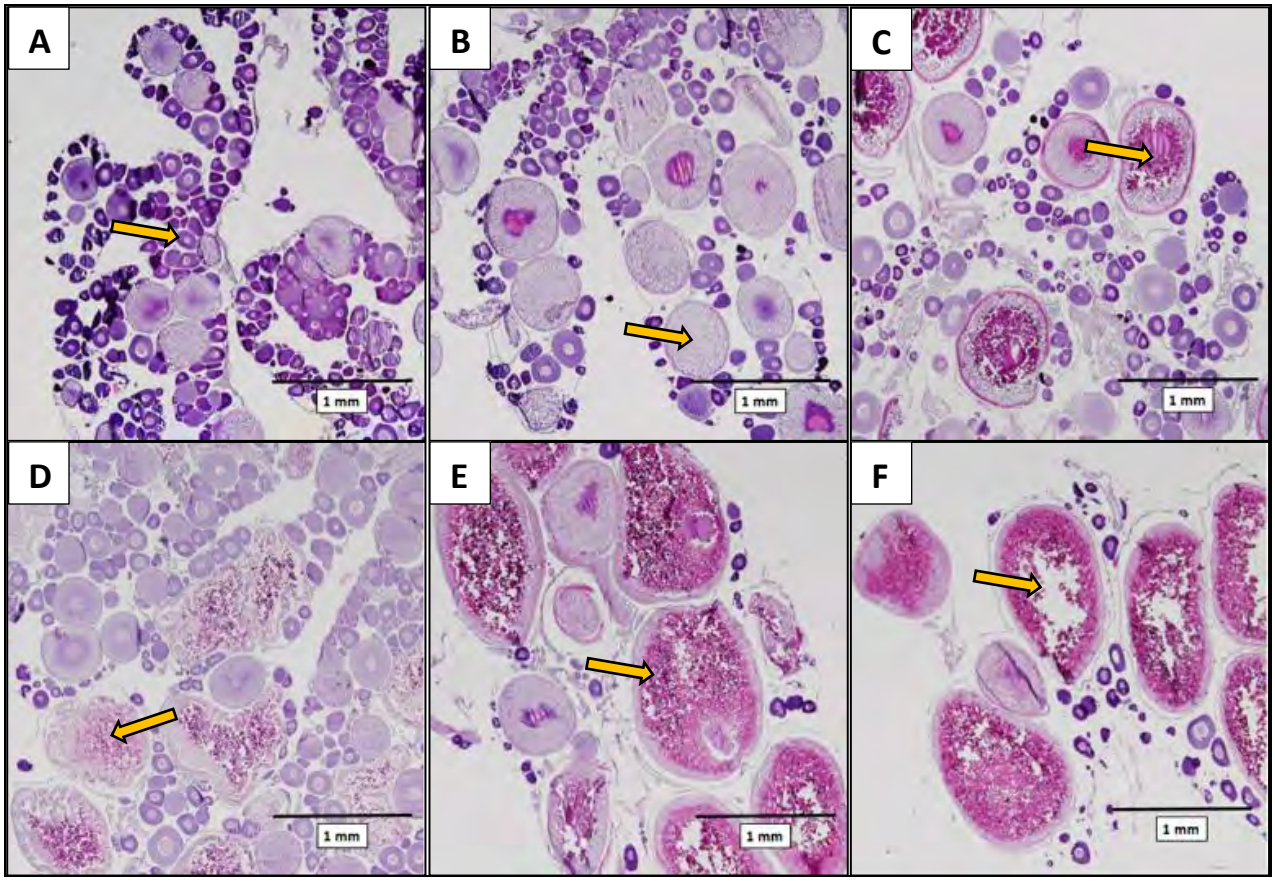


Figure 6. Histomicrographs of ovaries of northern pikeminnow representing early development stages. (Stage 1) consisting mainly of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E and F; Arrows).

Multivariate Analysis of Ovary Se Data

Analysis of historical ovary Se data for NPM suggests there are significant relationships with sampling location, fish size, and GSI (Figures 1-3). However, the historical data set lacks information on GSI for fish from the Montana side of the reservoir, may be confounded by correlations between fish size and GSI, and is limited for fish with a relatively high GSI (>5%). The sampling program for this study was designed to address these limitations and provide a robust dataset for evaluating the influence of multiple factors on ovary Se concentrations in NPM.

Historical sampling data (collected 2013-2018) were collated with data collected from 2019 (Appendix B). An initial exploratory analysis of natural log (ln)-transformed data was conducted by Principal Component Analysis (PCA) (prcomp, R) using z-scores of independent variables (total length, GSI, body weight, and gonad weight) to identify correlations among these variables and select the most appropriate variables for linear

modeling. The first two axes of the PCA with four input variables explained 99% of the variance in the four variables. Bivariate relationships among independent variables, and bivariate relationships between ovary Se and independent variables were plotted by area and year to help visualize effects of area and year on relationships. Natural log (ln) transformations of total length and body weight were highly correlated (R = 0.98), and a biplot from the first PCA with all four variables showed very similar relationship between body weight and total length and final PC scores. Because GSI includes body weight in its denominator, and body weight and total length were highly correlated, body weight was removed from the independent variables used in the MLR and total length was used as a measure of fish size in the model to reduce collinearity (Figure 8).

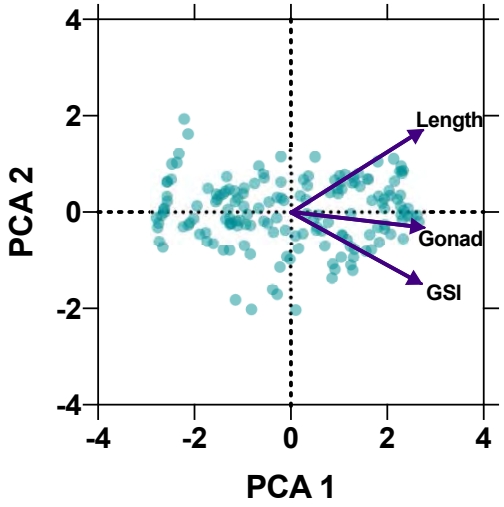


Figure 8. Biplot from PCA using z-scores of ln-transformed total length, gonad weight, and GSI.

After selecting initial independent variables (length, gonad weight, and GSI), exploratory linear and multiple linear regressions (MLR) were conducted to predict ln ovary Se for various subsets of the data. For example, models using one or more independent variables were developed for data sets for individual years, different combinations of years, individual locations, and different combinations of locations. These exploratory analyses were intended to gain a better understanding of how the data were distributed as a function of the independent variables, location, and sampling year. Based on these exploratory analyses, we concluded that the initial models should be developed using only the 2019 data because these data had been collected with a more balanced design of GSI and fish size classes than earlier data. Developing an initial model based on data from multiple years could introduce biases due to the incomplete sampling design with respect to the independent variables being evaluated.

The first model was developed to test for differences between area-specific slopes and intercepts with stepwise analysis using Bayesian Information Criterion (BIC) and to identify final models (Eq. 1). The contrasts used to test for area-specific intercepts tested for differences between Elk influenced sites and other sites.

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) +

Ln(GW) + area* Ln(GW) (Eq. 1)

where, OvSe = ovary Se, TL = total length (cm), GSI = gonadosomatic index, and GW = gonad weight. Variance inflation factors (VIFs) were relatively high for this model (>7) (Zuur et al., 2010) and gonad weight was not retained in the BIC version of the model, so gonad weight was removed and a second model was developed (Eq. 2).

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) (Eq. 2)

Area-specific slopes were not retained in the BIC model, resulting in a final model with area-specific intercepts and pooled slopes. Exclusion of area-specific slopes means that relationships between independent variables (total length and GSI) and ovary Se are statistically similar between sites. Retention of area-specific intercepts indicates that while differences in fish size and GSI between sites explains some of the observed differences in ovary Se concentrations, there are also statistically significant differences in ovary Se concentrations between some sites independent of the influence of fish size and GSI. This model performed reasonably well in terms of predicting ovary Se concentrations for the data on which it was based (Adj. R^2 = 0.72; Figure 9). Further, the predicted R^2 of 0.69 is just slightly lower than the adjusted R^2 of 0.72, indicating the model is not over-parameterized or unduly influenced by individual data points.

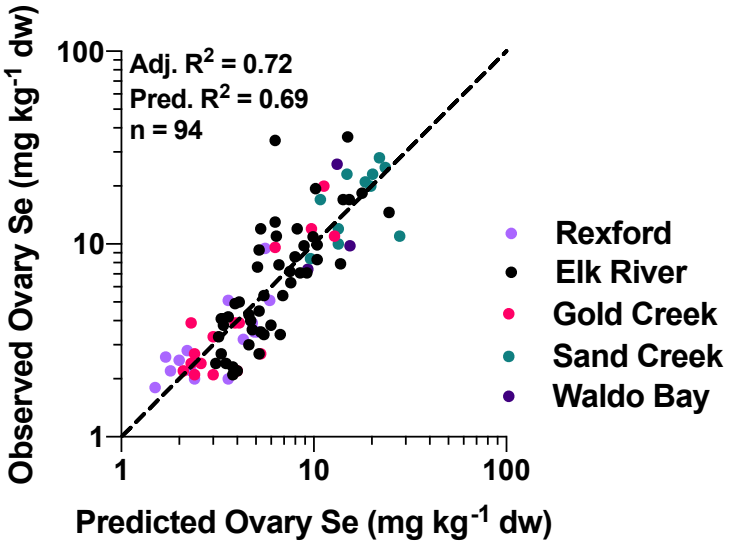


Figure 9. Ovary Se MLR model based on 2019 data only (see Eq. 2).

Once the MLR model based on data from 2019 only was developed, data from years prior to 2019 were then evaluated using the 2019 MLR model. Exploratory analyses indicated that data from 2014 and 2015 did not fit the model well. The majority of samples for 2014 were collected in February with the remaining 2014 samples and all 2015 samples collected in April. As would be expected given the sampling times, GSI was low in both data sets. The 2015 data set in particular consisted of fish with GSI <0.5%, which appears to introduce non-linearities into the overall relationship between GSI and ovary Se (Figure 10). Consequently, we opted to exclude the 2015 data from further analysis. It may have been possible to include the 2014 data set in the model, but given the limited amount of data (n=5) and limited ranged of GSI, we opted to exclude it from the analysis as well. Consequently, a model was fit using data from 2016-2019. The full model included "year" as a term to test for differences in ovary Se concentrations between years and as before was evaluated using BIC to select the most parsimonious variables for inclusion (Eq. 3).

$$\text{Ln(OvSe)} = \text{area} + \text{year} + \text{Ln(TL)} + \text{area*}\ \text{Ln(TL)} + \text{Ln(GSI)} + \text{area*}\ \text{Ln(GSI)} \quad \text{(Eq. 3)}$$

The final model selected by BIC using all data from 2016-2019 (n=141) retained the same variables as the model using only 2019 data with only slight differences in the model coefficients (Table 4). Adjusted and predicted R^2 for the BIC model were 0.67 and 0.65, respectively (Figure 11). The model tested for effects of year and area as well as area-specific slopes. Again, area-specific slopes were not retained in the BIC model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (ovary Se) across sites. Similarly, year was not retained as a factor in the model indicating there were no significant differences across the three sampling years (2016, 2018, and 2019) included in the analysis. Significant differences in area-specific intercepts were observed and retained in the model. The intercepts for both Gold Creek and Rexford were significantly lower than the Elk intercept ($p = 0.01$ and $p < 0.01$, respectively) indicating that after accounting for the influence of fish length and GSI, ovary Se concentrations in fish collected from Gold Creek and Rexford were significantly lower than those observed for fish collected near the Elk River (Table 4).

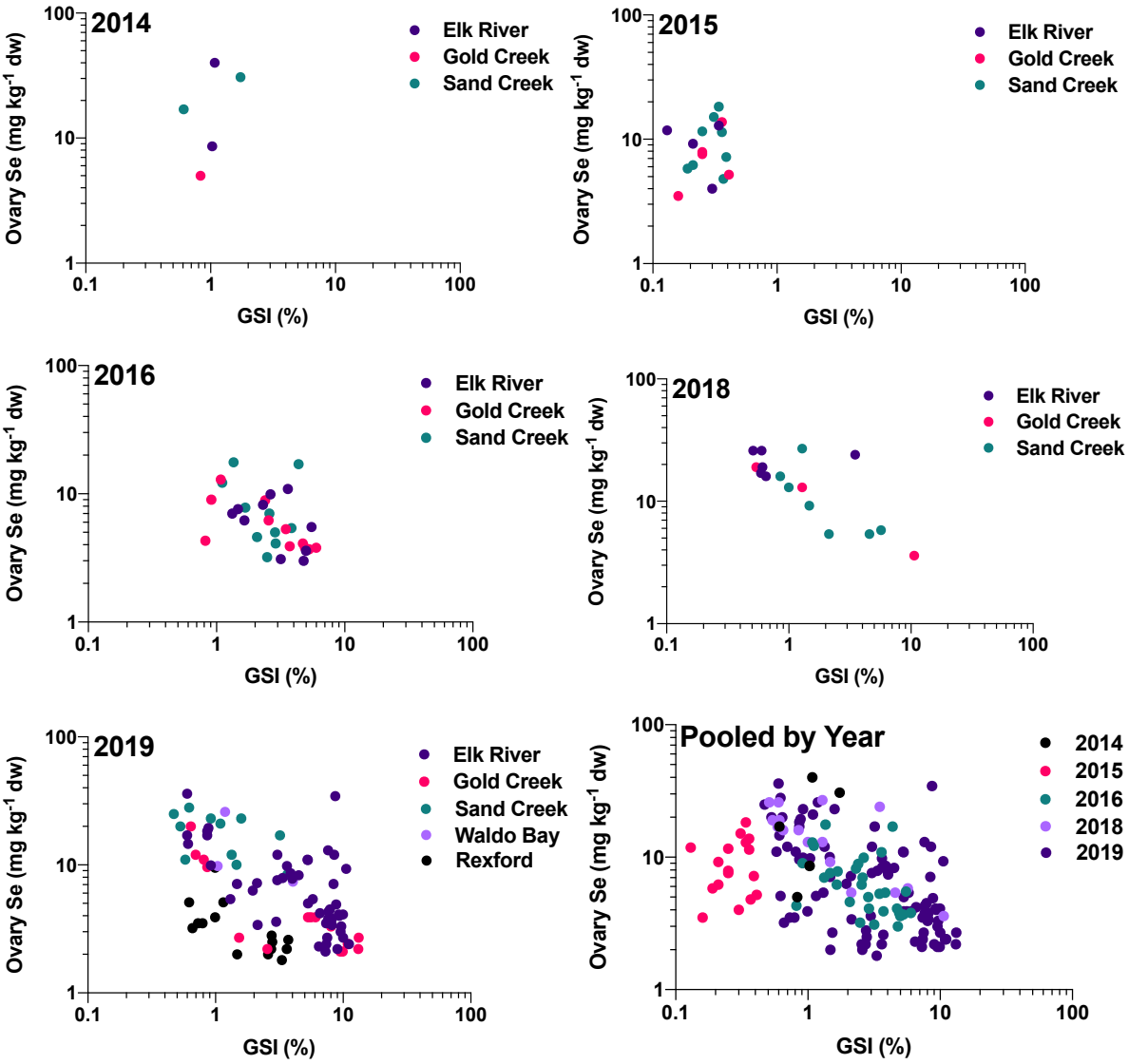


Figure 10. Relationship between GSI and Ovary Se by Year

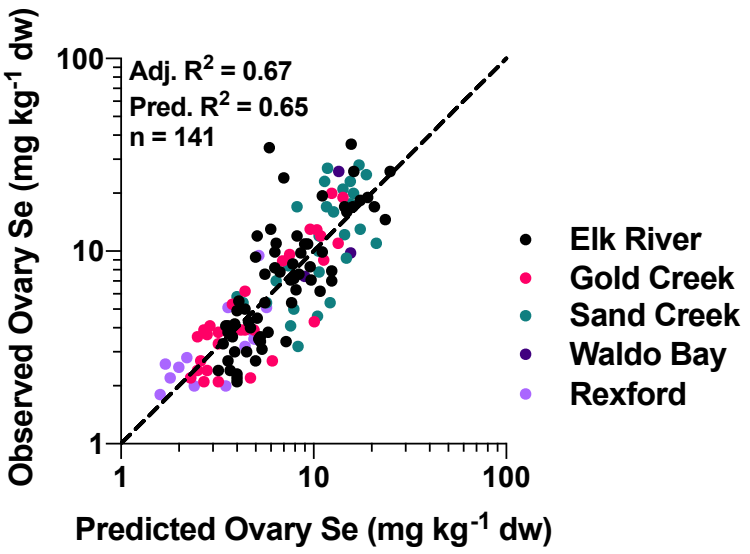


Figure 11. Final Ovary Se MLR model based on 2016-2019 data.

Table 4. Final ovary Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	7.94	0.96	8.31	-	
	Gold	7.66	0.10	-2.81	0.01	
	Sand	7.98	0.10	0.45	0.65	
	Waldo	8.02	0.26	0.33	0.75	
	Rexford	6.91	0.13	-7.62	<0.01	
Slopes	Ln Total Length	-1.45	0.26	-5.62	<0.01	-0.289
	Ln GSI	-0.39	0.05	-8.08	<0.01	-0.493

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that GSI (standardized slope = -0.49) has a stronger effect on ovary Se concentrations in the model than total length (standardized slope = -0.33) over the range sampled for these variables (Table 4). Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model appear to have equal variance (p = 0.145) but may not be normal (p = 0.031).

One potential caveat to this model is that the PCA analysis indicates a level of correlation between total length and GSI, as both variables have positive associations for PC1, though opposite associations for PC2 (Figure 8). A simple correlation analysis indicates these two variables are somewhat correlated (r=0.41; Figure 12). This correlation is primarily caused by the lack of data for fish with a total length >54 cm and GSI <3%. This observation is supported by the lack of a significant correlation (p >0.05) between total length and GSI for fish with a GSI >3%. It is unclear whether this data gap is due to sampling bias or some

mechanistic reason why fish in this category are not observed, though the former seems more likely. Regardless, this correlation introduces some uncertainty into the ovary Se model. While VIFs for the model were low (1.4 for both total length and GSI) suggesting the correlation is not unduly influencing the model, the full influence of this correlation can be difficult to characterize.

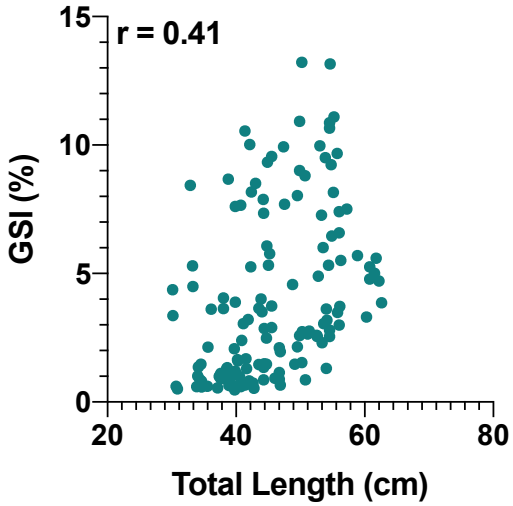


Figure 12. Correlation between total length and GSI in northern pikeminnow (2016-2019).

We further evaluated this issue by constraining the data set to only those data with GSI >3% (n = 69) where there is no correlation between fish length and GSI and re-parameterized the model. The resulting MLR model still retained both GSI and total length as variables (Adj. $R^2 = 0.55$), again supporting the premise that both variables are important predictors of ovary Se. However, the standardized model coefficients changed with total length (-0.49) now more important than GSI (-0.35). This reversal in relative importance of standardized model coefficients may simply be the result of constraining the original data set by ~50% or it could be an indication that the correlation between total length and GSI in the full data set is influencing the way variance is partitioned in the model.

Ultimately, the uncertainties associated with the correlation between total length and GSI appear to have relatively modest impacts on model predictions of ovary Se. Based on the final model using the full data set, differences in area-specific intercepts between sites would translate to predictions of ovary Se concentrations being, on average, 2.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold Creek and Elk River ovary Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.3 times higher than those from Gold Creek for a given fish length and GSI. Estimated mean ovary Se concentrations for small (30 cm) and large (60 cm) females with a

GSI of 6% (a conservatively low GSI for female fish ready to spawn) are below the BC ENV egg Se guideline (11 mg^{-1} kg dw) at all sampling locations using the MLR model based on the full data set. In comparison, the MLR model based on the data set constrained to a GSI >3% (i.e., the data set with no correlation between total length and GSI) provides higher estimates of mean ovary Se for small (30 cm) fish (Table 5), but estimates are generally within 30% of those using the MLR model based on the full data set. The somewhat larger increase in estimated mean ovary Se for Sand is the result of a higher intercept using the constrained data set.

Table 5. MLR model estimated mean ovary Se concentrations in female northern pikeminnow collected from different locations in Koocanusa Reservoir as a function of fish size and GSI. Estimated provided for the MLR model based on all data and the model based only on data where GSI was >3%.

Site	Fish Length (cm)	GSI (%)	Estimated Mean Ovary Se (mg kg^{-1} dw) All Data	Estimated Mean Ovary Se (mg kg^{-1} dw) Data with GSI >3%
Elk River	30	6	10.1	11.6
	60	6	3.7	3.5
Gold Creek	30	6	7.6	9.3
	60	6	2.8	2.8
Sand Creek	30	6	10.5	15.0
	60	6	3.8	4.6
Waldo Bay	30	6	10.9	9.0
	60	6	4.0	2.7
Rexford	30	6	3.6	5.0
	60	6	1.3	1.5

6.4 Multivariate Analysis of Muscle Se Data

Concurrent with ovary sampling, muscle samples have also been collected and analyzed for Se concentrations. The muscle Se data is a potential second line of evidence to support the observations and conclusions from the ovary Se analysis. As has been demonstrated in other species (USEPA, 2016), ovary Se and muscle Se concentrations in NPM are correlated (Figure 13). Consequently, observations based on ovary Se concentrations regarding the effects of fish size and sampling location are expected to also be observed for muscle Se data. While there is no identified mechanistic link between GSI and muscle Se concentrations, it is possible a correlation between GSI and muscle Se might be observed given the correlations between ovary Se and muscle Se, as well as total length and GSI in the data set.

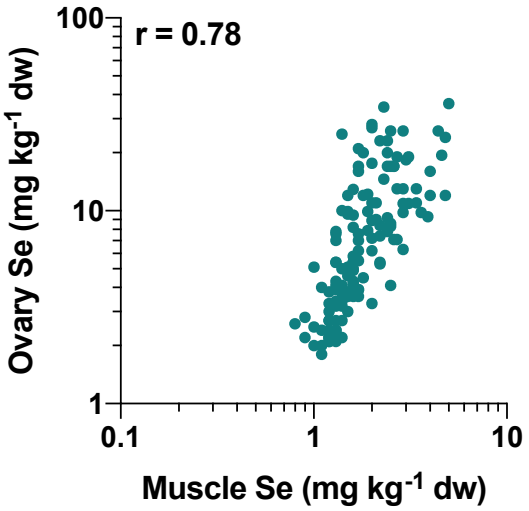


Figure 13. Correlation between muscle Se and ovary Se concentrations in NPM collected from Koocanusa Reservoir (2016-2019).

The multivariate analysis of muscle Se data used the same general multivariate approach described above for ovary Se data. The same data used in the ovary Se analysis was used in the muscle Se analysis for comparability except for a single fish collected from the mouth of the Elk River in 2016 for which no muscle data were collected (n = 140). Given the results of the ovary Se analysis, an initial model using only the 2019 data was not developed for muscle. Instead, the full data set (2016-2019) was used to evaluate the same general full model:

Ln(muscle Se) = area + year + Ln(TL) + area* Ln(TL) + Ln(GSI) +

area* Ln(GSI) (Eq. 4)

The model selected by BIC retained both total fish length and GSI as variables. Adjusted and predicted R^2 for the BIC muscle Se model were lower than for the ovary Se model at 0.47 and 0.45, respectively (Figure 14). The lower performance of the muscle Se model appears to be driven by underprediction of the relatively high muscle Se data for fish collected from the mouth of Elk River, although area-specific slopes were not retained in the model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (muscle Se) across sites (Table 6). Significant differences in area-specific intercepts were identified in the model. The intercepts for Gold Creek, Sand Creek and Rexford were all significantly lower than the Elk intercept ($p < 0.01$) indicating that after accounting for the influence of fish length and GSI, muscle Se concentrations in fish collected from all three locations were significantly lower than for fish collected near the Elk River (Table 6). Based on the final model, differences in area-specific intercepts between sites would translate to predictions of muscle Se concentrations being, on average, 1.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold and

Sand Creeks versus Elk River muscle Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.2-1.3 times higher than those from Gold and Sand Creeks for a given fish length and GSI.

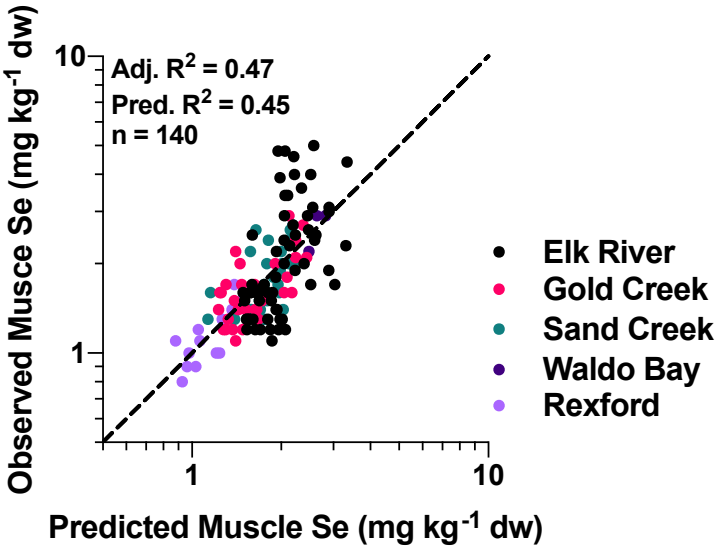


Figure 14. Final Muscle Se MLR model based on 2016-2019 data.

Table 6. Final muscle Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	4.197	0.607	6.92	-	
	Gold	4.022	0.063	-2.77	<0.01	
	Sand	3.919	0.066	-4.21	<0.01	
	Waldo	4.263	0.167	0.40	0.69	
	Rexford	3.619	0.085	-6.76	<0.01	
Slopes	Ln Total Length	-0.889	0.164	-5.41	<0.01	-0.406
	Ln GSI	-0.086	0.030	-2.81	<0.01	-0.215

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that total fish length (standardized slope = -0.41) has a stronger effect on muscle Se concentrations in the model than GSI (standardized slope = -0.21) over the range sampled for these variables (Table 5). This is the opposite of what was observed for ovary Se, but again, should be treated with caution given the correlation between total length and GSI. Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model have unequal variance (p = 0.001) and are not normally distributed (p = 0.001) again demonstrating the model has some systematic biases.

7. DISCUSSION AND CONCLUSIONS

The objectives of this study were to: 1.) determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations, and 2.) to evaluate the effects of GSI, fish size, and sampling location on ovary and muscle Se concentrations. The first objective was not achieved due to the inability to collect a sufficient number of ripe female NPM. In the remainder of this report, the success in achieving the second objective and implications of study findings are discussed.

7.1 Effects of GSI, Fish Size, and Sampling Location on Ovary Se Concentrations

Historical monitoring data suggest GSI, fish size, and sampling location may influence ovary Se concentrations, but the data are confounded by relatively small sample sizes, are unevenly distributed for some variables (e.g., GSI), and potentially auto-correlated. To address these limitations, a total of 94 additional ovary Se samples were collected in 2019 that were relatively evenly distributed across size classes and to a lesser extent GSIs.

Using 2019 data and incorporating most of the data from historical monitoring (total n = 141), an MLR model that characterizes ovary Se concentrations as a function of fish size (total length) and GSI was developed. While the model has some uncertainties related to the correlation between total fish length and GSI in the data set used for model development, the conclusion that total length and GSI are important predictors of ovary Se concentrations in NPM appears robust and predictions using a constrained data set with no correlation between independent variables are generally within 30% of the model based on the full data set.

There were several key findings from this model. First, the model indicates that fish with lower GSI have higher ovary Se concentrations independent of any other variables. This indicates that fish collected early in the year (e.g., February-May) have ovary Se concentrations that overestimate the egg Se concentrations that the fish will have at the time of spawning. The mechanism underlying this reduction in egg Se with development is currently unclear. Transfer of Se into the eggs is known to be associated with vitellogenesis (Janz et al., 2010) and the ovarian histology component of this study demonstrates vitellogenesis coincides with egg development and an increase in GSI, as is typical of most teleost fish. Consequently, an increase in egg Se rather than a decrease in egg Se would be expected with increasing GSI. However, there are many species-specific complexities to the process of vitellogenesis including variations in the use of multiple vitellogenin isoforms, variations in timing of primary and secondary vitellogenesis, and the level of processing of vitellogenin in the egg and associated level of water absorption (Hara et al., 2016). These processes could all influence how Se concentrations in eggs change during the course of egg development and to the best our knowledge, have not been studied in any detail in NPM or closely related species. However, regardless of the mechanism, the reduction in ovary Se concentrations with increasing GSI is important for assessing potential Se risks to NPM in the

reservoir as it is the ovary/egg Se concentration at the time of spawning that should be compared to an egg Se effect concentration.

Given this finding, ovary Se data from fish with low GSI (i.e., <5%) should be excluded from the data set when assessing potential risk to NPM. The oocyte maturation study demonstrated a strong positive relationship ($R^2 = 0.81$) between GSI and oocyte development. Fish where the majority of oocytes in an ovary were stage 3 (late vitellogenic) oocytes were associated with a GSI >5% (Figure 6). Consequently, only ovaries collected from fish with a GSI ≥5% should be used in assessing Se risks to NPM as these ovaries are likely to provide a relatively unbiased estimate of egg Se concentrations for comparison to egg Se toxicity thresholds. Using this data usability qualifier (GSI ≥5%) restricts the ovary Se data set. All data prior to 2016 are eliminated from assessment due to either low GSI or GSI not being reported and the distribution of ovary Se concentrations are significantly lower (Figure 15). Of all the samples collected from fish with a GSI >5% (n=45), only a single fish has exceeded the USEPA egg Se criteria of 15.1 mg kg^{-1} dw and only 4 fish have exceeded the BC ENV guideline of 11 mg kg^{-1} dw.

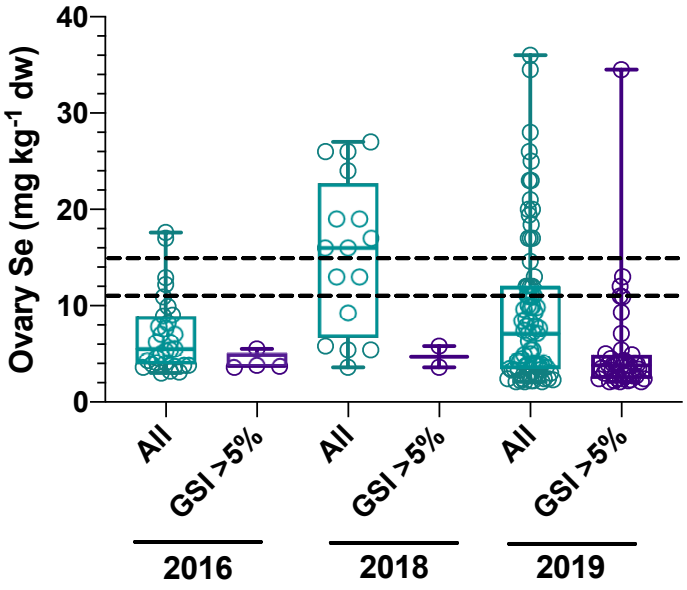


Figure 15. Comparison of ovary Se concentrations for all fish versus only fish with GSI ≥5%. Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines.

The second significant finding from development of the ovary MLR model was that fish size has a significant effect on ovary Se concentrations in NPM, with smaller fish having higher ovary Se concentrations. This is likely the result of differences in dietary preferences between small and large adult NPM. Small adult NPM (<30 cm) typically have a primarily insectivorous diet, but become increasingly piscivorous with increasing size, feeding

primarily on juvenile salmonids (Clarke et al., 2005; Petersen, 2001; Zimmerman, 1999). Whole body trophic transfer factors (TTFs) for fish (i.e., invertebrate to fish or fish to fish) are typically <1 except at very low (<1 mg kg^{-1} dw) dietary Se concentrations (DeForest et al., 2007). A consequence of TTFs <1 is that consumers at progressively higher trophic levels will have progressively lower whole body Se concentrations (i.e., biodilution). This may explain the size effect observed in the current analysis as small NPM feeding on insects would be expected to have higher Se exposure than large NPM which have a higher trophic level and are feeding on juvenile salmonids.

The third, and final, significant finding resulting from the ovary Se MLR model was identification of effects of sampling location on ovary Se concentrations. By accounting for the influence of fish size and GSI on ovary Se, the model was able to test for differences in ovary Se concentrations between sampling locations. Results from this analysis indicate that fish collected from Gold Creek and Rexford have significantly lower ovary Se concentrations than locations sampled higher in the reservoir. Locations higher in the reservoir are generally closer to the Se input from the Elk River although the Sand Creek sampling location is further from the Elk River than the Gold Creek sampling location (Figure 4).

7.2 Effects of GSI, Fish Size, and Sampling Location on Muscle Se Concentrations

The muscle Se MLR model was not as robust as the ovary Se MLR model (Figures 11 and 13). There are likely several reasons for this outcome. First, the range in muscle Se concentrations (0.8-5.0 mg kg^{-1} dw) is much less than observed for ovary Se concentrations (1.8-36 mg kg^{-1} dw). Consequently, small errors in analytical precision will introduce significantly more variance in the muscle Se MLR model. Second, although apparently not significant enough to be detected by the BIC analysis, the muscle Se data collected from the mouth of the Elk River qualitatively appear to have a systematic bias (i.e., different slope) with respect to the MLR model, over-predicting low muscle Se concentrations and under-predicting high muscle Se concentrations.

Despite the muscle Se model being less robust, it did generally support the observations of the ovary Se model. Specifically, the muscle model supports that fish size is an important variable in determining NPM Se concentrations in Koocanusa Reservoir (Table 5). It also supports observations that fish collected from the mouth of the Elk River have higher Se tissue concentrations than fish from most other locations sampled in the reservoir (Table 5).

The finding that GSI is a significant variable in the muscle Se model was somewhat unexpected. Mechanistically, there is no obvious reason why GSI would be an important determinant of muscle Se concentrations. It is possible that retention of GSI in the model is simply an artifact of GSI being an important variable in predicting ovary Se and ovary Se being generally strongly correlated to muscle Se, or that total length and GSI are somewhat correlated. Supporting this hypothesis is the observation that the standardized slope for GSI is

only half of the slope for fish size (Table 6), indicating it has proportionally less influence on muscle Se concentrations while whereas the opposite is true for ovary Se where the standardized slope for GSI is twice as steep as for fish size (Table 4).

7.3 Conclusions and Recommendations

A primary objective of this study was to determine the sensitivity of embryo-larval NPM to maternally transferred egg Se, which was not achieved due to the limited number of ripe female fish collected. However, the second objective of this study to evaluate the effects of fish size, GSI and sampling location on NPM ovary Se was successfully accomplished. Results from this effort indicates that all three variables influence ovary Se concentrations. Importantly, the study concludes that ovary Se data collected from fish with a GSI <5% should not be used to assess Se risks to NPM as these data over-estimate egg Se concentrations. However, the study also demonstrates that small adult NPM have higher egg Se concentrations than large NPM likely due to a predominantly insectivorous diet and that NPM near the Elk River and further upstream (i.e., Sand Creek) have higher egg Se concentrations than those collected further down the reservoir.

Based on these results, this sub-population (small adult fish that reside in the upper reservoir) of NPM likely exhibit higher egg Se concentrations than the overall NPM population in the reservoir, although the mean ovary Se concentration is still predicted to be below the BC ENV egg Se guideline. The relative size of this sub-population and distribution of egg Se concentrations within it is not well characterized, but current results suggests understanding the sensitivity of NPM to maternally transferred egg Se concentrations may still be important. Consequently, additional sampling to characterize the distribution of ovary Se concentrations in NPM in the upper reservoir and to conduct a toxicity study to determine their sensitivity to egg Se concentrations is recommended.

The main limitation of the 2019 Se toxicity study was an inability to capture a sufficient number of ripe female NPM. It is currently unclear why there was so much difficulty collecting a greater number of ripe females. Relatively large numbers of females were collected in the second half of June with GSIs in the range expected for ripe females (Table 2). This continued into early July, but despite relatively high GSIs, only a few fish manually expressed eggs. By mid-July, the CPUE began to drop rapidly and fish that had already spawned began to be captured. As the field season progressed, it was apparent that NPM were not continually congregating in the same areas during the presumed spawning period. Where abundant ripe males were found one day, no ripe males were present only two days later. It was expected that ripe females would be present in these congregations of males or join them days after they were located. This was not the case. Considerable effort (2,644 fishing hours) was invested using four different capture methods across a large spatial scale (~30 km reach of the reservoir). Although an abundance of mature females with high GSIs were captured in

the first four weeks of sampling, CPUE dropped drastically through the last two weeks without locating the desired numbers of ripe females.

A clearer understanding of where Koocanusa NPM populations are spawning is needed, including whether it occurs in congregations and whether tributaries are possible spawning habitat areas. Some changes in gear use, particularly setting gill nets during dusk and dawn, may increase capture rates, but this introduces new safety issues for crews, which will need to be addressed in planning. Greater capture success may result from investing efforts in tracking NPM movements within Koocanusa Reservoir. Understanding NPM movements provides possibilities for improving understanding of habitat use during spawning and allows more focused fishing efforts in suspected spawning sites.

8. CLOSURE

We trust this report provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact Kevin Brix at (305) 773-8347.

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APPENDIX A

UNIVERSITY OF SASKATCHEWAN SELENIUM TOXICITY REPORT

APPENDIX B

NORTHERN PIKEMINNOW OVARY SELENIUM, MUSCLE SELENIUM, AND GSI DATA FOR KOOCANUSA RESERVOIR: 2008-2019

APPENDIX C

NORTHERN PIKEMINNOW METAL TISSUE DATA: 2019

Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

Report

Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

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March 2020

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1 Study Rationale

Ongoing monitoring in the transboundary Koocanusa Reservoir located in British Columbia (BC) indicated a range of selenium (Se) concentrations in wild northern pikeminnow (NPM; *Ptychocheilus oregonensis*) that in some cases exceeded the U.S. Environmental Protection Agency (USEPA) criterion for fish egg/ovary of 15.1 mg/Kg dry weight [dw] and the British Columbia Ministry of the Environment and Climate Change Strategy (ENV) guideline of 11 mg/Kg dw (Brix et al. 2019). Embryonic life-stages of fishes are particularly susceptible to Se exposure via maternal transfer (Janz et al. 2010). However, to the best of our knowledge no studies have investigated the sensitivity of NPM to Se. Also, recent data suggest that there is a negative correlation between relative gonad size of females (as represented by gonadosomatic index [GSI]) and ovary Se concentrations. While this trend could indicate lower exposures of embryos under the assumption that GSI is directly related to maturation stage (later maturation stages are assumed to have greater GSIs, which were reported to have lower Se concentrations), little is known about gonadal phenotypes and their correlation with GSI in this species.

Therefore, this study aimed to investigate 1) the potential effects of maternal transfer of Se to embryos of NPM collected from several sites on the BC side of Koocanusa Reservoir, representing a gradient of Se concentrations, and 2) to characterize gonadal maturation phenotypes prior to and during the reproductive season of NPM in Koocanusa Reservoir. Unfortunately, despite extensive efforts, an insufficient number of female NPM in spawning condition were collected to properly characterize the relationship between egg Se concentrations and NPM embryo-larval development. Consequently, this report only presents the methods and results of the gonadal maturation characterization.

2 Objective

The main objective of this study was to determine whether egg Se concentrations found in NPM from different locations in Koocanusa Reservoir as well as the Elk River, BC may have effects on developing embryos and larvae of NPM. The secondary objective of this study was to characterize ovarian phenotypes of NPMs prior to and during their reproductive season using histology. To accomplish this,

Specific objectives to be addressed during the 2019 NPM early life stage (ELS) studies were:

- Characterize concentrations of Se in parent fish and embryos collected from the BC portion of Koocanusa Reservoir.
- Collect gonadal tissues (representing a range of GSIs) from NPM of different sizes prior to and during the reproductive season to characterize ovarian maturation and oocyte developmental stages.
- Establish a field-fertilization, and an on-site and laboratory culture protocol for NPM embryos and fry.

- Characterize survival, growth, and development of ELS of NPM related to tissue Se concentrations in ovaries of parent fish and eggs/embryos.
- Describe (if detectable) the toxicity threshold concentration (LC_{10} [mortality] and/or EC_{10} [time to hatch, time to swim-up, teratogenicity, growth]) of maternally transferred Se in NPM embryos.

Unfortunately, an insufficient number of female NPM in spawning condition were collected during the study to allow for full development of a protocol and characterization of the effect of maternally transferred Se on developing NPM embryos and larvae. Consequently, only the methods and results for the ovarian histology assessment are provided in this report.

3 Methods

3.1 Ovarian Histology to Assess Gonadal Maturation Stages

All methods for histology preparation followed the UofS Toxicology Centre's draft standard operating procedure (Appendix A). Field-collected NPM were dissected on site and gonads were excised, weighed and then immediately preserved in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed with an automated unit by the UofS Health Sciences Histology Core Facility, to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings, and cooled for 20 minutes to allow sufficient hardening. Because the ovary samples were fragile, blocks were pre-sectioned to expose the tissues and soaked in a glycerin-ethanol solution for 24 hours before section collection. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section, and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

Oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads (2009) - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish. Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

Gonadosomatic indices (GSIs) were calculated for all fish from which gonads were collected for histological assessment as follows (Eq. 1):

GSI = gonad weight [g] / body weight [g] *100 (1)

4 Results & Discussion

4.1 Ovarian Histology to Assess Gonadal Maturation Stages

Between July 8, 2019 and July 19, 2019, ovaries from a total of 15 NPM were collected for histological analyses of maturation stages of oocytes across fish of different sizes (weight range: 250 – 1800 g; fork length range: 33.2 – 61.8 cm), and GSIs (range 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (*Figure 4-1*; ***Table 4-1***). While there was no obvious relationship between the size of fish and GSIs, there was a clear positive correlation between GSI and ovarian maturation stage (*Figure 4-1*) with fish having GSIs greater than or equal to 5% all grouping in the final maturation stage (3) with one exception. Similarly, there was a significant and linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($R^2 = 0.81$), revealing that ovaries of mature fish with a GSI greater than 5% consisted of over 50% LVOs (*Figure 4-2B*). Finally, there was a negative relationship between ovarian Se concentrations and GSI as well as oocyte development stages (*Figure 4-3*).

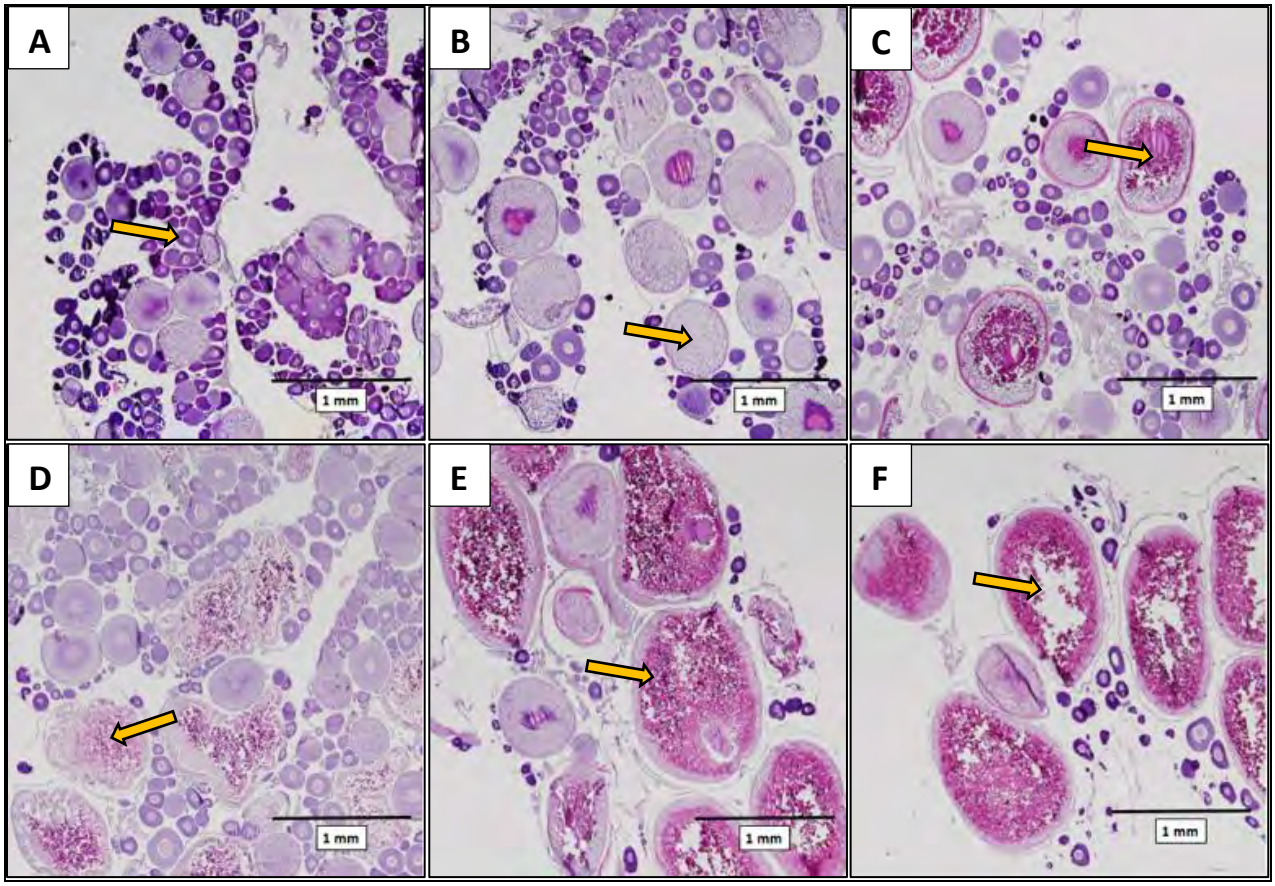


Figure 4-1. Histomicrographs of ovaries of northern pikeminnow representing early development stages (Stage 1) predominantly consisting of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E&F; Arrows).

Table 4-1. Summary of northern pikeminnow oocyte histology analysis detailing maternal morphometric characteristics, oocyte development counts, percent area covered by cell development categories, assessed gonadal developmental stage, and notes. Abbreviations are defined as follows: GSI – gonadosomatic index; PO – perinucleolar oocytes; CAO – cortical alveolar oocytes; EVO – early vitellogenic oocytes; LVO – late vitellogenic oocytes.

Sample ID	Maternal Assessment				Histological Assesment									
	Total Length (cm)	Total Weight (g)	GSI (%)	Ovary Se (µg/g)	Cell Type Count				Percent Area of Cell Type				Developmental Stage	Notes
					PO	CAO	EVO	LVO	PO	CAO	EVO	LVO		
SC-06	43.5	495	1.46	10	341	40	25	10	61.8	16.9	12.7	8.5	2	Atresia Present
GC-14	50.8	1030	0.86	9.6	364	38	4	0	67.7	28.9	3.4	0.0	1	
GC-15	61.8	1800	5.60	3.9	34	12	4	10	6.3	8.6	4.8	80.3	3	
ER-31	33.2	300	5.30	10.9	62	9	8	20	9.0	4.6	10.7	75.7	3	
ER-34	54.0	1470	1.31	5.4	156	46	10	8	37.4	27.6	13.1	21.9	2	
ER-35	40.7	530	7.65	3.8	47	9	4	18	11.2	3.7	5.3	79.7	3	
ER-36	41.4	650	10.54	9.3	19	8	5	14	3.9	6.9	9.8	79.4	3	
ER-37	45.2	750	5.77	5.4	67	8	3	3	32.5	14.2	17.6	35.7	2	
ER-38	41.1	560	3.05	12	54	11	12	9	11.5	11.6	38.5	38.4	2	
ER-39	42.1	580	10.02	2.7	42	10	4	12	10.6	7.4	7.5	74.4	3	Atresia Present
ER-40	34.4	250	0.86	18.4	367	37	0	0	73.5	26.5	0.0	0.0	1	
ER-41	42.4	530	8.17	3.4	48	6	3	10	18.3	5.2	6.8	69.7	3	
ER-42	42.3	620	5.25	11	35	7	4	5	12.0	10.9	22.8	54.3	3	
ER-44	40.7	610	0.60	36	255	64	0	0	41.7	58.3	0.0	0.0	1	
ER-45	49.9	1200	9.01	2.2	11	3	1	6	7.1	8.8	5.2	79.0	3	

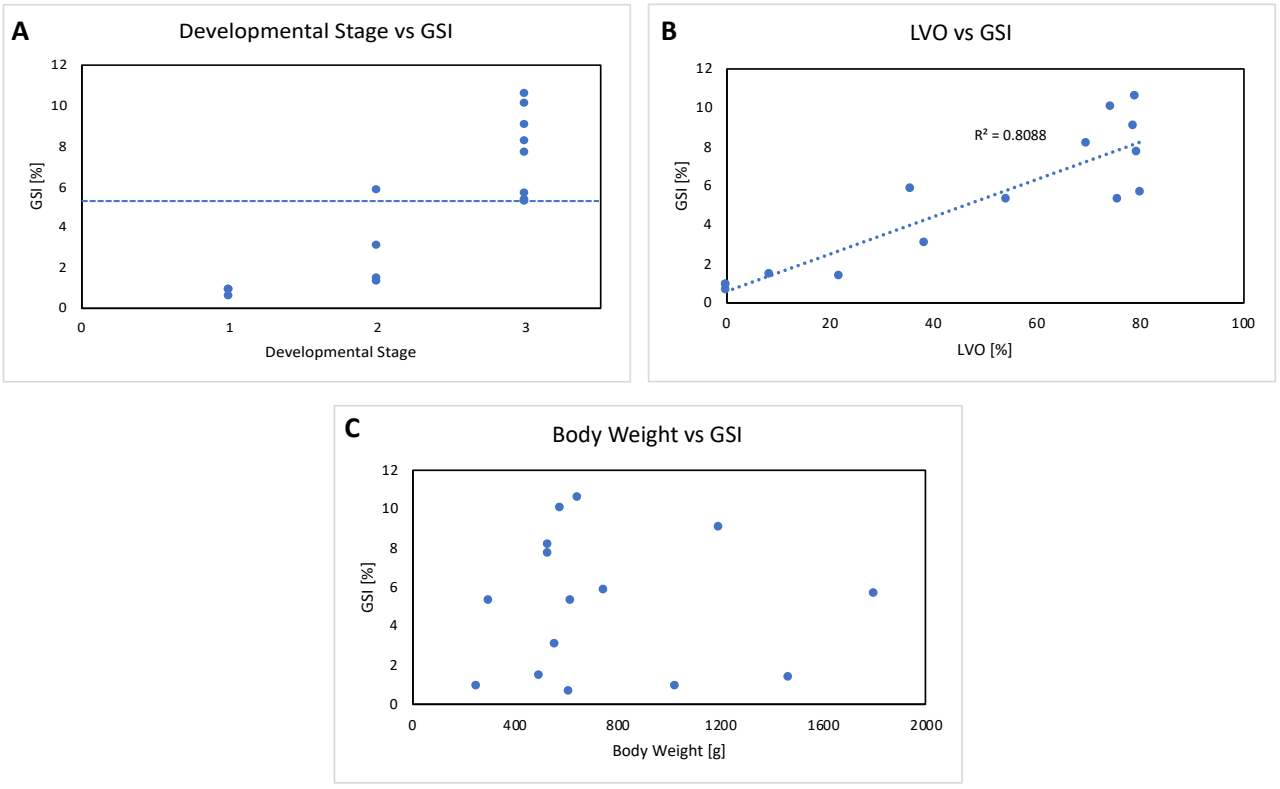


Figure 4-2. Relationships between gonadosomatic indices (GSIs; %) and A) body weight, B) LVO, and C) gonadal development stage in northern pikeminnow. Dotted line represents the 5% GSI level.

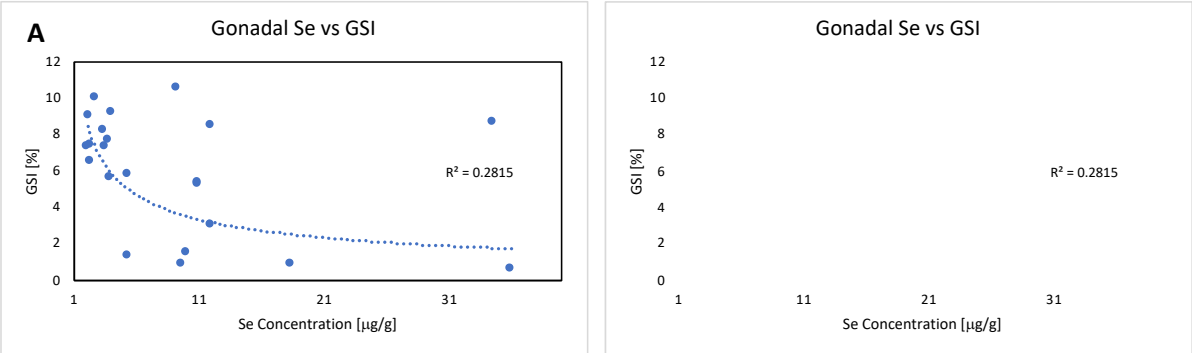


Figure 4-3. Relationship between ovarian Se concentration (µg/g d.w.) and A) gonadosomatic index (GSI), or B) Developmental Stage of oocytes in northern pikeminnow collected from the Koocanusa Reservoir. **Note:** Panel A includes data from 6 additional fish for which no histological evaluation was conducted.

5 Conclusions

This study successfully characterized, for the first time, the phenotypes of different ovarian maturation stage prior to spawning. Clear correlations between histologically determined proportion of follicular stages and GSI were described, demonstrating that fish in the final maturation stage (3) all had GSIs greater than or equal to 5%. Finally, there was significant, albeit weak, negative correlation between ovarian Se concentrations and maturation stage and GSI, indicating greater exposure of immature females. However, sample size and variability were such that future studies are required to confirm this relationship.

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Appendix A – Histological Procedures

University of Saskatchewan

Toxicology Centre

STANDARD OPERATING PROCEDURE

Histological Procedures

Draft

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DEFINITIONS AND ACRONYMS

PLEASE NOTE:

This protocol was produced for and by highly-qualified personnel of the Toxicology Centre of the University of Saskatchewan. It is therefore an in-house document and it should not be distributed without previous consent.

1.0 TISSUE SAMPLING & FIXATION

Morphometric measurements must be recorded as quickly as possible after the experimental animal is euthanized, because rapid degradation of tissues interferes with subsequent histological analyses. Record the following as applicable: individual ID#, sex, length (total length, fork length, standard length, snout-to-vent length, etc.), total weight, gonad weight, liver weight, appearance of secondary sex characteristics, and deformities or other external abnormalities.

There are several options for collecting histology samples, depending on the size of the organism:

1.1 Wole body/intact:

Fix the animal whole, and leave it intact for subsequent processing. In this case, the whole organism must be small enough to fit in a histocasette/standard paraffin block/microscope slide (e.g. fathead minnow at 30 days post-hatch);

1.2 Whole body/dissect

Fix the animal whole, and excise the tissues of interest at a later date prior to processing. For example, an adult fathead minnow or *Xenopus* metamorph can be fixed whole, transferred to 70% ethanol for storage, and then dissected to remove tissues of interest such as liver, gonad, thyroid etc. In this case, during sampling it is necessary to make an incision to expose the internal organs and allow rapid penetration of the fixative.

- Make a shallow mid-ventral incision through the body wall the entire length of the body cavity, being careful to not damage any of the internal organs;
- For adult fathead minnows, make a lateral incision up one side of the body wall to allow the fixative to penetrate the viscera. If possible, using fine forceps, gently move the viscera aside, grasp the swim bladder and discard;
- Attach an individual paper tag with ID# to the body using a needle, fishing line and a waterproof ‘rite-in-the-rain’ paper tag. Label with pencil only, because ethanol removes ink. Attach the tag by passing the needle and line through the body and tying it off.

1.3 Tissue necropsy:

Excise tissue samples from the freshly euthanized animal prior to fixation. This is done for large-bodied specimens that can’t be fixed whole;

- In some cases an entire organ can be excised completely intact and fixed whole for histological analysis. Alternately, it may be necessary to remove only a portion of the tissue of interest, which should be done in a standardised manner, e.g.:

- middle portion of the left or right gonad;
- a particular lobe of the liver;
- right 2nd gill arch;
- middle portion of the posterior kidney;

- When possible, tissue samples should not exceed 1 cm in any direction, although there are exceptions.

When collecting samples for histology, it is preferable to use chemical overdose, because physical methods of euthanasia can sometimes damage histological samples. It is possible to collect different types of samples from a given individual, e.g. remove the fresh liver for biochemical analysis, and then fix the remaining tissues for histology.

1.4 Fixation:

Proper fixation of tissues is one of the most crucial steps in routine histology, and should be kept consistent across samples. The histology samples (i.e. tissue samples in histocassettes, or whole body samples) should be placed in fixative within 2-3 minutes of euthanasia. Ensure that samples are fully submerged, using a minimum of 10 volumes of fixative to 1 volume of tissue. Use Nalgene wide-mouth leak-proof polyethylene containers.

Samples should remain submerged in fixative for 48 hours, and are then transferred to 70% ethanol for storage. Fixative cannot be re-used, and should be disposed appropriately. The 70% ethanol should be poured off and replenished two more times (minimum 1-2 days each) to remove excess fixative prior to tissue processing.

Commonly used histological fixatives include: 10% Neutral Buffered Formalin, Cal-Ex™ II (Fisher), Davidson's Fixative, and Bouin's fluid. Those containing acids have superior tissue penetration with the added advantage of de-calcifying bone, which can improve tissue sectioning. Cal-Ex is therefore preferred for whole body fixation. Davidson's Fixative is also popular; it can be prepared in advance using stock chemicals, and has a reasonable shelf life for longer-term storage.

Davidson's Fixative

Formalin	200 ml
100% Ethanol	300 ml
Glycerin	100 ml
Glacial Acetic Acid	100 ml
Distilled Water	300 ml

2.0 SPECIMEN GROSSING

Once fixed and stored in 70% ethanol, the specimens can be further trimmed if necessary prior to tissue processing. Whole body samples can be dissected to remove tissues of interest. In some cases, an entire organ can be excised (e.g. the gonad, liver), or alternately, a representative portion of the tissue of interest can be removed. Note: fixed weights and lengths can be used to generate condition factor, gonado- and hepatosomatic indices.

3.0 TISSUE PROCESSING and EMBEDDING

Fixed tissue specimens (stored in 70% ethanol) are loaded into a Vacuum Infiltration Processor (aka "**Tissue Processor**"). This programmable, automated unit contains reservoirs of various solvents as well as molten paraffin wax. The tissue processor can be programmed to control temperature, stir the solutions, and create pressure/vacuum cycles during sample processing, all of which can enhance the penetration of solutions through the tissues. The purpose of the process is: (1) **dehydration** - the tissues are bathed in a series of progressively stronger alcohols (70% up to 100%) to remove excess water from the cells, (2) **clearing** - the alcohol is flushed from the tissues and replaced with xylene or toluene (which are capable of dissolving paraffin) (3) **infiltration** - the tissues are infiltrated with molten paraffin. The final result is an intact tissue sample perfused with paraffin, which is immediately placed in a paraffin-filled mould and allowed to cool.

4.1 Tissue Processing:

It takes 14 hours to run a batch of samples through the tissues processor; this is typically done overnight, with sample embedding happening the following morning. Ensure that the samples are stored in a third rinse of 70% ethanol prior to loading them in the processor.

4.2 Embedding

1) Arrive at the Lab 20-30 minutes before the end of the processing run to prepare for embedding.

- Ensure that the Cryo station is turned on;
- Label an embedding ring for each sample to be embedded;
- Coat the embedding moulds with a thin layer of Mould Release, and place them on the warming console;

2) When the processing run is complete, remove the samples from the tissue processor and place them in the 'holding basin' full of molten paraffin wax in the embedding

console. Ensure that the samples do not cool down and solidify at this point, i.e. get the cassettes into the melted wax as quickly as possible, and keep the lid closed;

3) Using the heated paraffin dispenser, place a small amount of paraffin in the bottom of the mould. (Note that the paraffin dispenser flow rate can be adjusted);

4) Open a histocassette and spill the tissue sample out into the holding wax. Set the cassette aside.

5) Using heated forceps, gently grab the tissue and place in the bottom of the mould to attain the appropriate ***orientation for sectioning***. Place it on the cooling pad for 5-10 seconds, to ensure that the wax gels, and the tissue is held in place.

6) Place the labelled embedding ring on the mould with and fill with paraffin. The wax level should be above the rim of the embedding ring to account for shrinkage during cooling. Set it on the Cryo console to cool.

7) Repeat until all samples are embedded, working as quickly as possible.

8) Leave the blocks on the Cryo console for ~20 minutes. Gently pull the mould off and set the block on the benchtop to cool. Transport solidified blocks back to the Toxicology Centre, and let sit overnight prior to attempting any trimming or sectioning.

Table 1. Tissue processing program used at the University of Saskatchewan Histology Core Facility. Tissues are dehydrated in graded alcohols (Station 1 to 7), cleared in xylene (Station 8 to 10), and infiltrated with molten paraffin (Station 11 to 14).

Station	Reagent	Time	Temp (°C)	Pres/Vac Cycle	Mix
1	Ethanol 70%	1 hr	ambient	V	On
2	Ethanol 80%	1 hr	ambient	V	On
3	Ethanol 95%	1 hr	ambient	V	On
4	Ethanol 95%	1 hr	ambient	V	On
5	100% ethanol	1 hr	ambient	V	On
6	100% ethanol	1 hr	ambient	V	On
7	100% ethanol	1 hr	ambient	V	On
8	Ethanol/Xylene	1 hr	ambient	V	On
9	Xylene	1 hr	ambient	V	On
10	Xylene	1 hr	ambient	V	On
11	Paraffin	1 hr	60	V	On
12	Paraffin	1 hr	60	V	On
13	Paraffin	1 hr	60	V	On
14	Paraffin	1 hr	60	V	On

5.0 MICROTOMY (aka SECTIONING)

The embedding ring of the paraffin block is mounted on a rotary microtome. Ribbons of thin sections are created, and these are placed on glass microscope slides. The user can control the thickness of the sections (usually 5-7 μm, thinner is generally better), as well as the number and spacing of the sections retained on the microscope slide. There are several options for sectioning:

- **Single representative section** – one section is retained from each block, this is considered to be representative of the entire tissue;
- **Serial sectioning** - the user cuts through the entire tissue, and all sections are retained (labour-intensive);
- **Step sectioning** - the user cuts through the tissue and retains representative sections at pre-defined intervals;

5.1 General Methods for Sectioning:

- Turn on slide warming table, let it warm up to 40°C (temperature is generally pre-set, and shouldn't require adjustments);
- In a small beaker, prepare ~40mL of distilled water containing ~4 drops of Mayer's Albumin mounting medium, stored in fridge. This should be sufficient for 1 day of sectioning; fresh solution should be made up daily (1 drop Mayer's per 10 mL dH20);
- Use a razor blade to trim excess wax from the tissue blocks to within 2mm of the tissue edge. Maintain square sides on trimmed portion;
- Wipe down a fresh microtome blade with xylene to remove the oil coating, and mount it in the knife holder;
- Ensure microtome is clean and lubricated (see user manual);
- Pre-label a slide for the first paraffin block using solvent resistant marker (slides will be dipped in solvents during staining).
- Place the block firmly in the microtome chuck. Section the block according to the specific protocol (i.e. a single 'representative' section per block, step sections, or serial secions). The sections should come off the blade in continuous ribbons. Note that if the blocks are trimmed small, numerous sections and multiple rows of sections can be placed on a single slide;
- Place the labelled slide on the warming table and flood with the mounting medium. Float the sections of interest on the slide until they appear smooth and free of wrinkles;

- Once the sections are smooth, wipe away excess mounting medium from the slide (Kimwipe), and place it in a slide holder. Full racks of slides are stored in the 40C oven (minimum overnight) prior to staining;
- If scratches or nicks appear in the ribbons during sectioning, move the blade to an unused area, or replace entirely;

6.0 SLIDE STAINING - HEMATOXYLIN and EOSIN

Once the tissue sections have been allowed to dry overnight in a 40°C oven, they can be stained for light microscopy. Myriad staining techniques exist; Hematoxylin and Eosin ("**H&E**") is a common 2-part staining technique routinely used for basic paraffin sections. A rack full of slides is immersed in a series of solvents and stains, resulting in de-waxing of the sections and differential uptake of the 2 stains in various cellular components.

6.1 Staining:

- The stains and solvents can be used to stain app. 10 – 12 racks of slides, and then must be replaced. Check with other lab users regarding the status of the stain series, or if necessary check the quality of the most recently stained slides for fading or loss of contrast. Solvents can be topped up if they have evaporated down;
- Do not stain paraffin sections unless the slides have dried in 40°C oven for minimum 24 hours;
- Multiple racks can be stained at once. When the first boat is in the hematoxylin, a second rack can be started;
- It takes ~45 minutes to stain and coverglass one rack of slides;
- Staining and coverglassing are done in the fume hood;
- Before starting, check supplies of cytoseal and coverglass (use #1 thickness).

Table 2: Step-by-step staining process.

Station	Solution	Time	Notes
1	Xylene 1	2 min	
2	Xylene 2	2 min	
3	Xylene / 100% Ethanol	2 min	1:1 Ratio
4	100% Ethanol	2 min	
5	95% Ethanol	2 min	
6	70% Ethanol	2 min	
7	Tap Water	2 min	Replace often
8	Distilled Water	2 min	Replace often
9	Hematoxylin	5 min	
10	Tap water	rinse 4x	Water should run clear
11	Acid Alcohol (0.1%)	15 sec	0.1ml HCl/100ml 70% EtOH
12	Water	rinse 2x	
13	Phosphotungstic Acid (0.33%)	30 sec	(0.33 g/100ml Water)
14	Citric Acid (0.33%)	30 sec	(0.33 g/100ml Water)
15	Running Tap Water	5 min	
16	Eosin Y	2.5 min	
17	Tap Water	rinse 4x	Water should run clear
	70% Ethanol		
18	95% Ethanol	1 min	
19	100% Ethanol	2 min	
20	100% Ethanol	2 min	
21	Xylene / 100% Ethanol	2 min	1:1 Ratio
22	Xylene	2 min	
23	Xylene	Holding	

6.2 Coverglassing:

- Slides should be coverglassed as soon as possible after staining is completed. The slide rack is held in the last Xylene station until coverglassing is completed;
- Place a coverglass on a cork, add thin line of cytoseal full length;
- Remove slide from Xylene, blot slide edge on paper towel, do not allow Xylene to evaporate completely;
- Turn slide upside down, slowly lower it onto the coverglass at a slight angle, avoid trapping air bubbles in the cytoseal;
- Wipe off the back of the slide, and place flat on trays to dry, ensuring that the slide edges are not touching each other. Slides should air dry minimum 1 week before placing in slide boxes.

Appendix B. Northern Pikeminnow Ovary Selenium, Muscle Selenium, and GSI data for Koocanusa Reservoir: 2008-2019

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
MT	2008	May	14	-	Rexford	2.8	1.0	54.8	-	1973.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.5	1.0	48.3	-	1340.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.1	51.3	-	1347.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.1	51.8	-	1740.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.7	1.2	53.7	-	1708.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.2	1.2	56.2	-	1705.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.8	1.2	50.2	-	1592.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.2	50.0	-	1226.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.9	1.2	50.7	-	1306.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.9	1.3	53.1	-	1720.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.9	1.3	55.8	-	1789.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.3	49.5	-	1303.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.3	47.6	-	1183.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.4	50.9	-	1557.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.6	52.3	-	1586.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.7	51.8	-	1728.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.0	1.9	60.3	-	2259.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.5	1.9	47.0	-	1140.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.7	1.5	56.3	-	1851.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.4	1.5	48.6	-	1134.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.7	1.7	51.7	-	1297.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	1.7	47.0	-	1039.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.3	1.8	52.2	-	1465.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.4	1.8	53.8	-	1506.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.3	1.8	50.2	-	1190.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.4	1.9	61.2	-	2696.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	6.0	2.0	46.8	-	953.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	8.1	2.3	47.6	-	1043.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.1	2.3	51.0	-	1361.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.0	2.3	45.1	-	925.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	2.4	48.8	-	1052.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.5	56.2	-	1860.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.7	1.5	51.0	-	1343.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.3	1.6	50.4	-	1148.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.5	1.7	46.3	-	898.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.7	42.2	-	662.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.9	51.9	-	1134.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.2	1.9	44.4	-	776.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.9	1.9	46.4	-	1116.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.7	2.3	46.6	-	762.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	40.1	5.0	41.0	37.2	505.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	25.7	1.6	38.2	34.9	465.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	3.3	2.4	29.5	27.2	316.0	-	-	-	-	-
BC	2014	April	-	-	Elk River	21.9	4.6	39.3	35.2	440.0	-	-	-	-	-
BC	2014	April	-	ER-PM-14G-Apr-14	Elk River	40.1	6.2	34.1	30.8	312.0	-	3.4	-	-	1.08
BC	2014	April	-	ER-PM-11G-Apr-14	Elk River	8.6	2.5	37.3	33.5	438.0	-	4.5	-	-	1.03
BC	2014	February	-	-	Gold Creek	7.6	2.8	39.3	35.7	495.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	15.4	2.2	35.5	32.3	360.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	10.3	2.5	38.6	34.9	500.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	4.1	2.4	38.0	-	580.0	-	-	-	-	-
BC	2014	April	-	GC-PM-10G-Apr-14	Gold Creek	5.0	2.3	40.4	36.5	522.0	-	4.3	-	-	0.83

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2014	February	-	-	Sand Creek	13.7	1.6	35.5	32.1	320.0	-	-	-	-	-
BC	2014	February	-	-	Sand Creek	3.8	1.2	49.9	45.4	1200.0	-	-	-	-	-
BC	2014	April	-	SC-PM-10G-Apr-14	Sand Creek	17.0	1.6	34.4	31.2	300.0	-	1.8	-	-	0.61
BC	2014	April	-	SC-PM-01G-Apr-14	Sand Creek	30.7	2.9	36.1	32.8	355.0	-	6.2	-	-	1.74
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	4.0	-	35.8	32.3	380.0	-	1.1	-	-	0.30
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	12.9	4.1	34.2	30.8	320.0	13.0	1.1	4.01	315	0.34
BC	2015	April	-	ER-NSC-25-Apr-15	Elk River	9.2	3.1	40.4	36.5	560.0	13.0	1.2	7.81	551	0.21
BC	2015	April	-	ER-NSC-13-Apr-15	Elk River	11.8	6.0	46.7	42.7	885.0	13.0	1.1	12.97	871	0.13
BC	2015	April	-	GC-NSC-13-Apr-15	Gold Creek	5.2	1.9	32.1	28.9	252.0	9.0	1.0	5.50	245	0.41
BC	2015	April	-	GC-NSC-31-Apr-15	Gold Creek	13.8	2.0	33.0	30.2	261.0	12.0	0.9	2.30	258	0.36
BC	2015	April	-	GC-NSC-33-Apr-15	Gold Creek	7.9	2.3	37.4	34.0	390.0	10.0	1.0	2.41	387	0.25
BC	2015	April	-	GC-NSC-34-Apr-15	Gold Creek	7.6	1.6	37.5	34.0	390.0	9.0	1.0	2.10	387	0.25
BC	2015	April	-	GC-NSC-49-Apr-15	Gold Creek	3.5	1.3	44.8	40.1	880.0	12.0	1.4	17.22	861	0.16
BC	2015	April	-	SC-NSC-37-Apr-15	Sand Creek	7.2	1.7	32.6	29.4	261.0	9.0	1.0	3.22	257	0.39
BC	2015	April	-	SC-NSC-36-Apr-15	Sand Creek	4.8	1.9	33.5	30.0	275.0	10.0	1.0	2.51	271	0.37
BC	2015	April	-	SC-NSC-33-Apr-15	Sand Creek	11.4	1.4	34.0	30.2	300.0	8.0	1.1	3.30	296	0.36
BC	2015	April	-	SC-NSC-47-Apr-15	Sand Creek	18.3	2.4	34.4	30.8	325.0	13.0	1.1	3.66	320	0.34
BC	2015	April	-	SC-NSC-44-Apr-15	Sand Creek	15.1	2.4	35.2	31.7	370.0	13.0	1.2	5.49	363	0.31
BC	2015	April	-	SC-NSC-39-Apr-15	Sand Creek	11.6	2.0	38.0	34.0	445.0	12.0	1.1	3.34	441	0.25
BC	2015	April	-	SC-NSC-13-Apr-15	Sand Creek	6.2	1.5	40.0	36.3	492.0	14.0	1.0	8.33	483	0.21
BC	2015	April	-	SC-NSC-46-Apr-15	Sand Creek	5.8	1.7	41.6	37.5	630.0	13.0	1.2	14.58	614	0.19
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	3.1	-	54.1	49.5	1500.0		47.7			3.18
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	10.9	2.9	36.1	32.9	455.0	16.0	16.4	5.00	434	3.60
BC	2016	April	-	ER-NSC-19 O-Apr-16	Elk River	7.0	1.7	38.6	35.2	555.0	14.0	7.4	25.12	522	1.33
BC	2016	April	-	ER-NSC-17 O-Apr-16	Elk River	6.2	2.0	40.2	36.2	615.0	14.0	10.2	9.28	596	1.65
BC	2016	April	-	ER-NSC-27 O -Apr-16	Elk River	7.6	1.3	49.1	45.0	1200.0	15.0	17.6	24.42	1,158	1.47
BC	2016	April	-	ER-NSC-38 O-Apr-16	Elk River	9.9	1.5	51.1	47.0	1400.0	15.0	36.9	24.67	1,338	2.64
BC	2016	April	-	ER-NSC-16 O-Apr-16	Elk River	8.2	1.6	53.4	48.0	1540.0	14.0	35.5	17.35	1,487	2.31
BC	2016	April	-	ER-NSC-28 O-Apr-16	Elk River	5.5	1.7	56.3	50.8	1900.0	22.0	104.8	31.83	1,763	5.51
BC	2016	April	-	ER-NSC-15 O-Apr-16	Elk River	3.0	1.5	60.8	55.9	2600.0	20.0	124.3	46.89	2,429	4.78
BC	2016	April	-	ER-NSC-29 O-Apr-16	Elk River	3.6	1.6	61.5	56.7	2640.0	21.0	132.0	55.87	2,452	5.00
BC	2016	April	-	GC-NSC-17 O-Apr-16	Gold Creek	9.0	2.1	37.6	33.9	435.0	12.0	4.0	7.86	423	0.91
BC	2016	April	-	GC-NSC-01 O-Apr-16	Gold Creek	12.9	1.6	38.2	34.9	450.0	13.0	4.9	8.31	437	1.08
BC	2016	April	-	GC-NSC-14 O-Apr-16	Gold Creek	8.9	2.0	40.9	36.9	585.0	13.0	14.1	5.36	566	2.40
BC	2016	April	-	GC-NSC-16 O-Apr-16	Gold Creek	4.3	1.6	42.0	38.3	610.0	12.0	5.0	6.28	599	0.82
BC	2016	April	-	GC-NSC-12 O-Apr-16	Gold Creek	3.9	1.3	45.5	41.6	940.0	14.0	35.1	15.21	890	3.73
BC	2016	April	-	GC-NSC-26 O-Apr-16	Gold Creek	3.8	1.5	53.5	48.0	1600.0	15.0	96.2	42.73	1,461	6.01
BC	2016	April	-	GC-NSC-24 O-Apr-16	Gold Creek	3.8	1.4	52.8	48.2	1640.0	14.0	80.3	25.92	1,534	4.89
BC	2016	April	-	GC-NSC-06 O-Apr-16	Gold Creek	6.2	1.7	54.5	48.8	1400.0	15.0	35.7	22.15	1,342	2.55
BC	2016	April	-	GC-NSC-08 O-Apr-16	Gold Creek	5.3	2.2	55.8	50.6	1870.0	17.0	65.1	30.61	1,774	3.48
BC	2016	April	-	GC-NSC-13 O -Apr-16	Gold Creek	3.7	1.6	60.8	55.6	2360.0	17.0	124.2	62.56	2,173	5.26
BC	2016	April	-	GC-NSC-05 O-Apr-16	Gold Creek	4.1	1.6	62.2	57.0	2500.0	15.0	117.8	58.55	2,324	4.71
BC	2016	April	-	SC-NSC-25 O-Apr-16	Sand Creek	17.0	2.6	30.1	27.2	257.0	12.0	11.2	3.32	242	4.37
BC	2016	April	-	SC-NSC-09 O-Apr-16	Sand Creek	17.6	2.0	34.1	30.5	315.0	13.0	4.3	3.97	307	1.36
BC	2016	April	-	SC-NSC-21 O-Apr-16	Sand Creek	12.2	1.9	37.6	33.8	520.0	13.0	5.8	9.69	505	1.11
BC	2016	April	-	SC-NSC-29 O-Apr-16	Sand Creek	4.6	1.5	39.7	35.9	550.0	10.0	11.4	13.65	525	2.07
BC	2016	April	-	SC-NSC-03 O-Apr-16	Sand Creek	7.8	1.3	41.5	37.5	760.0	14.0	12.7	9.50	738	1.68
BC	2016	April	-	SC-NSC-35 O-Apr-16	Sand Creek	5.0	1.4	44.4	39.8	935.0	14.0	26.8	20.35	888	2.86
BC	2016	April	-	SC-NSC-36 O-Apr-16	Sand Creek	3.2	1.4	44.7	40.4	875.0	14.0	21.7	21.84	831	2.48
BC	2016	April	-	SC-NSC-32 O-Apr-16	Sand Creek	4.1	1.4	45.5	41.2	996.0	15.0	28.8	20.28	947	2.89
BC	2016	April	-	SC-NSC-34 O-Apr-16	Sand Creek	7.0	1.3	52.5	47.8	1420.0	15.0	36.8	19.82	1,363	2.59

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2016	April	-	SC-NSC-33 O-Apr-16	Sand Creek	5.4	1.3	62.6	57.4	2530.0	21.0	97.4	48.72	2,384	3.85
BC	2018	June	7	RG_ER_NSC06O_20180607	Elk River	26.0	4.4	30.9	27.7	205.0	6.0	1.1	2.93	201	0.51
BC	2018	June	7	RG_ER_NSC03O_20180607	Elk River	17.0	1.7	33.9	30.2	275.0	10.0	1.6	2.06	271	0.59
BC	2018	June	6	RG_ER_NSC02O_20180606	Elk River	19.0	3.1	35.5	31.8	315.0	9.0	1.9	2.70	310	0.61
BC	2018	June	7	RG_ER_NSC05O_20180607	Elk River	26.0	2.5	39.9	35.7	445.0	11.0	2.7	5.16	437	0.60
BC	2018	June	6	RG_ER_NSC01O_20180606	Elk River	16.0	4.0	41.4	37.0	545.0	10.0	3.6	6.42	535	0.65
BC	2018	June	7	RG_ER_NSC04O_20180607	Elk River	24.0	4.8	44.0	39.8	755.0	12.0	26.4	4.40	724	3.50
BC	2018	June	7	RG_GC_NSC02O_20180607	Gold Creek	19.0	2.7	37.1	33.5	350.0	9.0	1.9	4.32	344	0.54
BC	2018	June	7	RG_GC_NSC01O_20180607	Gold Creek	13.0	2.9	38.5	34.7	475.0	9.0	6.1	8.25	461	1.29
BC	2018	June	7	RG_GC_NSC03O_20180607	Gold Creek	3.6	1.7	54.5	50.1	1800.0	15.0	191.6	47.82	1,561	10.65
BC	2018	June	5	RG_SC_NSC05O_20180605	Sand Creek	13.0	2.7	34.0	31.0	280.0	8.0	2.8	2.76	274	1.00
BC	2018	June	5	RG_SC_NSC03O_20180605	Sand Creek	9.2	2.4	34.5	31.2	330.0	10.0	4.9	2.93	322	1.47
BC	2018	June	5	RG_SC_NSC04O_20180605	Sand Creek	5.4	1.6	35.6	32.4	340.0	9.0	7.2	3.65	329	2.13
BC	2018	June	10	RG_SC_NSC06O_20180610	Sand Creek	27.0	2.0	41.6	37.7	530.0	11.0	6.8	4.65	519	1.29
BC	2018	June	5	RG_SC_NSC01O_20180605	Sand Creek	16.0	1.7	44.3	40.4	685.0	12.0	5.8	10.55	669	0.85
BC	2018	June	5	RG_SC_NSC02O_20180605	Sand Creek	5.4	1.3	48.8	44.8	1140.0	13.0	52.1	24.00	1,064	4.57
BC	2018	June	10	RG_SC_NSC07O_20180610	Sand Creek	5.8	1.6	58.9	53.9	1690.0	17.0	96.2	29.16	1,565	5.69
MT	2018	May	8	-	Rexford	3.5	1.1	49.0	-	1215.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.2	1.1	47.4	-	1090.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	5.5	1.3	52.5	-	1575.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	4.6	1.4	48.3	-	1155.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.5	1.4	45.3	-	1110.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.7	1.4	51.6	-	1290.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.4	1.6	53.6	-	1360.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	6.7	1.6	44.2	-	760.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.9	1.9	51.3	-	1395.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.3	1.4	48.8	-	1220.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.5	1.1	49.3	-	1190.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.9	1.1	46.8	-	915.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.4	1.1	44.1	-	960.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.1	1.2	48.2	-	1070.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	1.8	1.2	56.4	-	1575.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.3	49.8	-	1145.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.6	48.2	-	1150.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.8	1.6	47.1	-	955.0	-	-	-	-	-
BC	2019	June	14	6/14/2019 RG_ER-NPM-01_20190614	Elk River	3.8	1.6	55.1	50.2	1680.0	-	137.0	-	-	8.15
BC	2019	June	14	6/14/2019 RG_ER-NPM-02_20190614	Elk River	2.7	1.3	57.2	53.0	1520.0	-	114.0	-	-	7.50
BC	2019	June	17	6/17/2019 RG_ER-NPM-03_20190617	Elk River	3.3	1.2	55.7	50.5	1550.0	-	149.9	-	-	9.67
BC	2019	June	17	6/17/2019 RG_ER-NPM-04_20190617	Elk River	3.0	1.2	45.5	41.6	1050.0	-	100.3	-	-	9.55
BC	2019	June	18	6/18/2019 RG_ER-NPM-05_20190618	Elk River	4.9	1.6	50.7	46.6	1140.0	-	100.3	-	-	8.80
BC	2019	June	18	6/18/2019 RG_ER-NPM-06_20190618	Elk River	5.0	1.6	54.4	49.6	1540.0	-	81.9	-	-	5.32
BC	2019	June	18	6/18/2019 RG_ER-NPM-07_20190618	Elk River	4.3	1.3	47.5	43.0	1140.0	-	87.7	-	-	7.69
BC	2019	June	18	6/18/2019 RG_ER-NPM-08_20190618	Elk River	4.2	1.6	56.0	51.2	1400.0	-	92.1	-	-	6.58
BC	2019	June	19	6/19/2019 RG_ER-NPM-09_20190619	Elk River	7.2	2.0	46.7	42.4	880.0	-	18.6	-	-	2.11
BC	2019	June	19	6/19/2019 RG_ER-NPM-10_20190619	Elk River	9.9	1.9	46.0	42.2	720.0	-	6.6	-	-	0.92
BC	2019	June	19	6/19/2019 RG_ER-NPM-11_20190619	Elk River	17.0	2.5	40.2	36.5	620.0	-	3.7	-	-	0.60
BC	2019	June	20	6/20/2019 RG_ER-NPM-12_20190620	Elk River	2.4	1.3	55.2	50.3	1950.0	-	216.3	-	-	11.09
BC	2019	June	20	6/20/2019 RG_ER-NPM-13_20190620	Elk River	3.6	1.5	56.0	51.5	1590.0	-	47.6	-	-	2.99
BC	2019	June	20	6/20/2019 RG_ER-NPM-14_20190620	Elk River	7.6	1.7	53.6	48.8	1520.0	-	46.2	-	-	3.04
BC	2019	June	20	6/20/2019 RG_ER-NPM-15_20190620	Elk River	8.6	2.5	39.9	36.2	480.0	-	18.6	-	-	3.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-16_20190620	Elk River	17.0	2.4	39.0	34.9	430.0	-	3.7	-	-	0.86

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_ER-NPM-17_20190620	Elk River	4.5	1.8	44.2	40.0	840.0	-	66.2	-	-	7.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-18_20190620	Elk River	4.1	2.5	53.0	48.0	1540.0	-	153.5	-	-	9.97
BC	2019	June	25	6/25/2019 RG_ER-NPM-19_20190625	Elk River	7.9	1.9	30.2	28.3	200.0	-	6.7	-	-	3.35
BC	2019	June	25	6/25/2019 RG_ER-NPM-20_20190625	Elk River	6.3	2.9	46.9	42.4	740.0	-	14.5	-	-	1.96
BC	2019	June	27	6/27/2019 RG_ER-NPM-21_20190627	Elk River	13.0	3.4	39.9	36.9	710.0	-	54.0	-	-	7.61
BC	2019	June	28	6/28/2019 RG_ER-NPM-22_20190628	Elk River	7.1	2.6	32.9	29.5	295.0	-	24.9	-	-	8.43
BC	2019	June	28	6/28/2019 RG_ER-NPM-23_20190628	Elk River	9.8	3.6	38.0	34.2	440.0	-	16.0	-	-	3.63
BC	2019	June	28	6/28/2019 RG_ER-NPM-24_20190628	Elk River	7.8	2.4	43.9	39.6	740.0	-	29.6	-	-	4.00
BC	2019	June	28	6/28/2019 RG_ER-NPM-25_20190628	Elk River	8.3	2.5	33.3	30.4	340.0	-	15.3	-	-	4.49
BC	2019	June	28	6/28/2019 RG_ER-NPM-26_20190628	Elk River	4.0	1.1	44.9	41.5	915.0	-	85.3	-	-	9.33
BC	2019	June	28	6/28/2019 RG_ER-NPM-27_20190628	Elk River	14.6	2.3	30.7	27.6	180.0	-	1.1	-	-	0.61
BC	2019	July	3	7/3/2019 RG_ER-NPM-28_20190703	Elk River	34.5	2.3	38.8	35.7	550.0	-	47.7	-	-	8.67
BC	2019	July	4	7/4/2019 RG_ER-NPM-29_20190704	Elk River	19.4	4.6	46.7	42.4	700.0	-	6.1	-	-	0.88
BC	2019	July	4	7/4/2019 RG_ER-NPM-30_20190704	Elk River	7.1	2.7	44.7	40.5	760.0	-	11.1	-	-	1.47
BC	2019	July	8	7/8/2019 RG_ER-NPM-31_20190708	Elk River	10.9	3.1	33.2	29.9	300.0	-	15.9	-	-	5.30
BC	2019	July	9	7/9/2019 RG_ER-NPM-32_20190709	Elk River	4.1	1.3	54.8	50.0	1550.0	-	143.1	-	20.09	9.23
BC	2019	July	9	7/9/2019 RG_ER-NPM-33_20190709	Elk River	3.5	1.4	44.3	41.6	780.0	-	57.3	-	-	7.35
BC	2019	July	9	7/9/2019 RG_ER-NPM-34_20190709	Elk River	5.4	1.6	54.0	49.0	1470.0	-	19.3	-	-	1.31
BC	2019	July	9	7/9/2019 RG_ER-NPM-35_20190709	Elk River	3.8	1.2	40.7	36.0	530.0	-	40.6	-	-	7.65
BC	2019	July	9	7/9/2019 RG_ER-NPM-36_20190709	Elk River	9.3	3.9	41.4	37.9	650.0	-	68.5	-	-	10.54
BC	2019	July	9	7/9/2019 RG_ER-NPM-37_20190709	Elk River	5.4	2.2	45.2	40.5	750.0	-	43.3	-	-	5.77
BC	2019	July	10	7/10/2019 RG_ER-NPM-38_20190710	Elk River	12.0	4.0	41.1	37.1	560.0	-	17.1	-	-	3.05
BC	2019	July	10	7/10/2019 RG_ER-NPM-39_20190710	Elk River	2.7	1.2	42.1	38.3	580.0	-	58.1	-	-	10.02
BC	2019	July	10	7/10/2019 RG_ER-NPM-40_20190710	Elk River	18.4	3.0	34.4	30.4	250.0	-	2.1	-	-	0.86
BC	2019	July	10	7/10/2019 RG_ER-NPM-41_20190710	Elk River	3.4	1.3	42.4	38.3	530.0	-	43.3	-	-	8.17
BC	2019	July	10	7/10/2019 RG_ER-NPM-42_20190710	Elk River	11.0	3.4	42.3	38.8	620.0	-	32.6	-	-	5.25
BC	2019	July	11	7/11/2019 RG_ER-NPM-43_20190711	Elk River	12.0	4.8	43.0	39.3	825.0	-	70.1	-	-	8.50
BC	2019	July	12	7/12/2019 RG_ER-NPM-44_20190712	Elk River	36.0	5.0	40.7	36.6	610.0	-	3.7	-	-	0.60
BC	2019	July	12	7/12/2019 RG_ER-NPM-45_20190712	Elk River	2.2	1.2	49.9	45.3	1200.0	-	108.1	-	-	9.01
BC	2019	July	13	7/13/2019 RG_ER-NPM-46_20190713	Elk River	2.4	1.3	56.0	50.6	1575.0	-	116.5	-	-	7.40
BC	2019	July	15	7/15/2019 RG_ER-NPM-47_20190715	Elk River	2.1	1.2	53.3	48.2	1200.0	-	87.2	-	-	7.27
BC	2019	July	16	7/16/2019 RG_ER-NPM-48_20190716	Elk River	2.3	1.3	54.9	50.5	1240.0	-	80.1	-	-	6.46
BC	2019	July	26	7/26/2019 RG_ER-NPM-49_20190726	Elk River	3.4	1.4	49.5	45.0	590.0	-	12.6	-	-	2.14
BC	2019	June	26	6/26/2019 RG_GC-NPM-01_20190626	Gold Creek	2.4	1.2	54.5	49.0	1375.0	-	149.5	-	-	10.87
BC	2019	June	26	6/26/2019 RG_GC-NPM-02_20190626	Gold Creek	2.1	1.3	53.9	49.3	1425.0	-	135.5	-	-	9.51
BC	2019	June	26	6/26/2019 RG_GC-NPM-03_20190626	Gold Creek	2.1	1.2	47.4	43.1	1075.0	-	106.7	-	-	9.93
BC	2019	June	26	6/26/2019 RG_GC-NPM-04_20190626	Gold Creek	20.0	2.4	38.9	34.9	460.0	-	2.9	-	-	0.64
BC	2019	June	26	6/26/2019 RG_GC-NPM-05_20190626	Gold Creek	3.9	1.7	44.8	40.4	915.0	-	55.5	-	-	6.07
BC	2019	June	26	6/26/2019 RG_GC-NPM-06_20190626	Gold Creek	2.4	1.1	49.9	44.9	1060.0	-	115.8	-	-	10.92
BC	2019	June	26	6/26/2019 RG_GC-NPM-07_20190626	Gold Creek	11.0	2.1	34.6	31.3	375.0	-	3.0	-	-	0.81
BC	2019	June	27	6/27/2019 RG_GC-NPM-08_20190627	Gold Creek	2.2	1.2	54.6	49.8	1600.0	-	210.4	-	-	13.15
BC	2019	June	27	6/27/2019 RG_GC-NPM-09_20190627	Gold Creek	2.2	1.4	52.6	47.8	1060.0	-	27.0	-	-	2.55
BC	2019	June	27	6/27/2019 RG_GC-NPM-10_20190627	Gold Creek	2.7	1.2	50.2	45.4	1280.0	-	169.2	-	-	13.22
BC	2019	June	27	6/27/2019 RG_GC-NPM-11_20190627	Gold Creek	12.0	1.8	41.6	37.4	600.0	-	4.2	-	-	0.70
BC	2019	June	27	6/27/2019 RG_GC-NPM-12_20190627	Gold Creek	3.9	1.4	45.0	40.5	920.0	-	48.9	-	-	5.32
BC	2019	June	27	6/27/2019 RG_GC-NPM-13_20190627	Gold Creek	3.3	2.0	49.5	44.8	1150.0	-	92.4	-	-	8.03
BC	2019	July	18	7/18/2019 RG_GC-NPM-14_20190718	Gold Creek	9.6	1.5	50.8	46.2	1030.0	-	8.8	-	-	0.86
BC	2019	July	19	7/19/2019 RG_GC-NPM-15_20190719	Gold Creek	3.9	1.4	61.8	57.0	1800.0	-	100.9	-	-	5.60
BC	2019	July	25	7/25/2019 RG_GC-NPM-16_20190725	Gold Creek	2.7	1.4	50.2	46.0	1200.0	-	18.3	-	-	1.53
BC	2019	June	20	6/20/2019 RG_SC-NPM-01_20190620	Sand Creek	8.4	2.2	43.5	39.3	740.0	-	26.9	-	-	3.64
BC	2019	June	20	6/20/2019 RG_SC-NPM-02_20190620	Sand Creek	20.0	1.8	42.8	38.0	600.0	-	3.2	-	-	0.53

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_SC-NPM-03_20190620	Sand Creek	11.0	2.0	34.6	31.6	340.0	-	2.0	-	-	0.58
BC	2019	June	20	6/20/2019 RG_SC-NPM-04_20190620	Sand Creek	17.0	2.6	41.9	38.9	640.0	-	20.5	-	-	3.20
BC	2019	June	20	6/20/2019 RG_SC-NPM-05_20190620	Sand Creek	28.0	2.0	39.2	35.5	490.0	-	3.1	-	-	0.62
BC	2019	July	24	7/24/2019 RG_SC-NPM-06_20190724	Sand Creek	10.0	1.4	43.5	34.2	495.0	-	7.2	-	-	1.46
BC	2019	July	25	7/25/2019 RG_SC-NPM-07_20190725	Sand Creek	21.0	1.7	38.4	35.0	525.0	-	5.7	-	-	1.09
BC	2019	July	25	7/25/2019 RG_SC-NPM-08_20190725	Sand Creek	23.0	2.4	40.3	37.3	540.0	-	8.6	-	-	1.59
BC	2019	July	25	7/25/2019 RG_SC-NPM-09_20190725	Sand Creek	12.0	1.5	44.3	39.9	790.0	-	10.6	-	-	1.34
BC	2019	July	26	7/26/2019 RG_SC-NPM-10_20190726	Sand Creek	25.0	1.4	39.8	35.7	510.0	-	2.4	-	-	0.47
BC	2019	July	26	7/26/2019 RG_SC-NPM-11_20190726	Sand Creek	23.0	2.2	37.9	34.2	495.0	-	4.6	-	-	0.92
BC	2019	June	21	6/21/2019 RG_WB-NPM-01_20190621	Waldo Bay	7.4	2.2	38.0	34.5	406.0	-	16.4	-	-	4.04
BC	2019	June	26	6/26/2019 RG-WB-NPM-02_20190626	Waldo Bay	26.0	2.9	39.9	35.9	480.0	-	5.7	-	-	1.19
BC	2019	June	26	6/26/2019 RG-WB-NPM-03_20190626	Waldo Bay	9.8	2.9	37.5	33.7	440.0	-	4.6	-	-	1.04
MT	2019	May	15	Rexford_NSC_01	Rexford	3.9	1.7	40.5	37.0	540.0	-	5.3	7.4	527.28	0.99
MT	2019	May	15	Rexford_NSC_02	Rexford	2.5	1.0	54.5	50.3	1785.0	-	49.7	32.04	1703.24	2.79
MT	2019	May	15	Rexford_NSC_03	Rexford	5.1	1.5	39.9	36.2	495.0	-	3.1	7.74	484.17	0.62
MT	2019	May	15	Rexford_NSC_04	Rexford	2.2	1.2	50.3	46.0	1500.0	-	41.0	29.007	1430.033	2.73
MT	2019	May	15	Rexford_NSC_05	Rexford	1.8	1.1	60.3	54.8	2060.0	-	68.2	29.69	1962.14	3.31
MT	2019	May	15	Rexford_NSC_06	Rexford	3.5	1.4	42.6	38.8	760.0	-	5.5	27.71	726.76	0.73
MT	2019	May	15	Rexford_NSC_07	Rexford	3.2	1.3	46.9	42.5	1070.0	-	7.1	20.16	1042.76	0.66
MT	2019	May	15	Rexford_NSC_08	Rexford	3.5	1.4	40.9	36.4	610.0	-	4.8	13.94	591.22	0.79
MT	2019	May	15	Rexford_NSC_09	Rexford	2.6	0.8	56.1	51.3	1620.0	-	60.3	28.73	1530.992	3.72
MT	2019	May	15	Rexford_NSC_10	Rexford	2.0	1.1	49.9	44.9	1200.0	-	30.9	14.03	1155.03	2.58
MT	2019	May	15	Rexford_NSC_11	Rexford	9.5	1.6	37.4	33.5	475.0	-	4.7	7.27	463.01	0.99
MT	2019	May	15	Rexford_NSC_12	Rexford	5.1	1.0	46.7	42.0	940.0	-	10.8	16.07	913.11	1.15
MT	2019	May	15	Rexford_NSC_13	Rexford	2.0	1.0	44.4	40.1	890.0	-	13.1	22.17	854.689	1.48
MT	2019	May	15	Rexford_NSC_14	Rexford	2.8	0.9	51.4	47.2	1540.0	-	42.4	20.822	1476.813	2.75
MT	2019	May	15	Rexford_NSC_15	Rexford	2.2	0.9	54.0	49.3	1490.0	-	53.9	21.269	1414.834	3.62

Appendix C. Northen Pikeminnow Tissue Data: 2019

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG-ER-NPM-32-BF-20190709	Eggs	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	0.01	2.2	21	<0.01	0.6	0.037	0.03	<0.05	2.8	<0.01	0.10	<0.005	<0.05	<0.2	<0.005	<0.1	66	64.02
7/9/2019 RG-ER-NPM-33-BF-20190709	Eggs	<5	<0.02	<0.05	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.6	56	<0.05	0.9	0.04	<0.05	<0.5	3.0	<0.02	0.2	<0.01	<0.2	<0.5	<0.02	<0.2	80	65.11
7/12/2019 RG-ER-NPM-43-BF-20190712	Eggs	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.02	2.9	45	<0.01	2.4	0.046	0.04	0.07	11.7	<0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	69	64.87
7/13/2019 RG-ER-NPM-46-BF-20190713	Eggs	<2	<0.01	0.11	0.13	<0.01	<1	<0.01	<0.05	0.02	2.1	30	<0.01	0.3	0.042	0.03	<0.05	2.7	<0.01	0.10	0.010	<0.05	<0.2	<0.005	<0.1	98	63.44
7/16/2019 RG-ER-NPM-48-BF-20190716	Eggs	<2	<0.01	0.10	0.36	<0.01	<1	<0.01	0.06	0.02	2.6	22	<0.01	0.8	0.051	0.04	<0.05	2.3	<0.01	0.15	0.009	<0.05	<0.2	<0.005	<0.1	77	69.18
7/3/2019 RG-ER-NPM-28-AF-20190703	Eggs	120	<0.01	0.04	1.2	<0.01	<1	<0.01	0.06	0.04	2.4	74	0.07	2.9	0.019	0.04	0.10	8.4	<0.01	2.9	<0.005	<0.05	1.0	0.031	<0.1	77	95.04
7/9/2019 RG-ER-NPM-32-AF-20190709	Eggs	260	<0.02	0.17	2.6	<0.02	<2	<0.02	<0.1	0.04	2.2	140	0.51	7.4	0.03	<0.05	0.2	2.9	<0.02	3.0	<0.01	<0.1	4.3	0.05	<0.2	68	96.25
7/13/2019 RG-ER-NPM-46-AF-20190713	Eggs	100	<0.01	0.08	3.3	<0.01	<1	<0.01	0.07	0.04	2.2	130	0.20	12	0.041	0.05	0.10	2.3	<0.01	2.6	0.011	<0.05	3.8	0.028	<0.1	88	94.15
7/15/2019 RG-ER-NPM-47-AF-20190715	Eggs	60	<0.1	<0.5	<5	<0.02	<50	<0.02	<5	<5	<5	<50	<0.5	<5	0.07	<0.5	<5	1.8	<0.02	1	<0.1	<2	<5	<0.1	<1	70	94.79
7/16/2019 RG-ER-NPM-48-AF-20190716	Eggs	560	0.01	0.31	15	0.02	<1	0.01	0.09	0.11	2.6	760	1.1	85	0.048	0.12	0.21	2.7	0.01	6.7	0.015	<0.05	24	0.14	0.4	86	91.84
7/3/2019 RG-ER-NPM-28-PF_20190703	Eggs	<5	<0.02	0.25	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.1	34	<0.05	2.3	0.02	<0.05	<0.5	9.6	<0.02	0.1	<0.01	<0.2	<0.5	<0.02	<0.2	62	66.93
6/14/2019 RG_ER-NPM-01-M_20190614	Muscle	2	<0.01	0.15	0.25	<0.01	<1	<0.01	0.24	0.01	2.0	25	<0.01	0.3	1.3	<0.02	0.05	1.6	<0.01	3.8	0.016	<0.05	<0.2	<0.005	<0.1	19	72.64
6/14/2019 RG_ER-NPM-02-M_20190614	Muscle	<2	<0.01	0.21	0.95	<0.01	<1	<0.01	<0.05	0.02	2.6	24	0.02	0.8	2.2	<0.02	<0.05	1.3	<0.01	6.2	0.014	<0.05	<0.2	<0.005	<0.1	33	72.76
6/17/2019 RG_ER-NPM-03-M_20190617	Muscle	<2	<0.01	0.22	0.92	<0.01	<1	<0.01	0.15	0.02	3.5	33	0.06	0.6	1.2	<0.02	<0.05	1.2	<0.01	3.6	0.013	<0.05	<0.2	<0.005	<0.1	28	71.78
6/17/2019 RG_ER-NPM-04-M_20190617	Muscle	<2	<0.01	0.16	0.90	<0.01	<1	<0.01	<0.05	0.02	2.6	26	<0.01	0.6	0.66	<0.02	<0.05	1.2	<0.01	3.0	0.021	<0.05	0.2	<0.005	<0.1	22	71.16
6/18/2019 RG_ER-NPM-05-M_20190618	Muscle	<2	<0.01	0.04	0.72	<0.01	<1	<0.01	0.20	0.01	0.85	9	<0.01	0.6	1.7	<0.02	<0.05	1.6	<0.01	3.6	0.010	<0.05	<0.2	<0.005	<0.1	19	77.76
6/18/2019 RG_ER-NPM-06-M_20190618	Muscle	<2	<0.01	0.07	0.58	<0.01	<1	<0.01	<0.05	0.03	0.79	8	<0.01	0.8	2.1	<0.02	<0.05	1.6	<0.01	3.0	0.008	<0.05	<0.2	<0.005	<0.1	17	78.52
6/18/2019 RG_ER-NPM-07-M_20190618	Muscle	<2	<0.01	0.17	1.2	<0.01	<1	<0.01	<0.05	0.02	2.4	23	<0.01	1.0	0.90	<0.02	0.08	1.3	<0.01	5.7	0.013	<0.05	<0.2	<0.005	<0.1	24	72.59
6/18/2019 RG_ER-NPM-08-M_20190618	Muscle	<2	<0.01	0.03	0.75	<0.01	<1	<0.01	<0.05	0.01	0.95	13	0.01	0.4	1.9	<0.02	<0.05	1.6	<0.01	3.1	0.009	<0.05	<0.2	<0.005	<0.1	22	78.06
6/19/2019 RG_ER-NPM-09-M_20190619	Muscle	3	<0.01	0.06	1.7	<0.01	<1	<0.01	<0.05	0.03	2.5	27	0.09	1.2	0.94	<0.02	0.07	2.0	<0.01	8.5	0.017	<0.05	<0.2	<0.005	<0.1	38	76.79
6/19/2019 RG_ER-NPM-10-M_20190619	Muscle	<2	<0.01	0.03	0.95	<0.01	<1	<0.01	<0.05	0.02	1.7	16	0.02	0.7	0.98	<0.02	<0.05	1.9	<0.01	3.0	0.009	<0.05	<0.2	<0.005	<0.1	29	76.48
6/19/2019 RG_ER-NPM-11-M_20190619	Muscle	<2	<0.01	0.07	0.36	<0.01	<1	<0.01	<0.05	0.01	1.5	16	0.03	0.6	0.87	<0.02	<0.05	2.5	<0.01	1.4	0.012	<0.05	<0.2	<0.005	<0.1	18	78.43
6/20/2019 RG_ER-NPM-12-M_20190620	Muscle	<2	<0.01	0.17	0.20	<0.01	<1	<0.01	<0.05	<0.01	0.96	9	0.01	0.2	1.5	<0.02	<0.05	1.3	<0.01	0.61	0.010	<0.05	<0.2	<0.005	<0.1	15	72.41
6/20/2019 RG_ER-NPM-13-M_20190620	Muscle	<2	<0.01	0.08	0.78	<0.01	<1	<0.01	<0.05	0.02	1.1	11	0.01	0.7	1.5	<0.02	<0.05	1.5	<0.01	4.5	0.006	<0.05	<0.2	<0.005	<0.1	19	75.51
6/20/2019 RG_ER-NPM-14-M_20190620	Muscle	<2	<0.01	0.14	0.65	<0.01	<1	<0.01	<0.05	0.01	1.1	11	<0.01	0.6	1.3	<0.02	<0.05	1.7	<0.01	2.6	0.010	<0.05	<0.2	<0.005	<0.1	16	74.20
6/20/2019 RG_ER-NPM-15-M_20190620	Muscle	<2	<0.01	0.02	0.85	<0.01	<1	<0.01	<0.05	0.01	0.92	14	0.02	0.9	1.1	<0.02	<0.05	2.5	<0.01	2.0	0.008	<0.05	<0.2	<0.005	<0.1	24	79.46
6/20/2019 RG_ER-NPM-16-M_20190620	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	0.09	0.03	1.9	29	0.01	1.3	1.0	<0.02	<0.05	2.4	<0.01	4.8	0.011	<0.05	<0.2	<0.005	<0.1	34	80.14
6/20/2019 RG_ER-NPM-17-M_20190620	Muscle	<2	<0.01	0.08	1.0	<0.01	<1	<0.01	<0.05	0.02	2.2	22	<0.01	1.0	1.1	<0.02	<0.05	1.8	<0.01	3.7	0.013	<0.05	<0.2	<0.005	<0.1	26	78.24
6/20/2019 RG_ER-NPM-18-M_20190620	Muscle	<2	<0.01	0.07	0.46	<0.01	<1	<0.01	<0.05	<0.01	0.83	7	<0.01	0.4	1.7	<0.02	<0.05	2.5	<0.01	1.7	<0.005	<0.05	<0.2	<0.005	<0.1	20	74.74
6/25/2019 RG_ER-NPM-19-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.02	1.8	17	<0.01	1.0	0.41	<0.02	<0.05	1.9	<0.01	2.2	0.021	<0.05	<0.2	<0.005	<0.1	38	78.49
6/25/2019 RG_ER-NPM-20-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.03	2.7	27	0.01	1.4	0.92	<0.02	<0.05	2.9	<0.01	7.1	0.018	<0.05	<0.2	<0.005	<0.1	29	76.00
6/27/2019 RG_ER-NPM-21-M_20190627	Muscle	<2	<0.01	0.04	1.3	<0.01	<1	<0.01	<0.05	0.02	2.6	35	<0.01	1.9	0.78	<0.02	<0.05	3.4	<0.01	7.1	0.011	<0.05	<0.2	<0.005	<0.1	28	76.67
6/28/2019 RG_ER-NPM-22-M_20190628	Muscle	<2	<0.01	0.02	0.84	<0.01	<1	<0.01	<0.05	0.02	0.92	9	<0.01	1.2	0.61	<0.02	0.05	2.6	<0.01	3.3	0.016	<0.05	<0.2	<0.005	<0.1	18	76.61
6/28/2019 RG_ER-NPM-23-M_20190628	Muscle	<2	<0.01	0.01	2.4	<0.01	<1	<0.01	0.08	0.02	2.3	21	<0.01	1.3	1.9	<0.02	<0.05	3.6	<0.01	3.4	0.012	<0.05	<0.2	<0.005	<0.1	25	74.79
6/28/2019 RG_ER-NPM-24-M_20190628	Muscle	<2	<0.01	0.06	2.0	<0.01	<1	<0.01	0.22	0.02	1.5	20	<0.01	1.4	0.88	<0.02	<0.05	2.4	<0.01	8.4	0.008	<0.05	<0.2	<0.005	<0.1	28	78.90
6/28/2019 RG_ER-NPM-25-M_20190628	Muscle	<2	<0.01	0.02	0.50	<0.01	<1	<0.01	0.06	<0.01	1.1	10	<0.01	0.7	1.0	<0.02	<0.05	2.5	<0.01	1.5	0.012	<0.05	<0.2	<0.005	<0.1	17	75.15
6/28/2019 RG_ER-NPM-26-M_20190628	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	0.07	0.02	1.7	21	<0.01	1.5	1.1	<0.02	<0.05	1.1	<0.01	7.1	0.010	<0.05	<0.2	<0.005	<0.1	24	77.37
6/28/2019 RG_ER-NPM-27-M_20190628	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	0.02	0.83	11	<0.01	1.2	0.35	<0.02	<0.05	2.3	<0.01	4.7	0.013	<0.05	<0.2	<0.005	<0.1	22	77.97
7/3/2019 RG_ER-NPM-28-M_20190703	Muscle	<2	<0.01	0.03	1.1	<0.01	<1	<0.01	<0.05	0.01	1.2	12	0.02	1.4	0.86	<0.02	<0.05	2.3	<0.01	4.7	0.010	<0.05	<0.2	<0.005	<0.1	19	76.85
7/4/2019 RG_ER-NPM-29-M_20190704	Muscle	<2	<0.01	0.03	0.37	<0.01	<1	<0.01	<0.05	0.01	1.7	21	<0.01	0.6	0.82	<0.02	<0.05	4.6	<0.01	0.75	0.011	<0.05	<0.2	<0.005	<0.1	21	78.32

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/4/2019 RG_ER-NPM-30-M_20190704	Muscle	<2	<0.01	0.03	0.58	<0.01	<1	<0.01	<0.05	<0.01	0.73	9	0.02	0.8	1.6	<0.02	<0.05	2.7	<0.01	2.3	0.006	<0.05	<0.2	<0.005	<0.1	15	77.17
7/8/2019 RG_ER-NPM-31-M_20190708	Muscle	3	<0.01	0.04	1.4	<0.01	<1	<0.01	13	0.05	2.3	170	0.02	1.6	0.72	<0.02	0.40	3.1	<0.01	2.7	0.019	<0.05	<0.2	<0.005	<0.1	33	78.99
7/9/2019 RG_ER-NPM-32-M_20190709	Muscle	<2	<0.01	0.12	0.77	<0.01	<1	<0.01	0.77	0.02	1.8	26	<0.01	0.5	1.2	<0.02	<0.05	1.3	<0.01	3.2	0.010	<0.05	<0.2	<0.005	<0.1	25	72.74
7/9/2019 RG_ER-NPM-33-M_20190709	Muscle	<2	<0.01	0.11	0.92	<0.01	<1	<0.01	0.11	0.02	2.2	21	<0.01	0.8	0.76	<0.02	<0.05	1.4	<0.01	3.9	0.021	<0.05	<0.2	<0.005	<0.1	26	75.45
7/9/2019 RG_ER-NPM-34-M_20190709	Muscle	<2	<0.01	0.12	1.5	<0.01	<1	<0.01	0.17	0.02	2.0	20	<0.01	1.1	1.4	<0.02	<0.05	1.6	<0.01	5.4	0.011	<0.05	<0.2	<0.005	<0.1	28	74.77
7/9/2019 RG_ER-NPM-35-M_20190709	Muscle	<2	<0.01	0.32	1.8	<0.01	<1	<0.01	0.14	0.02	2.2	24	<0.01	1.3	1.0	<0.02	<0.05	1.2	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	31	77.08
7/9/2019 RG_ER-NPM-36-M_20190709	Muscle	<2	<0.01	0.06	1.4	<0.01	<1	<0.01	0.17	0.03	2.7	25	<0.01	1.4	2.0	<0.02	<0.05	3.9	<0.01	4.3	0.020	<0.05	<0.2	<0.005	<0.1	34	78.53
7/9/2019 RG_ER-NPM-37-M_20190709	Muscle	4	<0.01	0.11	1.8	<0.01	<1	<0.01	0.07	0.03	2.2	26	0.02	2.0	0.87	<0.02	<0.05	2.2	<0.01	6.6	0.009	<0.05	<0.2	<0.005	<0.1	33	79.86
7/10/2019 RG_ER-NPM-38-M_20190710	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	0.10	0.02	2.4	25	0.02	1.4	0.80	<0.02	0.05	4.0	<0.01	4.0	0.011	<0.05	<0.2	<0.005	<0.1	29	78.21
7/10/2019 RG_ER-NPM-39-M_20190710	Muscle	<2	<0.01	0.11	0.46	<0.01	<1	<0.01	0.06	0.01	1.6	15	<0.01	0.5	0.92	<0.02	<0.05	1.2	<0.01	1.7	0.027	<0.05	<0.2	<0.005	<0.1	20	76.75
7/10/2019 RG_ER-NPM-40-M_20190710	Muscle	5	<0.01	0.09	0.74	<0.01	<1	<0.01	0.11	0.02	1.6	27	0.04	0.9	0.66	<0.02	0.09	3.0	<0.01	1.2	0.010	<0.05	<0.2	<0.005	<0.1	32	79.51
7/10/2019 RG_ER-NPM-41-M_20190710	Muscle	<2	<0.01	0.12	0.91	<0.01	<1	<0.01	0.18	0.01	1.0	11	<0.01	0.8	0.87	<0.02	<0.05	1.3	<0.01	3.1	0.024	<0.05	<0.2	<0.005	<0.1	21	76.75
7/10/2019 RG_ER-NPM-42-M_20190710	Muscle	<2	<0.01	0.03	1.5	<0.01	<1	<0.01	0.08	0.02	1.7	15	<0.01	1.4	0.92	<0.02	<0.05	3.4	<0.01	5.2	0.009	<0.05	<0.2	<0.005	<0.1	29	78.08
7/11/2019 RG_ER-NPM-43-M_20190711	Muscle	<2	<0.01	0.05	1.1	<0.01	<1	<0.01	0.13	0.02	2.6	17	0.02	1.3	0.69	<0.02	<0.05	4.8	<0.01	4.0	0.022	<0.05	<0.2	<0.005	<0.1	26	79.26
7/12/2019 RG_ER-NPM-44-M_20190712	Muscle	<2	<0.01	0.05	0.14	<0.01	<1	<0.01	<0.05	<0.01	0.73	10	<0.01	0.5	1.1	<0.02	<0.05	5.0	<0.01	0.10	0.008	<0.05	<0.2	<0.005	<0.1	17	78.91
7/12/2019 RG_ER-NPM-45-M_20190712	Muscle	<2	<0.01	0.10	0.19	<0.01	<1	<0.01	0.05	<0.01	0.77	6	<0.01	0.2	1.0	<0.02	<0.05	1.2	<0.01	0.80	0.015	<0.05	<0.2	<0.005	<0.1	14	76.31
7/13/2019 RG_ER-NPM-46-M_20190713	Muscle	2	<0.01	0.19	0.60	<0.01	<1	<0.01	0.06	0.02	2.2	23	0.01	0.4	1.3	<0.02	<0.05	1.3	<0.01	2.8	0.022	<0.05	<0.2	<0.005	<0.1	28	75.43
7/15/2019 RG_ER-NPM-47-M_20190715	Muscle	<2	<0.01	0.17	1.0	<0.01	<1	<0.01	0.07	0.02	1.6	16	<0.01	0.7	1.1	<0.02	0.12	1.2	<0.01	5.2	0.014	<0.05	<0.2	<0.005	<0.1	26	76.36
7/16/2019 RG_ER-NPM-48-M_20190716	Muscle	<2	<0.01	0.14	1.2	<0.01	<1	<0.01	<0.05	0.02	1.4	12	<0.01	0.8	1.7	<0.02	<0.05	1.3	<0.01	4.9	0.021	<0.05	<0.2	<0.005	<0.1	27	78.26
7/26/2019 RG_ER-NPM-49-M_20190726	Muscle	<2	<0.01	0.09	0.71	<0.01	<1	<0.01	0.06	0.02	1.5	15	<0.01	0.6	1.6	<0.02	<0.05	1.4	<0.01	3.6	0.014	<0.05	<0.2	<0.005	<0.1	24	77.86
6/26/2019 RG_GC-NPM-01-M_20190626	Muscle	<2	<0.01	0.18	0.17	<0.01	<1	<0.01	0.05	<0.01	0.84	8	<0.01	0.1	2.6	<0.02	<0.05	1.2	<0.01	0.51	0.007	<0.05	<0.2	<0.005	<0.1	12	75.39
6/26/2019 RG_GC-NPM-02-M_20190626	Muscle	<2	<0.01	0.07	0.07	<0.01	<1	<0.01	<0.05	<0.01	0.75	7	<0.01	0.1	1.5	<0.02	<0.05	1.3	<0.01	0.12	0.015	<0.05	<0.2	<0.005	<0.1	15	77.80
6/26/2019 RG_GC-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	0.54	<0.01	<1	<0.01	<0.05	<0.01	0.68	7	<0.01	0.5	1.1	<0.02	<0.05	1.2	<0.01	2.3	0.010	<0.05	<0.2	<0.005	<0.1	18	77.49
6/26/2019 RG_GC-NPM-04-M_20190626	Muscle	<2	<0.01	0.06	0.60	<0.01	<1	<0.01	<0.05	<0.01	0.82	12	<0.01	0.8	1.1	<0.02	<0.05	2.4	<0.01	2.3	0.011	<0.05	<0.2	<0.005	<0.1	22	78.87
6/26/2019 RG_GC-NPM-05-M_20190626	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.8	20	0.01	1.4	1.1	<0.02	<0.05	1.7	<0.01	3.4	0.009	<0.05	<0.2	<0.005	<0.1	25	77.49
6/26/2019 RG_GC-NPM-06-M_20190626	Muscle	<2	<0.01	0.12	1.0	<0.01	<1	<0.01	<0.05	<0.01	1.7	17	<0.01	0.7	0.92	<0.02	<0.05	1.1	<0.01	3.8	0.021	<0.05	<0.2	<0.005	<0.1	22	75.90
6/26/2019 RG_GC-NPM-07-M_20190626	Muscle	<2	<0.01	0.02	1.1	<0.01	<1	<0.01	0.06	<0.01	1.3	20	0.01	1.2	1.1	<0.02	<0.05	2.1	<0.01	4.6	0.015	<0.05	<0.2	<0.005	<0.1	26	79.07
6/27/2019 RG_GC-NPM-08-M_20190627	Muscle	2	<0.01	0.10	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.4	12	0.23	0.7	1.2	<0.02	<0.05	1.2	<0.01	6.6	0.022	<0.05	0.4	<0.005	<0.1	31	75.59
6/27/2019 RG_GC-NPM-09-M_20190627	Muscle	2	<0.01	0.04	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.91	12	<0.01	0.2	1.5	<0.02	<0.05	1.4	<0.01	0.78	0.017	<0.05	<0.2	<0.005	<0.1	14	78.43
6/27/2019 RG_GC-NPM-10-M_20190627	Muscle	<2	<0.01	0.09	0.92	<0.01	<1	<0.01	<0.05	<0.01	1.6	13	<0.01	0.7	1.2	<0.02	<0.05	1.2	<0.01	4.8	0.022	<0.05	<0.2	<0.005	<0.1	20	75.35
6/27/2019 RG_GC-NPM-11-M_20190627	Muscle	<2	<0.01	0.09	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.63	10	<0.01	0.3	1.1	<0.02	<0.05	1.8	<0.01	0.60	0.005	<0.05	<0.2	<0.005	<0.1	14	77.35
6/27/2019 RG_GC-NPM-12-M_20190627	Muscle	<2	<0.01	0.10	1.3	<0.01	<1	<0.01	<0.05	<0.01	2.2	21	<0.01	1.4	1.0	<0.02	<0.05	1.4	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	26	78.38
6/27/2019 RG_GC-NPM-13-M_20190627	Muscle	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	<0.01	0.82	11	<0.01	0.3	1.6	<0.02	<0.05	2.0	<0.01	0.57	<0.005	<0.05	<0.2	<0.005	<0.1	14	78.91
7/18/2019 RG_GC-NPM-14-M_20190718	Muscle	<2	<0.01	0.11	0.78	<0.01	<1	<0.01	<0.05	<0.01	2.9	34	<0.01	0.4	1.7	<0.02	<0.05	1.5	<0.01	1.3	0.014	<0.05	<0.2	<0.005	<0.1	27	76.81
7/19/2019 RG_GC-NPM-15-M_20190719	Muscle	2	<0.01	0.04	0.44	<0.01	<1	<0.01	<0.05	<0.01	0.84	6	<0.01	0.3	2.6	<0.02	<0.05	1.4	<0.01	1.8	0.011	<0.05	<0.2	<0.005	<0.1	20	80.92
7/25/2019 RG_GC-NPM-16-M_20190725	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	<0.01	1.2	9	<0.01	0.8	1.3	<0.02	<0.05	1.4	<0.01	5.6	0.016	<0.05	<0.2	<0.005	<0.1	27	77.46
6/20/2019 RG_SC-NPM-01-M_20190620	Muscle	<2	<0.01	0.05	0.70	<0.01	<1	<0.01	<0.05	<0.01	2.5	31	<0.01	0.9	1.0	<0.02	<0.05	2.2	<0.01	2.7	0.013	<0.05	<0.2	<0.005	<0.1	27	78.73
6/20/2019 RG_SC-NPM-02-M_20190620	Muscle	<2	<0.01	0.03	0.69	<0.01	<1	<0.01	1.2	<0.01	1.2	24	<0.01	0.9	0.99	0.04	0.12	1.8	<0.01	3.8	0.010	<0.05	<0.2	<0.005	<0.1	23	80.68
6/20/2019 RG_SC-NPM-03-M_20190620	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	<0.05	<0.01	2.1	21	0.02	1.6	0.55	<0.02	<0.05	2.0	<0.01	5.1	0.012	<0.05	<0.2	<0.005	<0.1	34	79.58
6/20/2019 RG_SC-NPM-04-M_20190620	Muscle	<2	<0.01	0.07	0.77	<0.01	<1	<0.01	<0.05	<0.01	2.1	25	<0.01	0.8	1.1	<0.02	<0.05	2.6	<0.01	2.9	0.016	<0.05	<0.2	<0.005	<0.1	34	79.13
6/20/2019 RG_SC-NPM-05-M_20190620	Muscle	<2	<0.01	0.04	0.93	<0.01	<1	<0.01	<0.05	0.01	1.1	13	<0.01	0.9	0.81	<0.02	<0.05	2.0	<0.01	3.4	0.011	<0.05	<0.2	<0.005	<0.1	24	79.40

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/24/2019 RG_SC-NPM-06-M_20190724	Muscle	<2	<0.01	0.01	0.79	<0.01	<1	<0.01	0.32	0.01	0.85	17	<0.01	1.3	1.4	<0.02	<0.05	1.4	<0.01	4.8	<0.005	<0.05	<0.2	<0.005	<0.1	18	79.65
7/25/2019 RG_SC-NPM-07-M_20190725	Muscle	3	<0.01	0.06	1.1	<0.01	<1	<0.01	0.06	0.01	0.95	15	0.02	1.6	1.3	<0.02	<0.05	1.7	<0.01	4.7	0.007	<0.05	<0.2	<0.005	<0.1	44	80.43
7/25/2019 RG_SC-NPM-08-M_20190725	Muscle	<2	<0.01	0.10	1.0	<0.01	<1	<0.01	0.05	0.02	1.0	15	<0.01	1.8	1.3	<0.02	0.05	2.4	<0.01	6.6	0.014	<0.05	<0.2	<0.005	<0.1	24	78.64
7/25/2019 RG_SC-NPM-09-M_20190725	Muscle	<2	<0.01	0.02	0.24	<0.01	<1	<0.01	<0.05	0.01	1.8	19	<0.01	0.5	1.2	<0.02	<0.05	1.5	<0.01	1.1	0.006	<0.05	<0.2	<0.005	<0.1	19	76.51
7/26/2019 RG_SC-NPM-10-M_20190726	Muscle	<2	<0.01	0.07	0.39	<0.01	<1	<0.01	<0.05	0.01	0.76	10	<0.01	0.6	1.0	<0.02	0.06	1.4	<0.01	1.4	0.006	<0.05	<0.2	<0.005	<0.1	17	79.18
7/26/2019 RG_SC-NPM-11-M_20190726	Muscle	<2	<0.01	0.06	0.28	<0.01	<1	<0.01	<0.05	<0.01	1.1	14	<0.01	0.4	0.90	<0.02	<0.05	2.2	<0.01	1.1	0.013	<0.05	<0.2	<0.005	<0.1	21	79.53
6/21/2019 RG_WB-NPM-01-M_20190621	Muscle	<2	<0.01	0.02	0.97	<0.01	<1	<0.01	<0.05	0.02	1.7	23	0.04	0.9	1.1	<0.02	<0.05	2.2	<0.01	2.8	0.010	<0.05	<0.2	<0.005	<0.1	28	77.69
6/26/2019 RG-WB-NPM-02-M_20190626	Muscle	<2	<0.01	0.09	2.4	<0.01	<1	<0.01	<0.05	0.02	2.6	28	0.01	1.7	0.99	<0.02	<0.05	2.9	<0.01	9.4	0.015	<0.05	<0.2	<0.005	<0.1	44	79.29
6/26/2019 RG-WB-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	1.5	<0.01	<1	<0.01	<0.05	0.02	2.5	26	<0.01	1.5	1.0	<0.02	<0.05	2.9	<0.01	3.9	0.006	<0.05	<0.2	<0.005	<0.1	32	79.01
6/14/2019 RG_ER-NPM-01-O_20190614	Ovary	<2	<0.01	0.07	0.12	<0.01	<1	<0.01	<0.05	0.02	3.2	43	<0.01	0.6	0.10	0.05	<0.05	3.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	97	64.38
6/14/2019 RG_ER-NPM-02-O_20190614	Ovary	<2	<0.01	0.07	0.14	<0.01	<1	<0.01	<0.05	0.03	3.1	38	<0.01	3.0	0.16	0.06	<0.05	2.7	0.01	0.19	0.007	<0.05	<0.2	<0.005	<0.1	110	65.57
6/17/2019 RG_ER-NPM-03-O_20190617	Ovary	<2	<0.01	0.06	0.22	<0.01	<1	<0.01	<0.05	0.03	2.8	45	0.01	1.3	0.10	0.05	<0.05	3.3	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	94	65.63
6/17/2019 RG_ER-NPM-04-O_20190617	Ovary	<2	<0.01	0.09	0.13	<0.01	<1	<0.01	<0.05	0.02	2.8	41	<0.01	0.7	0.051	0.04	<0.05	3.0	<0.01	0.11	0.009	<0.05	<0.2	<0.005	<0.1	87	61.36
6/18/2019 RG_ER-NPM-05-O_20190618	Ovary	<2	<0.01	0.04	0.21	<0.01	<1	<0.01	<0.05	0.03	3.0	37	0.01	2.2	0.12	0.04	<0.05	4.9	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	78	62.62
6/18/2019 RG_ER-NPM-06-O_20190618	Ovary	<2	<0.01	0.26	0.14	<0.01	<1	<0.01	<0.05	0.03	2.6	52	<0.01	2.2	0.14	0.07	<0.05	5.0	<0.01	0.20	0.006	<0.05	<0.2	<0.005	<0.1	120	67.92
6/18/2019 RG_ER-NPM-07-O_20190618	Ovary	<2	<0.01	0.06	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	41	<0.01	1.3	0.11	0.04	<0.05	4.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	87	63.03
6/18/2019 RG_ER-NPM-08-O_20190618	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.03	2.3	91	0.01	2.6	0.13	0.08	<0.05	4.2	<0.01	0.27	0.009	<0.05	<0.2	<0.005	<0.1	120	67.65
6/19/2019 RG_ER-NPM-09-O_20190619	Ovary	3	<0.01	0.06	0.16	<0.01	<1	<0.01	<0.05	0.03	2.8	60	0.18	2.2	0.054	0.07	<0.05	7.2	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	68.24
6/19/2019 RG_ER-NPM-10-O_20190619	Ovary	2	<0.01	0.04	0.26	<0.01	<1	<0.01	0.21	0.05	2.8	96	0.13	3.4	0.17	0.14	<0.05	9.9	<0.01	0.32	0.023	<0.05	<0.2	<0.005	<0.1	260	76.39
6/19/2019 RG_ER-NPM-11-O_20190619	Ovary	4	<0.01	0.06	0.32	<0.01	<1	0.01	0.11	0.06	4.1	130	0.18	2.7	0.096	0.12	0.06	17	<0.01	0.41	0.049	<0.05	<0.2	<0.005	<0.1	500	79.40
6/20/2019 RG_ER-NPM-12-O_20190620	Ovary	<2	<0.01	0.07	0.15	<0.01	<1	<0.01	<0.05	0.02	3.0	33	<0.01	0.5	0.081	0.04	<0.05	2.4	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	84	63.59
6/20/2019 RG_ER-NPM-13-O_20190620	Ovary	<2	<0.01	0.06	0.14	<0.01	<1	<0.01	<0.05	0.03	2.4	54	<0.01	1.9	0.23	0.06	<0.05	3.6	<0.01	0.22	0.008	<0.05	<0.2	<0.005	<0.1	120	71.25
6/20/2019 RG_ER-NPM-14-O_20190620	Ovary	<2	<0.01	0.06	0.12	<0.01	<1	<0.01	<0.05	0.04	2.6	60	<0.01	1.8	0.21	0.08	<0.05	7.6	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	72.31
6/20/2019 RG_ER-NPM-15-O_20190620	Ovary	<2	<0.01	0.04	0.36	<0.01	<1	<0.01	<0.05	0.05	2.9	95	<0.01	5.5	0.078	0.10	<0.05	8.6	<0.01	0.24	0.009	<0.05	<0.2	<0.005	<0.1	150	70.05
6/20/2019 RG_ER-NPM-16-O_20190620	Ovary	2	<0.01	0.08	0.35	<0.01	<1	0.01	0.09	0.07	3.6	150	0.02	2.5	0.13	0.12	0.06	17	<0.01	0.26	0.033	<0.05	<0.2	<0.005	<0.1	520	83.66
6/20/2019 RG_ER-NPM-17-O_20190620	Ovary	<2	<0.01	0.07	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	2.3	0.085	0.04	<0.05	4.5	<0.01	0.16	0.005	<0.05	<0.2	<0.005	<0.1	74	65.19
6/20/2019 RG_ER-NPM-18-O_20190620	Ovary	<2	<0.01	0.05	0.22	<0.01	<1	<0.01	<0.05	0.02	1.8	32	<0.01	2.5	0.14	0.04	<0.05	4.1	<0.01	0.17	<0.005	<0.05	<0.2	<0.005	<0.1	80	65.88
6/25/2019 RG_ER-NPM-19-O_20190625	Ovary	<2	<0.01	0.08	0.38	<0.01	<1	<0.01	<0.05	0.04	2.6	66	<0.01	8.3	0.024	0.07	<0.05	7.9	<0.01	0.17	0.018	<0.05	<0.2	<0.005	<0.1	120	66.61
6/25/2019 RG_ER-NPM-20-O_20190625	Ovary	<2	<0.01	0.04	0.08	<0.01	<1	<0.01	<0.05	0.06	3.2	110	<0.01	2.6	0.12	0.08	<0.05	6.3	<0.01	0.21	0.030	<0.05	<0.2	<0.005	<0.1	180	75.96
6/27/2019 RG_ER-NPM-21-O_20190627	Ovary	<2	<0.01	0.03	0.11	<0.01	<1	<0.01	<0.05	0.02	2.7	49	<0.01	2.3	0.039	0.03	<0.05	13	<0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	80	60.36
6/28/2019 RG_ER-NPM-22-O_20190628	Ovary	<2	<0.01	0.05	0.17	<0.01	<1	<0.01	<0.05	0.02	2.2	37	<0.01	2.5	0.021	0.03	<0.05	7.1	<0.01	0.09	0.008	<0.05	<0.2	<0.005	<0.1	79	61.41
6/28/2019 RG_ER-NPM-23-O_20190628	Ovary	<2	<0.01	0.01	0.32	<0.01	<1	<0.01	<0.05	0.03	2.7	44	<0.01	4.1	0.12	0.04	<0.05	9.8	0.02	0.14	0.006	<0.05	<0.2	<0.005	<0.1	100	65.42
6/28/2019 RG_ER-NPM-24-O_20190628	Ovary	<2	<0.01	0.05	0.19	<0.01	<1	<0.01	<0.05	0.04	3.1	55	<0.01	1.5	0.12	0.05	<0.05	7.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	120	67.86
6/28/2019 RG_ER-NPM-25-O_20190628	Ovary	<2	<0.01	0.02	0.22	<0.01	<1	<0.01	<0.05	0.03	3.3	51	<0.01	4.7	0.050	0.06	<0.05	8.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	97	63.20
6/28/2019 RG_ER-NPM-26-O_20190628	Ovary	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	0.02	3.0	54	<0.01	2.1	0.037	0.04	<0.05	4.0	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	77	62.47
6/28/2019 RG_ER-NPM-27-O_20190628	Ovary	<2	<0.01	0.86	1.5	<0.01	<1	0.10	0.06	0.06	1.5	47	0.07	0.4	0.023	<0.02	0.06	14.6	<0.01	0.10	0.021	<0.05	<0.2	<0.005	<0.1	300	56.47
7/3/2019 RG_ER-NPM-28-O_20190703	Ovary	4	<0.01	0.03	0.20	<0.01	<1	0.01	<0.05	0.04	2.8	170	0.06	2.8	0.088	0.08	<0.05	34.5	<0.01	0.27	0.018	<0.05	<0.2	<0.005	<0.1	180	77.60
7/4/2019 RG_ER-NPM-29-O_20190704	Ovary	6	<0.01	0.05	0.39	<0.01	<1	0.04	0.22	0.07	3.9	150	0.06	2.8	0.097	0.15	0.10	19.4	<0.01	0.57	0.034	<0.05	0.3	<0.005	<0.1	330	77.92
7/4/2019 RG_ER-NPM-30-O_20190704	Ovary	14	<0.01	0.04	0.44	<0.01	<1	0.02	0.05	0.05	4.2	110	0.15	3.7	0.11	0.07	<0.05	7.1	<0.01	0.36	0.014	<0.05	0.3	<0.005	0.1	200	76.76
7/8/2019 RG_ER-NPM-31-O_20190708	Ovary	<2	<0.01	0.04	0.20	<0.01	<1	<0.01	0.09	0.03	3.3	64	<0.01	3.5	0.068	0.05	<0.05	10.9	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	100	65.74
7/9/2019 RG_ER-NPM-32-O_20190709	Ovary	<2	<0.01	0.08	0.13	<0.01	<1	<0.01	0.10	0.04	3.3	81	0.01	1.2	0.13	0.08	<0.05	4.1	<0.01	0.36	0.016	<0.05	<0.2	<0.005	<0.1	160	74.41

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG_ER-NPM-33-O_20190709	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.6	47	<0.01	0.9	0.030	<0.02	<0.05	3.5	<0.01	0.18	0.010	<0.05	<0.2	<0.005	<0.1	97	63.66
7/9/2019 RG_ER-NPM-34-O_20190709	Ovary	3	<0.01	0.07	0.13	<0.01	<1	0.01	<0.05	0.05	3.2	77	<0.01	3.1	0.21	0.08	<0.05	5.4	<0.01	0.24	0.029	<0.05	<0.2	<0.005	0.1	260	76.24
7/9/2019 RG_ER-NPM-35-O_20190709	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.02	2.4	57	<0.01	1.1	0.056	0.04	<0.05	3.8	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	82	65.42
7/9/2019 RG_ER-NPM-36-O_20190709	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	0.02	3.0	43	<0.01	2.4	0.12	0.04	<0.05	9.3	0.01	0.10	0.006	<0.05	<0.2	<0.005	<0.1	78	62.62
7/9/2019 RG_ER-NPM-37-O_20190709	Ovary	<2	<0.01	0.06	0.23	<0.01	<1	<0.01	0.21	0.03	3.1	48	<0.01	1.5	0.050	0.05	<0.05	5.4	<0.01	0.24	<0.005	<0.05	<0.2	<0.005	<0.1	92	63.31
7/10/2019 RG_ER-NPM-38-O_20190710	Ovary	3	<0.01	0.04	0.19	<0.01	<1	<0.01	<0.05	0.04	3.6	57	<0.01	5.9	0.071	0.08	<0.05	12	0.01	0.28	0.007	<0.05	<0.2	<0.005	<0.1	130	69.92
7/10/2019 RG_ER-NPM-39-O_20190710	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.02	3.3	48	<0.01	2.0	0.038	0.04	<0.05	2.7	0.01	0.14	0.010	<0.05	<0.2	<0.005	<0.1	110	62.30
7/10/2019 RG_ER-NPM-40-O_20190710	Ovary	5	<0.01	0.06	0.35	<0.01	<1	0.02	0.12	0.04	4.0	110	0.07	1.4	0.060	0.07	0.06	18.4	<0.01	0.24	0.036	<0.05	0.2	<0.005	<0.1	540	79.88
7/10/2019 RG_ER-NPM-41-O_20190710	Ovary	<2	<0.01	0.07	0.17	<0.01	<1	<0.01	<0.05	0.02	2.6	52	<0.01	1.3	0.030	0.05	<0.05	3.4	<0.01	0.13	0.015	<0.05	<0.2	<0.005	<0.1	110	64.12
7/10/2019 RG_ER-NPM-42-O_20190710	Ovary	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.03	3.2	55	<0.01	5.1	0.079	0.07	<0.05	11	0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	100	65.47
7/11/2019 RG_ER-NPM-43-O_20190711	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.03	3.0	42	<0.01	2.5	0.049	0.04	<0.05	12	0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	78	64.30
7/12/2019 RG_ER-NPM-44-O_20190712	Ovary	3	<0.01	0.05	0.15	<0.01	<1	0.01	0.11	0.06	3.6	110	0.02	2.9	0.068	0.14	<0.05	36	<0.01	0.34	0.032	<0.05	<0.2	<0.005	<0.1	410	78.06
7/12/2019 RG_ER-NPM-45-O_20190712	Ovary	<2	<0.01	0.09	0.22	<0.01	<1	<0.01	<0.05	0.02	2.7	33	<0.01	0.5	0.049	0.04	<0.05	2.2	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	91	62.46
7/13/2019 RG_ER-NPM-46-O_20190713	Ovary	<2	<0.01	0.12	0.07	<0.01	<1	<0.01	<0.05	0.04	3.3	91	<0.01	0.6	0.17	0.09	<0.05	2.4	<0.01	0.22	0.046	<0.05	<0.2	<0.005	<0.1	210	75.90
7/15/2019 RG_ER-NPM-47-O_20190715	Ovary	<2	<0.01	0.12	0.14	<0.01	<1	<0.01	<0.05	0.02	2.5	31	<0.01	0.7	0.091	0.03	<0.05	2.1	<0.01	0.12	0.011	<0.05	<0.2	<0.005	<0.1	99	66.82
7/16/2019 RG_ER-NPM-48-O_20190716	Ovary	<2	<0.01	0.11	0.06	<0.01	<1	0.01	<0.05	0.05	3.7	69	<0.01	1.4	0.37	0.11	<0.05	2.3	<0.01	0.33	0.059	<0.05	<0.2	<0.005	<0.1	230	81.71
7/26/2019 RG_ER-NPM-49-O_20190726	Ovary	2	<0.01	0.15	0.32	<0.01	<1	0.02	0.06	0.05	3.7	240	<0.01	1.2	0.23	0.07	<0.05	3.4	<0.01	0.43	0.051	<0.05	<0.2	0.008	0.1	380	82.16
6/26/2019 RG_GC-NPM-01-O_20190626	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	41	<0.01	0.6	0.14	0.05	<0.05	2.4	<0.01	0.17	0.005	<0.05	<0.2	<0.005	<0.1	100	64.51
6/26/2019 RG_GC-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	0.6	0.096	0.06	<0.05	2.1	<0.01	0.14	0.012	<0.05	<0.2	<0.005	<0.1	110	65.08
6/26/2019 RG_GC-NPM-03-O_20190626	Ovary	2	<0.01	0.05	0.18	<0.01	<1	<0.01	0.40	0.04	3.1	47	<0.01	0.6	0.049	0.04	<0.05	2.1	<0.01	0.12	0.006	<0.05	<0.2	<0.005	<0.1	77	63.05
6/26/2019 RG_GC-NPM-04-O_20190626	Ovary	<2	<0.01	0.17	0.19	<0.01	<1	<0.01	0.07	<0.01	2.5	75	0.01	1.1	0.096	0.04	<0.05	20	<0.01	0.21	0.032	<0.05	<0.2	<0.005	<0.1	360	72.82
6/26/2019 RG_GC-NPM-05-O_20190626	Ovary	<2	<0.01	0.03	0.26	<0.01	<1	<0.01	<0.05	<0.01	2.2	46	<0.01	1.9	0.076	0.03	<0.05	3.9	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	83	63.49
6/26/2019 RG_GC-NPM-06-O_20190626	Ovary	<2	<0.01	0.06	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.7	40	<0.01	1.9	0.048	0.04	<0.05	2.4	<0.01	0.12	0.012	<0.05	<0.2	<0.005	<0.1	100	63.02
6/26/2019 RG_GC-NPM-07-O_20190626	Ovary	<2	<0.01	0.11	0.13	<0.01	<1	0.01	0.12	<0.01	3.2	120	0.01	1.4	0.082	0.08	<0.05	11	<0.01	0.19	0.047	<0.05	<0.2	<0.005	<0.1	360	77.70
6/27/2019 RG_GC-NPM-08-O_20190627	Ovary	<2	<0.01	0.15	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	30	<0.01	0.4	0.078	0.03	<0.05	2.2	<0.01	0.11	0.012	<0.05	<0.2	<0.005	<0.1	100	62.90
6/27/2019 RG_GC-NPM-09-O_20190627	Ovary	<2	<0.01	0.10	0.06	<0.01	<1	<0.01	<0.05	<0.01	3.8	68	<0.01	1.5	0.25	0.10	<0.05	2.2	<0.01	0.30	0.036	<0.05	<0.2	<0.005	<0.1	180	77.62
6/27/2019 RG_GC-NPM-10-O_20190627	Ovary	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	40	<0.01	1.1	0.093	0.04	<0.05	2.7	<0.01	0.11	0.010	<0.05	<0.2	<0.005	<0.1	90	61.14
6/27/2019 RG_GC-NPM-11-O_20190627	Ovary	<2	<0.01	0.04	0.12	<0.01	<1	<0.01	0.11	0.01	2.8	92	<0.01	2.4	0.13	0.10	<0.05	12	<0.01	0.29	0.018	<0.05	<0.2	<0.005	<0.1	340	78.10
6/27/2019 RG_GC-NPM-12-O_20190627	Ovary	<2	<0.01	0.04	0.25	<0.01	<1	<0.01	<0.05	<0.01	2.3	47	<0.01	3.3	0.070	0.03	<0.05	3.9	<0.01	0.12	<0.005	<0.05	<0.2	<0.005	<0.1	95	64.74
6/27/2019 RG_GC-NPM-13-O_20190627	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.1	33	<0.01	1.3	0.078	0.03	<0.05	3.3	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	65	61.68
7/18/2019 RG_GC-NPM-14-O_20190718	Ovary	7	<0.01	0.09	0.18	<0.01	<1	0.02	<0.05	<0.01	3.3	140	0.02	1.5	0.28	0.08	<0.05	9.6	<0.01	0.27	0.045	<0.05	0.2	<0.005	0.1	430	79.54
7/19/2019 RG_GC-NPM-15-O_20190719	Ovary	2	<0.01	0.10	0.21	<0.01	<1	<0.01	<0.05	<0.01	2.2	37	<0.01	0.5	0.27	0.04	<0.05	3.9	<0.01	0.11	0.008	<0.05	<0.2	<0.005	<0.1	120	67.63
7/25/2019 RG_GC-NPM-16-O_20190725	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.8	39	<0.01	1.1	0.083	0.03	<0.05	2.7	<0.01	0.11	0.011	<0.05	0.4	<0.005	<0.1	91	64.09
6/20/2019 RG_SC-NPM-01-O_20190620	Ovary	<2	<0.01	0.04	0.13	<0.01	<1	<0.01	<0.05	<0.01	3.1	56	<0.01	3.3	0.071	0.06	<0.05	8.4	<0.01	0.20	0.008	<0.05	<0.2	<0.005	<0.1	120	68.02
6/20/2019 RG_SC-NPM-02-O_20190620	Ovary	7	<0.01	0.06	0.26	<0.01	<1	<0.01	0.05	<0.01	2.6	130	0.03	1.2	0.12	0.06	<0.05	20	<0.01	0.84	0.041	<0.05	<0.2	<0.005	<0.1	490	80.46
6/20/2019 RG_SC-NPM-03-O_20190620	Ovary	<2	<0.01	0.09	0.07	<0.01	<1	<0.01	<0.05	<0.01	3.0	93	0.02	2.1	0.041	0.08	<0.05	11	<0.01	0.20	0.025	<0.05	<0.2	<0.005	<0.1	320	73.78
6/20/2019 RG_SC-NPM-04-O_20190620	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	<0.01	3.6	110	0.02	3.0	0.098	0.09	<0.05	17	<0.01	0.72	0.012	<0.05	<0.2	<0.005	<0.1	140	69.48
6/20/2019 RG_SC-NPM-05-O_20190620	Ovary	<2	<0.01	0.10	0.33	<0.01	<1	<0.01	<0.05	0.05	3.0	98	<0.01	1.8	0.088	0.10	0.05	28	<0.01	0.23	0.034	<0.05	<0.2	<0.005	<0.1	360	75.91
7/24/2019 RG_SC-NPM-06-O_20190724	Ovary	<2	<0.01	0.08	0.18	<0.01	<1	<0.01	<0.05	0.05	2.5	120	<0.01	1.9	0.11	0.04	0.09	10	<0.01	0.78	0.008	<0.05	<0.2	<0.005	<0.1	250	77.04
7/25/2019 RG_SC-NPM-07-O_20190725	Ovary	<2	<0.01	0.07	0.09	<0.01	<1	<0.01	<0.05	0.04	3.0	100	<0.01	8.9	0.14	0.16	<0.05	21	<0.01	0.38	0.018	<0.05	<0.2	<0.005	<0.1	280	77.16
7/25/2019 RG_SC-NPM-08-O_20190725	Ovary	<2	<0.01	0.15	0.15	<0.01	<1	<0.01	<0.05	0.05	2.8	86	<0.01	5.1	0.092	0.10	<0.05	23	<0.01	0.57	0.018	<0.05	<0.2	<0.005	<0.1	200	74.33

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/25/2019 RG_SC-NPM-09-O_20190725	Ovary	<2	<0.01	0.08	0.07	<0.01	<1	<0.01	<0.05	0.05	2.7	75	<0.01	3.5	0.18	0.12	0.08	12	<0.01	0.33	0.007	<0.05	<0.2	<0.005	<0.1	220	75.90
7/26/2019 RG_SC-NPM-10-O_20190726	Ovary	3	<0.01	0.13	0.62	<0.01	<1	<0.01	0.09	0.05	3.2	100	<0.01	1.6	0.072	0.07	0.07	25	<0.01	0.28	0.023	<0.05	<0.2	<0.005	<0.1	440	75.75
7/26/2019 RG_SC-NPM-11-O_20190726	Ovary	<2	<0.01	0.12	0.15	<0.01	<1	<0.01	0.08	0.07	4.2	160	<0.01	2.8	0.12	0.05	<0.05	23	<0.01	0.62	0.028	<0.05	<0.2	<0.005	<0.1	320	80.08
6/21/2019 RG_WB-NPM-01-O_20190621	Ovary	5	<0.01	0.02	0.23	<0.01	<1	<0.01	<0.05	0.04	3.6	77	0.06	4.5	0.069	0.09	0.06	7.4	<0.01	0.40	0.006	<0.05	<0.2	<0.005	<0.1	120	67.47
7/13/2019 RG_ER-NPM-47-O_20190713	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	45	<0.01	0.9	0.045	0.03	<0.05	3.5	<0.01	0.10	0.009	<0.05	<0.2	<0.005	<0.1	94	64.10
6/26/2019 RG-WB-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.05	3.0	83	<0.01	4.4	0.12	0.13	<0.05	26	<0.01	0.28	0.030	<0.05	<0.2	<0.005	<0.1	260	76.76
6/26/2019 RG-WB-NPM-03-O_20190626	Ovary	<2	<0.01	0.14	0.12	<0.01	<1	<0.01	<0.05	0.03	2.0	65	<0.01	1.6	0.18	0.06	<0.05	9.8	<0.01	0.14	0.007	<0.05	<0.2	<0.005	<0.1	240	70.13

ATTACHMENT 2

Analysis of Historical Mountain Whitefish Data Memorandum

EcoTox

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MEMORANDUM

Date: June 10, 2020

To: Mariah Arnold

From: Kevin Brix (EcoTox), David DeForest (Windward), and Lucinda Tear (Windward)

Subject: Analysis of Historical Mountain Whitefish Data

This memorandum describes the methods and results of a multivariate analysis of historical monitoring data for mountain whitefish (MW) collected in the Elk Valley. The first objective of this analysis was to determine whether factors such as gonado-somatic index (GSI), fish size (as measured by fork length), and fish sampling location influence observed ovary selenium (Se) concentrations in MW in the Elk Valley. The second objective of the analysis was to identify sampling locations and estimate the sampling intensity required (i.e., number of fish) to have a high probability of collecting MW in spawning condition with egg Se concentrations >33 mg kg^{-1} dw, the maximum egg Se concentration observed in previous efforts to conduct a selenium toxicity study with this species (Nautilus Environmental 2017).

Introduction

Historical and ongoing monitoring of MW in the Elk Valley indicate individual, and in some cases mean, ovary Se concentrations exceed the BC ENV guideline of 11 mg kg^{-1} dw, as well as the Level 1 Benchmark (18 mg kg^{-1} dw) and in some cases the Interim Screening Benchmark (29 mg kg^{-1} dw). This is true for ovaries collected from both reference and mine-exposed locations, although ovaries collected from fish in mine-exposed areas are on average higher than those collected from reference areas.

Given the ovary Se concentrations observed historically, Teck conducted three rounds (2010, 2011, and 2013) of toxicity testing in an attempt to estimate the sensitivity of the embryo-larval stage of MW to maternally transferred Se (Nautilus Environmental 2017). Combined, these studies demonstrated no effects on MW embryo-larval survival, growth, or development up to the highest egg Se concentration obtained (33 mg kg dw^{-1}). However, through 2018, ovary Se concentrations as high as 81 mg kg^{-1} dw have been observed through sampling under the Regional Aquatics Effect Monitoring Program (RAEMP) (Figure 1). Consequently, it is

important to understand whether effects are occurring in MW with egg Se concentrations >33 mg kg^{-1} dw and Teck anticipates conducting a fourth toxicity study on MW in the Fall of 2020.

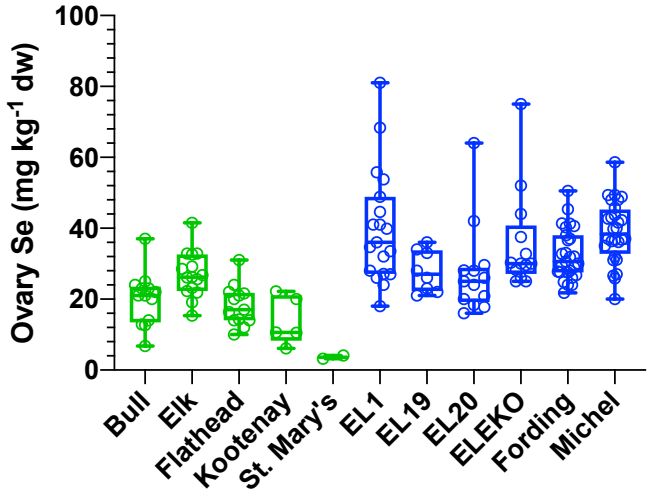


Figure 1. Ovary Se concentrations in mountain whitefish by location (2006-2018).

A preliminary review of the MW ovary Se data collected in 2018 demonstrates a negative correlation between GSI and ovary Se concentrations (Figure 2). The relationship is similar to that observed for northern pikeminnow (*Ptychocheilus oregonensis*; NPM). In NPM, females in spawning condition have a GSI >5%, while much of the historical ovary Se data were from fish with a GSI <5%, leading to an overestimation of egg Se concentrations likely to occur in NPM from Koocanusa Reservoir (EcoTox et al. 2020).

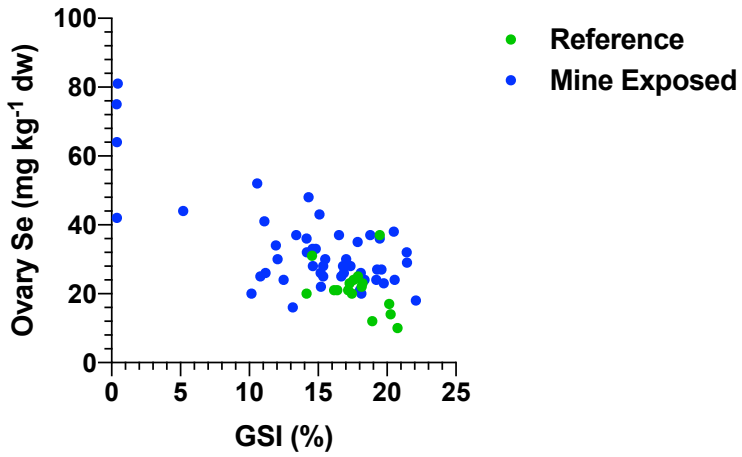


Figure 2. Relationship between GSI and ovary Se in mountain whitefish collected from Elk Valley in 2018.

Based on the preliminary analysis of the 2018 MW data, it is possible a similar bias exists in the ovary Se monitoring data for this species. This is a critical issue to understand with respect to developing a study plan for the 2020 MW Se toxicity study. If this negative relationship between GSI and ovary Se occurs across historical monitoring data, it will be important to understand the distribution of ovary Se data associated only with fish in spawning condition. This will inform the study design with respect to the sampling intensity required (i.e., number of females needing to be sampled) to have a high likelihood of collecting fish with egg Se concentrations >33 mg kg^{-1} dw. Similar to NPM, it is possible other factors (e.g., fish size and sampling location) also influence ovary Se concentrations (EcoTox et al. 2020). Understanding the importance of these factors can improve the sampling design for the 2020 MW Se toxicity study.

Data for Analysis

Historical monitoring data from 2006-2018 collected in the Elk River drainage and reference locations were considered in evaluating potential relationships between ovary Se concentrations and GSI, fish size (fork length), or sampling location. Data from 2012 were excluded from the analysis because information on GSI was not collected that year. Similarly, data from Koocanusa Reservoir were excluded from the analysis as GSI data were not available for fish collected from this location. In total, data from 156 fish were available for the analysis (Table 1).

Table 1. Ovary Se Sample Size by Location

	Year				
Location	2006	2009	2015	2018	Total
Mine Exposed Sites					
EL1	5	5		9	19
EL19				8	8
EL20			5	8	13
ELEKO			5	8	13
Fording	5	10	4	8	27
Michel	10	5	5	8	28
References Sites					
Bull River			5		5
Elk River	5	10			15
Flathead River			5	8	13
Kootenay River			5		5
St. Mary's River			2		2

Exploratory Analysis

An initial exploratory analysis of data was conducted using scatter plots and Principal Component Analysis (PCA) to provide visual and quantitative understanding of correlations among the variables of interest and how the relationships among those variables varied geographically. Fork length, GSI, and ovary Se were natural log (ln)-transformed, centered, and scaled for the PCA.

Scatter plots suggest that, similar to the 2018 data set (Figure 2), a relationship exists between GSI and ovary Se, while there does not appear to be a relationship with fish length (Figure 3).

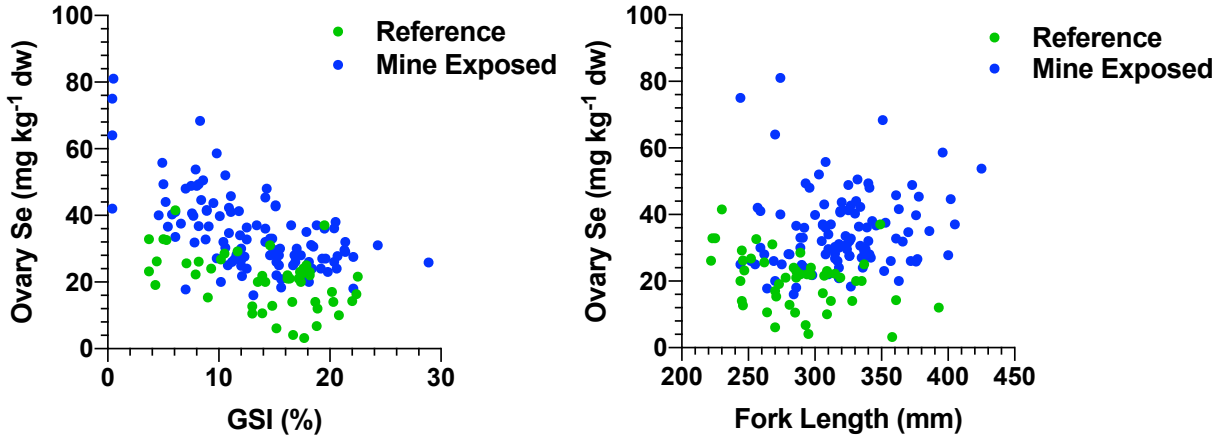


Figure 3. Relationships between GSI and fish length versus ovary Se using data summarized in Table 1.

The first two axes of the PCA explained approximately 88% of the variance. Axis 1 was most highly correlated with GSI (Figure 4) and explained 49% of the variance. Axis 2 was somewhat equally correlated with fork length and ovary Se and explained 38% of the variance. The biplot of PCA scores shows each of the variables having similar effects on the spread of the data (arrows indicating direction of increasing value of the associated variable are approximately equal length), and identifies four samples with very low GSI as quite separate from the rest of the data (far left of Axis 1).

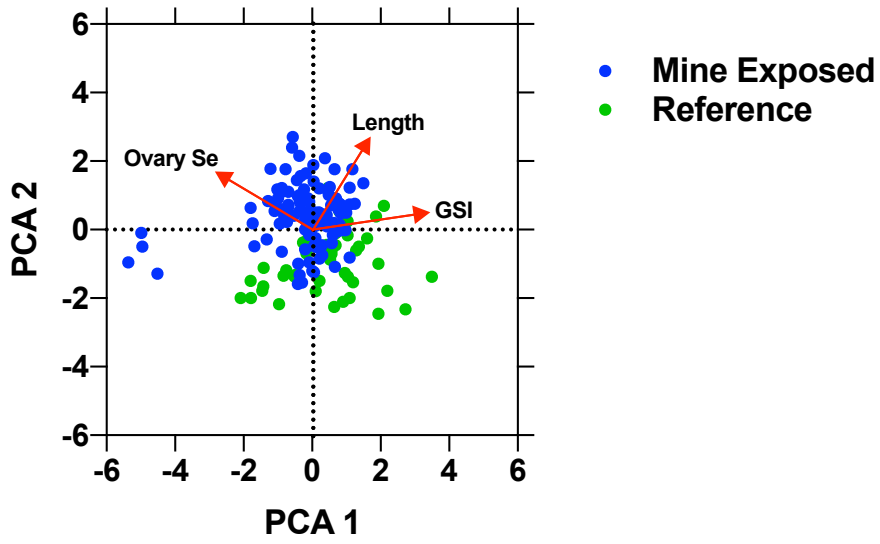


Figure 4. Biplot of Principal Component Analysis using ln-transformed fork length, GSI, and ovary Se. Arrows indicate direction of increasing values of the associated variable.

Multi-linear Regression (MLR) Analysis

Although the exploratory analyses indicated four data points differed from the bulk of the data, all data were retained in the initial MLR analyses. As in the exploratory analyses, all data were ln-transformed. A step-wise MLR model testing for linear effects of fork length and GSI with area-specific slopes and intercepts was run using the Akaike and Bayesian Information Criteria (AIC, BIC) to identify the most parsimonious models (R, stepAIC) (Equation 1).

$$Ln(OvSe) = area + Ln(FL) + area^* Ln(FL) + Ln(GSI) + area^* Ln(GSI) \quad \text{(Eq. 1)}$$

where, OvSe = ovary Se, FL = fork length (mm), and GSI = gonadosomatic index.

Model residuals were tested for normality using Shapiro Wilks (shapiro.test, R) and Nonconstant Variance (ncv, R).

The models identified by both AIC and BIC were identical. Fish size, as measured by fork length, was not retained in the model, indicating it does not have a significant influence on ovary Se. In contrast, GSI was highly significant ($p < 0.001$), but no area-specific slopes were retained, indicating the relationship between GSI and ovary Se is similar across sites. Area-specific intercepts were retained in the model, indicating significant differences in ovary Se among sites after correcting for the effect of GSI.

Two samples with low ovary Se collected from St. Mary's River (reference location) did not fit the model well. These are not part of the four samples with very low GSI identified in the PCA. The two samples from St. Mary's River were removed and the model was rerun to evaluate how these two outliers might be unduly influencing model parameterization. Results were similar to the first run, with GSI highly significant ($p < 0.001$) and area-specific intercepts with the same differences as the previous model.

Adjusted and predicted R^2 for the final model were 0.59 and 0.58, respectively (Figure 5). Model residuals were not normally distributed and did not have constant variance across the range of predicted values (Shapiro Wilks $p < 0.001$, Nonconstant Variance Test $p < 0.001$). Overall, the model appears to predict the mine-exposed sites reasonably well, but performs more poorly for the reference locations.

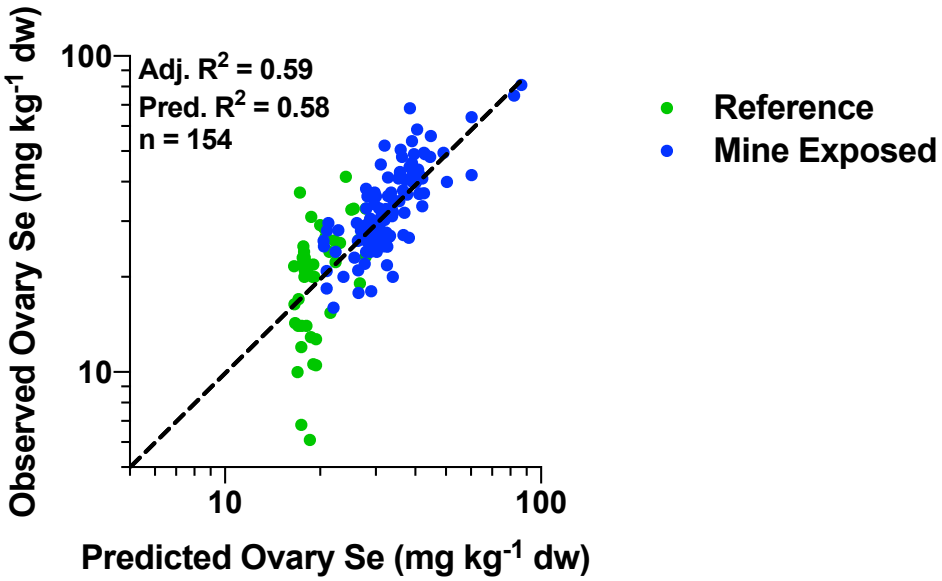


Figure 5. Final Linear Model Excluding Data from St. Mary's River.

The final model has a ln GSI slope of -0.288 and area-specific intercepts (Table 2). Treatment contrasts comparing Michel Creek to other locations indicate, with the exception of EL1, all areas have a significantly ($p < 0.05$) lower intercept than Michel Creek (Table 2). This means that after accounting for the effect of GSI on ovary Se concentrations, Michel Creek has significantly higher ovary Se concentrations than all locations except EL1.

Table 2. Final ovary Se model coefficients and significance. Note: The intercept t values and *p* values relate to testing for significant differences in intercepts relative to Michel Creek.

		Estimate	Std. Error	t Value	*p* Value
Intercepts	Reference	3.705	0.065	-10.10	<0.001
	EL1	4.261	0.081	-1.22	0.223
	EL19	4.106	0.109	-2.33	0.021
	EL20	3.836	0.092	-5.69	<0.001
	ELEKO	4.145	0.091	-2.34	0.020
	Fording	4.203	0.073	-2.15	0.033
	Michel	4.360	0.097	-	-
Slope	ln GSI	-0.288	0.033	-8.80	<0.001

Sample Size Analysis

The negative relationship between GSI and ovary Se provides evidence that this variable needs to be considered in evaluating sampling effort for future toxicity studies. Information on the GSI typically associated with MW in spawning conditioning is limited, but a recent study indicates a GSI of >15% is associated with spawning in this species (Irvine et al. 2017). The model also demonstrated that significant differences in ovary Se between sampling locations exist, with Michel Creek having the highest ovary Se on average. Given this information, we focused our sample size analysis on MW with a GSI >15% collected from Michel Creek.

The objective of the sample size analysis was to estimate the sampling intensity required to obtain egg Se concentrations with a specific level of confidence. Specifically, we wanted to estimate the number of female MW that will need to be sampled from Michel Creek to obtain eggs from at least 3 females with a given egg Se concentration with 90% confidence. Recalling that the highest egg Se concentration evaluated in previous toxicity testing efforts was 33 mg kg^{-1} dw, we estimated samples sizes needed to collect egg Se concentrations of 34, 36, 40, and 43 mg kg^{-1} dw. First, we calculated the proportion of fish captured with both a GSI >15% and specified ovary Se concentration as:

P(ovary Se >X and GSI >15%) = Count(ovary Se >X and GSI >15%)/Count(GSI >15%)

The proportions ranged from 0 to 0.50 with the highest proportions (as well as the highest number of fish) in Michel and Fording drainages. To estimate the sample size needed to collect at least 3 fish with a specific ovary Se, we assumed the proportion of fish with the specified P fit the binomial distribution.

P ~ Binomial(n,p)

where p = observed proportion of ovary Ovary Se fish = observed P, and n = sample size.

We estimated the number of samples required to be 90% confident of collecting at least 3 fish with the specified ovary Se fish for a range of p levels [P = 0.1, 0.2, 0.3, 0.4, 0.5] using the inverse of the binomial distribution [Excel function =BINOM.INV(*n, p, alpha*) or R function qbinom(1-*alpha*, *n*, *p*]. This was done by submitting a range of possible sample sizes (n = 5:100) to each function to find the minimum sample size at which 3 ovary Se samples would be collected with the desired confidence (*alpha* = 0.10). In parallel, we estimated the probability of collecting at least 3 ovary Se samples for the same range of sample sizes and p levels (Excel function =1-BINOM.DIST(2, P, *n*, cumulative = 1), pbinom(2, *n*, *alpha*, lower.tail = FALSE).

Results from this analysis indicate that even a relatively modest increase in the range of ovary Se concentrations of 5 mg kg^{-1} dw over the maximum concentration of the existing data can only be accomplished by sampling Michel Creek and would require sampling at least 28 female fish (Table 1). The sample size remains constant for ovary Se >38 mg kg^{-1} dw because the probability of capturing a fish remains constant. This is because the sample size for this analysis is small and unevenly distributed (n=11 for Michel Creek fish with GSI >15%). Consequently, there is some uncertainty in these estimates and caution should be exercised in using these values for sample design, i.e., sampling more fish than estimated by this analysis is recommended.

Table 1. Estimated Sample Size Required to Obtain 3 Female Mountain Whitefish with Specified Ovary Se Concentration with 90% Probability of Success Assuming GSI = 15%.

	Target Ovary Se Concentration				
Location	**34 mg kg^{-1} dw**	**36 mg kg^{-1} dw**	**38 mg kg^{-1} dw**	**40 mg kg^{-1} dw**	**43 mg kg^{-1} dw**
Michel Creek	9	13	28	28	28
Fording River	24	28	UC[1]	UC[1]	UC[1]

[1] Unable to calculate as no ovary samples with this Se concentration from fish with a GSI of at least 15% have been collected.

Conclusions

This analysis was conducted to provide information on the sampling design for the planned MW Se toxicity study. The objective of the toxicity study is to extend the range of concentration-response data above 33 mg kg dw^{-1}, the highest concentration observed over the course of three previous rounds of testing. This analysis demonstrated that ovary Se concentrations in MW are related to the GSI, but not the size, of female MW from which they are collected. After correcting for the effect of GSI using a linear model, differences in ovary Se between mine-

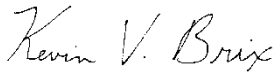
exposed and reference locations were detected, with fish collected from Michel Creek having the highest ovary Se.

A subsequent sample size analysis used historical monitoring data including only fish with a GSI >15%, the likely GSI associated with fish in spawning condition. The sample size analysis indicates that at least 28 female fish will need to be collected to have a high (90%) probability of collecting at least 3 fish that increase the range of the concentration-response data by 5-10 mg kg dw^{-1}. Based on this analysis, we recommend that at least 30 female fish from Michel Creek be sampled for the 2020 mountain whitefish toxicity study. We also recommend 5-10 fish from 2 reference locations be collected to provide controls for the study.

Closing

I trust that this analysis provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact me at (305) 773-8347.

Sincerely,



Kevin V. Brix, Ph.D.
Principal Scientist
EcoTox LLC

References

EcoTox, University of Saskatchewan and Minnow Environmental (2020). Draft Report - Evaluation of selenium concentrations in ovary of northern pikeminnow (*Ptychochelius oregonensis*). Miami, Florida, EcoTox LLC**:** 27 pp. + appendices.

Irvine, R. L., J. L. Thorley and L. Porto (2017). "When do mountain whitefish (*Prosopium williamsoni*) spawn? A comparison of estimates based on gonadosomatic indices and spawner and egg counts." Open Fish Sci. J. **10**: 12-22.

Nautilus Environmental (2017). Evaluation of the effects of selenium on early lifestage development of mountain whitefish from the Elk Valley, BC. Burnaby, British Columbia, Nautilus Environmental**:** 561 pp.

APPENDICES

2. Appendix B – Monitoring and Research Committee written recommendations

From:	Gildea, Jason
To:	Sullivan, Lauren; Reddekopp, Sheldon ENV:EX
Cc:	Myla Kelly; Epps, Deb ENV:EX; Schmit, Ayn; McGrath, Patricia; McLaughlin, Julianne; Beaman, Joe
Subject:	2020 08 28 J.Gildea-USEPA_recmds
Date:	Friday, August 28, 2020 4:10:24 PM
Attachments:	image003.jpg

[EXTERNAL] This email came from an external source. Only open attachments or links that you are expecting from a known sender.

Disclaimer: *Please note the comments and recommendations contained in this document are strictly for Montana's and British Columbia's consideration. The views expressed in these comments and recommendations are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Regarding Montana's submission, the comments do not constitute approval or disapproval decisions under CWA Section 303(c). Neither are these comments a determination by the EPA Administrator under CWA Section 303(c)(4)(B) that revised or new standards are necessary to meet the requirements of the Act. These comments and recommendations do not impose any binding requirements, determine the obligations of the regulated community, change or substitute for any statutory provision or regulation requirement, represent, change or substitute for any Agency policy or guidance, or control in any case of conflict between this discussion and statute, regulation, policy or guidance.*

Lauren and Sheldon,
Thank you for the opportunity to provide comments on the technical analysis for setting a site-specific criterion for Lake Koocanusa. Please consider the following as my formal submission of comments as a member of the Lake Koocanusa Monitoring and Research Committee (MRC) to the chairs of the Selenium Technical Subcommittee (SeTSC).

First, I would like to acknowledge the extensive, well vetted process for getting to this point in the criterion development. The Lake Koocanusa Monitoring and Research Working Group (LKMRWG), which includes the MRC and SeTSC, has been meeting regularly for six years with the primary goal of developing and implementing a site-specific selenium criterion for Lake Koocanusa. Multiple in-person and phone-based meetings occurred during this time period allowing committee members and other stakeholders ample opportunity to discuss technical issues, provide input, collect data, analyze data, understand reservoir hydrodynamics and chemistry, assimilate information, and to develop and understand the selenium model. Specifically during this time period, I note that the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019) were produced and significantly added to the group's understanding of the process and available data. These reports, along with all data and information from the meetings, are well organized and archived on a website maintained by the Montana Department of Environmental Quality (DEQ) and B.C. Ministry of Environment and Climate Change Strategies (ENV) (available at http://lakekoocanusaconservation.pbworks.com/

w/page/100633354/FrontPage). The six-year process has provided all stakeholders with substantial background information and has prepared us to fully comment on the site-specific criterion model in a timely manner.

I note that it is seven years since B.C. ENV issued Ministerial Order #M113, which states that, "the Minister of Environment wishes to reduce calcite formation and to manage water quality to stabilize and reverse increasing trends in the water contaminant concentrations." Despite this order, pollutant concentrations and loads continue to increase into Lake Koocanusa, as demonstrated at the B.C. water quality monitoring site located at the Elk River Hwy 93 Crossing (Figure 1). This information is alarming and highlights the need to immediately stabilize and reverse pollutant loads into Lake Koocanusa.

Given the six-year process leading up to this point, I believe that it is now imperative for DEQ and B.C. to quickly adopt and implement a site-specific selenium criterion for Lake Koocanusa. I fully support Montana DEQ's proposed schedule to propose and adopt the criterion by December 2020 and I believe that the currently available science allows you to do that. The CWA and EPA's regulations at 40 CFR Part 131 require states and authorized tribes to establish water quality criteria that are protective of the designated use and scientifically sound, and then require those states and tribes to periodically (at least once every three years) review and revise the criteria as needed. Therefore, I encourage you to use the science available to you now to develop a site-specific selenium criterion. As you know, Lake Koocanusa currently has a site-specific selenium objective of 2 µg/L in B.C. and a water column chronic criterion of 5 µg/L in Montana. The modeling results provided by USGS in their 2020 report demonstrate that protective selenium concentrations for Lake Koocanusa are likely lower than either of these values, necessitating the need for immediate action to revise the criterion to a protective value. Presser and Naftz, 2020, note that, "78 percent of predictions are <1.5 µg/L and at least 46 percent of predictions are <1 µg/L for protection of this community of core benthic feeders." Delaying criterion development in any way will exacerbate on-going degradation of the Lake Koocanusa ecosystem, particularly given that pollutant loads are increasing.

Thank you again for the opportunity to comment on the site-specific selenium criterion and I look forward to working with you in the future on Lake Koocanusa issues.

Jason Gildea
Sent via email August 28, 2020
Hydrologist, USEPA Region 8, Montana Office
Gildea.Jason@epa.gov
406-457-5028

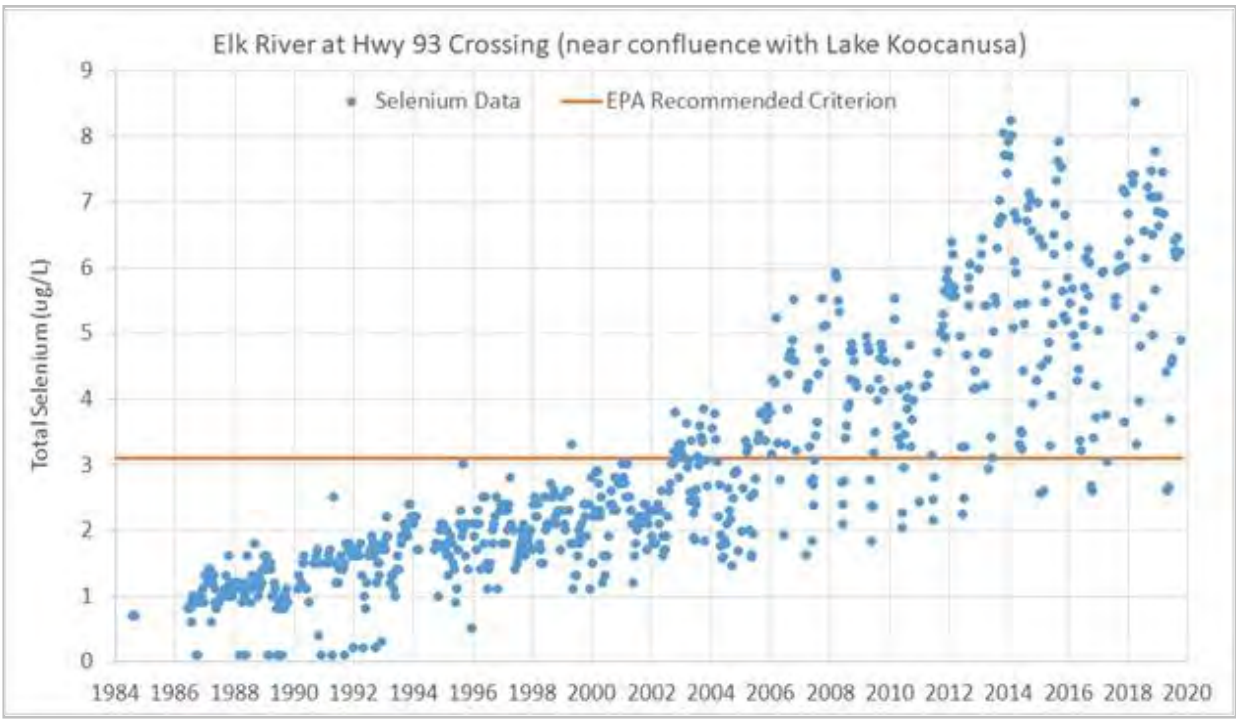


Figure 1. Selenium concentrations in the Elk River at HWY 93 crossing with EPA's recommended selenium criterion for lentic waters for comparison.

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Delivered via email to : TimDavis@mt.gov

August 28, 2020

Dear Mr. Davis and Mr. Moore:

Headwaters Montana works in northwest Montana to protect the water, wildlife, and traditional quiet outdoor recreation. We have over 2,000 subscribers. For the past several years we have been engaged in the Lake Koocanusa Monitoring and Research Committee (MRC) and the MRC's Selenium Technical Committee (SeTC) as an observer. We have appreciated being a part of the process and send this letter to provide you with our views and recommendation for the Montana selenium standard setting process that is now in its final phase.

First, we want to thank the DEQ and your dedicated staff for stepping up and taking on the significant task of working with BC to set this standard. It has proven to be an expensive and time-consuming process, but one which was necessary. Scientists are learning more and more about the toxic effects of selenium, and it has become apparent to us that the LKMRC process was necessary. Montana could have adopted the US EPA national standard. However, it was understood by many of the SeTC that selenium in Koocanusa had potentially more harmful and toxic effects. We think that the USGS model for selenium in Koocanusa proves this point and that the US EPA national standard would not have been protective of fish and aquatic life in the reservoir, and or would have had to be adjusted downward rather quickly after adoption.

Second, we would like to acknowledge that the standard-setting process is both a scientific and political one.

With respect to the science, it is clear that more and better data would have been helpful to the USGS modeling process. The USGS model was built with the scientific expertise of the most knowledgeable selenium experts in North America. We are confident that the essential components of the model reflect good scientific judgement. Headwaters Montana has played a role in securing congressional appropriations for the water quality monitoring on Lake Koocanusa, in particular the funding for the "super sipper" buoy deployed at the border. Our continuing efforts to secure addition federal funds for water quality monitoring should help ensure that the USGS has more robust data in the future.

The important point is that the USGS model represents the best available science at this point in time, and that future data will only make the model's predictions more reliable. Given that we can now expect BC and Teck to export even higher levels of selenium in the near future, we support the model and the goal of setting a selenium standard at the border in 2020 that is protective of aquatic life throughout the reservoir and downstream in the Kootenai River, including in Idaho and beyond.

During the August 25 MRC virtual meeting, some confusion was expressed by some on the MRC as to the "placement" of the selenium standard. Both BC and MT have used the motto, "one lake, one number" to describe the goal of the six-year effort. We want to be clear that we understand and expect the Montana standard will be set for the international border and does not represent a 'reservoir average' number.

With respect to the political decisions to be made by Montana on setting a selenium standard for the border, we understand that Montana will be in communication with both BC and Teck. We can anticipate some of their arguments and would like to address and correct several of them here. We think BC and Teck will argue for as high a number as possible, and one that will not protect US/Montana downstream interests. They will:

- Argue that the USGS is not scientifically valid. That no site-specific egg-ovary data or fish data were used. That 'generic' fish data was used. They will try to argue that the 'uncertainty' of the model should require additional data collection, study and analysis…. And delay. Our response to this, should BC and Teck make these arguments, is that 'uncertainty' argues for a more protective standard rather than a weak standard or delay, and that the standard should be set in 2020 and not delayed. The US and Montana will continue to collect data and the USGS model predictions will improve over time. After six years, further delay is not needed. It is better to approve now a protective standard that protects all aquatic life than study and delay.

In closing we would like to emphasize that:

- We support the MT-BC process
- We support the USGS model
- We support the timeframe for setting a selenium standard at the border before the end of 2020
- We support a standard that is conservative and protective of all aquatic life given the uncertainties of increased selenium loading, proposed new mines in the Elk Valley, and the future opportunity to refine the model – and the standard – as time goes by.
- We also support the Confederated Salish and Kootenai Tribes, the Kootenai Tribe of Idaho, and the Ktunaxa National Council's recommended number(s), that we anticipate will be at or below 1.0 ug/l.

Thank you for the opportunity to comment.

Sincerely,



Dave Hadden, Ex. Dir.
Headwaters Montana, Inc.
406-270-3184 / info@headwatersmontana.org

Deborah Epps
British Columbia Ministry of Environment
Deb.Epps@gov.bc.ca

Myla Kelly
Montana Department of Environmental Quality
MKelly@mt.gov

August 28, 2020

RE: development of site-specific selenium criteria for Lake Koocanusa

Dear Deborah and Myla,

Montana Department of Fish, Wildlife & Parks (MFWP) has been involved in monitoring and researching selenium (Se) in Lake Koocanusa fish since 2008. MFWP has participated in the Lake Koocanusa Monitoring and Research Working Group since its inception in 2015, and has been an active member of the Monitoring and Research Committee (MRC), including the collection of fish and macro-invertebrate samples on the reservoir. To that end, MFWP has been actively involved in the effort to develop a site specific Se criteria for Lake Koocanusa and is fully supportive of the process led by British Columbia (BC) and MDEQ.

Like many large rivers in Montana, Lake Koocanusa is a complex system that has many anthropogenic stressors to fish. However, despite alterations to natural flow, temperature and sediment regimes, the reservoir still supports a largely native fish component. Burbot are a native species in the reservoir that are listed as a species of concern and have high cultural, sport-fish, and ecological values. Their populations have been declining steadily since 1989, however the reason for their decline is unclear. Burbot are difficult to capture, especially females with eggs, resulting in limited data for this species. Unfortunately, not much is known about burbot sensitivity to Se since there is no surrogate species to reference. These uncertainties warrant a conservative approach for the development of a Se criteria for Lake Koocanusa.

Due to the uncertainties surrounding the variability of the data used in the models to develop a site-specific criteria and unknown sensitivities of fish species, a conservative approach to criteria setting is imperative. The tissue concentrations of Se found in several fish species in Lake Koocanusa currently exceed EPA criteria and BC guidelines. With tissue exceedances occurring from waterborne concentration of ~1 ug/L, it is evident that a site-specific criterion would need to be less than 1 ug/L to be protective based on the best available science. Montana cannot wait any longer to adopt site specific criteria for Lake Koocanusa, and as more data becomes available this number could be revised (if needed) during triennial reviews. Waterborne concentration of Se downstream of Libby Dam have also

been observed ~1 ug/L. Therefore, applying a conservative approach for the Lake Koocanusa criteria would also provide protection of downstream fish populations where tissue exceedances have also been documented and where the most sensitive species to Se toxicity, the endangered white sturgeon, is present.

Thank you for the opportunity to comment on the adoption of site-specific criteria for Lake Koocanusa and for all the efforts MDEQ and BCMOE has made toward this process.

Trevor Selch

Fisheries Pollution Biologist
Montana Fish, Wildlife & Parks

Cc:

Lauren Sullivan (MDEQ)
Sheldon Reddekopp (BC MOE)
Tim Davis (MDEQ)
Sean Moore (BC MOE)

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August 31, 2020

Ms. Myla Kelly
Water Quality Standards Section Supervisor
Water Quality Planning Bureau
Department of Environmental Quality
1520 East Sixth Avenue
Helena, MT 59620-0901

Ms. Deb Epps
A/Regional Director
Monitoring, Assessment and Stewardship
Ministry of Environment and Climate Change Strategy
PO Box 9334 Stn Prov Govt
Victoria, BC V8W 9N3

Re: Teck response to the USGS Sampling and Analysis Plan for the collection of selenium and associated water quality parameters in Lake Koocanusa

Dear Ms. Kelly and Ms. Epps,

In your roles as Co-Chairs of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG) and as requested during the working group meeting on August 26, 2020, I write to provide Teck's response to the recently published USGS Sampling and Analysis Plan and the process that will inform a draft selenium criteria for aquatic life in Koocanusa Reservoir. Given the nature of Teck's response, the Co-Chairs of the Selenium Technical Subcommittee (SeTSC) have been copied on this letter.

Teck has been operating for over 100 years to become a leading Canadian diversified natural resource company, committed to responsible mining and mineral development. Strong sustainability practices are core to how we do business and Teck's leadership in this area is recognized globally. For instance, Teck was named to the Dow Jones Sustainability Index in 2019 for our 10th straight year, and we were the top ranked mining company in the world on this index. Central to this is Teck's commitment to responsible management of water resources and to protecting water quality where we operate.

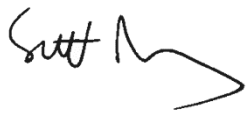
Over the last five years, Teck has made significant progress towards achieving the objectives of the Elk Valley Water Quality Plan (EVWQP), which is a long-term approach to addressing the management of selenium, nitrate and other substances released by current and historic mining activities in the Elk Valley. We forecast that our total investment in water quality management will be $1.2 billion through to 2024. This includes the water treatment facility at our Fording River Operations that is scheduled to complete in 2021. Additionally, Teck has more than 25 research and development projects underway, including the advancement of smaller, in-situ water treatment facilities that can be built closer to where treatment is needed. We are already seeing reductions in selenium and nitrate concentrations downstream of the Line Creek treatment facility, and we expect to see further significant reductions in other areas as future facilities come online. Details and information on our efforts can be found in the data and reports available online on Teck's website[1] and on the U.S. Environmental Protection Agency WQX Portal[2].

[1] Teck Resources Limited: "Responsible Mining in the Elk Valley".
[2] United States Environmental Protection Agency: "Water Quality Data (WQX)"

As members of the LKMRWG and observers to the SeTSC, we have made best efforts to understand and to provide input into the committee's work to inform a science-based and site-specific selenium recommended value for the Koocanusa Reservoir. This is a complex and multifaceted initiative and we appreciate the efforts that have gone into this work to date. It is our hope that the expertise and input of LKMRWG and SeTSC members and observers to this process is fully considered in your deliberations. We look forward to further engagement with the LKMRWG, SeTSC, Montana Department of Environmental Quality and the BC Ministry of Environment and Climate Change Strategy as this process continues to advance.

If you have any questions about our response to the USGS Sampling and Analysis Plan or Teck's approach to water quality management, please do not hesitate to contact me at Scott.Maloney@teck.com. We thank you for the level of engagement to date and for this opportunity to provide input directly to you for this process.

Sincerely,



Scott Maloney
Vice President, Environment

Cc: Tim Davis, Administrator, Water Quality Division, Montana Department of Environmental Quality
Lauren Sullivan, Water Quality Standards and Modeling, Montana Department of Environmental Quality
Kevin Jardine, Deputy Minister, Ministry of Environment and Climate Change Strategy
James Mack, Assistant Deputy Minister, Ministry of Environment and Climate Change Strategy
Sheldon Reddekopp, Sr. Environmental Assessment Biologist, Ministry of Environment and Climate Change Strategy
Katherine Gizikoff, General Manager, Environment and Social Responsibility, Teck Resources Limited
Carla Fraser, Manager, Water, Environment, Teck Resources Limited
Tom Syer, Head, Government Affairs, Teck Resources Limited
Meera Bawa, Manager, Regulatory Affairs, Teck Resources Limited

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Teck comments on the U.S. Geological Survey Lake Koocanusa Open-File Report 2020–1098

Overview

The comments below are Teck's response to the above-referenced open-file report developed for Lake Koocanusa by the U.S. Geological Survey (Presser and Naftz 2020). In summary, we believe there are a number of misleading and inaccurate statements within the report, and, despite the importance of site-specific fish tissue data, it was not used as a means to validate the model. As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the Koocanusa Reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion.

Misleading and inaccuracies within the report

The U.S. Geological Survey (USGS) fails to acknowledge that Lake Koocanusa has been listed under section 303(d) of the U.S. *Clean Water Act* as "impaired" since 2002 due to hydro-modifications, specifically Libby Dam[3]. By focusing on the 2012 reporting year, Presser and Naftz (2020) obscure this fact and create the potential for readers to inaccurately conclude that the listing is due to selenium concentrations. This is further exasperated by an incomplete analysis of data collected at the mouth of the Elk River by Environment and Climate Change Canada.

As noted by Presser and Naftz (2020), aqueous selenium concentrations measured at the mouth of the Elk River above Highway 93 (BC08NK0003)[4] have increased during the period of record, but incorrectly identify this trend as *continuing* to increase. Rather and as illustrated within Figure 1 below, selenium concentrations at the mouth of the Elk River have not increased since 2014. On the contrary, not only have selenium concentrations stabilized but a negative slope for the best-fit linear regression line suggests that concentrations are decreasing. This trend is wholly consistent with the goals and intentions of the Area Based Management Plan to "stabilize and reverse increasing trends in water contaminant concentrations". Furthermore, the suggestion that selenium loads are and will continue to increase is not supported by data.

[3] https://ofmpub.epa.gov/waters10/attains_waterbody.control?p_au_id=MT76D003_010&p_cycle=2002.

[4] http://aquatic.pyr.ec.gc.ca/webdataonlinenational/en/SiteDetails/BC08NK0003/Projects/PYLTM/Regions/0

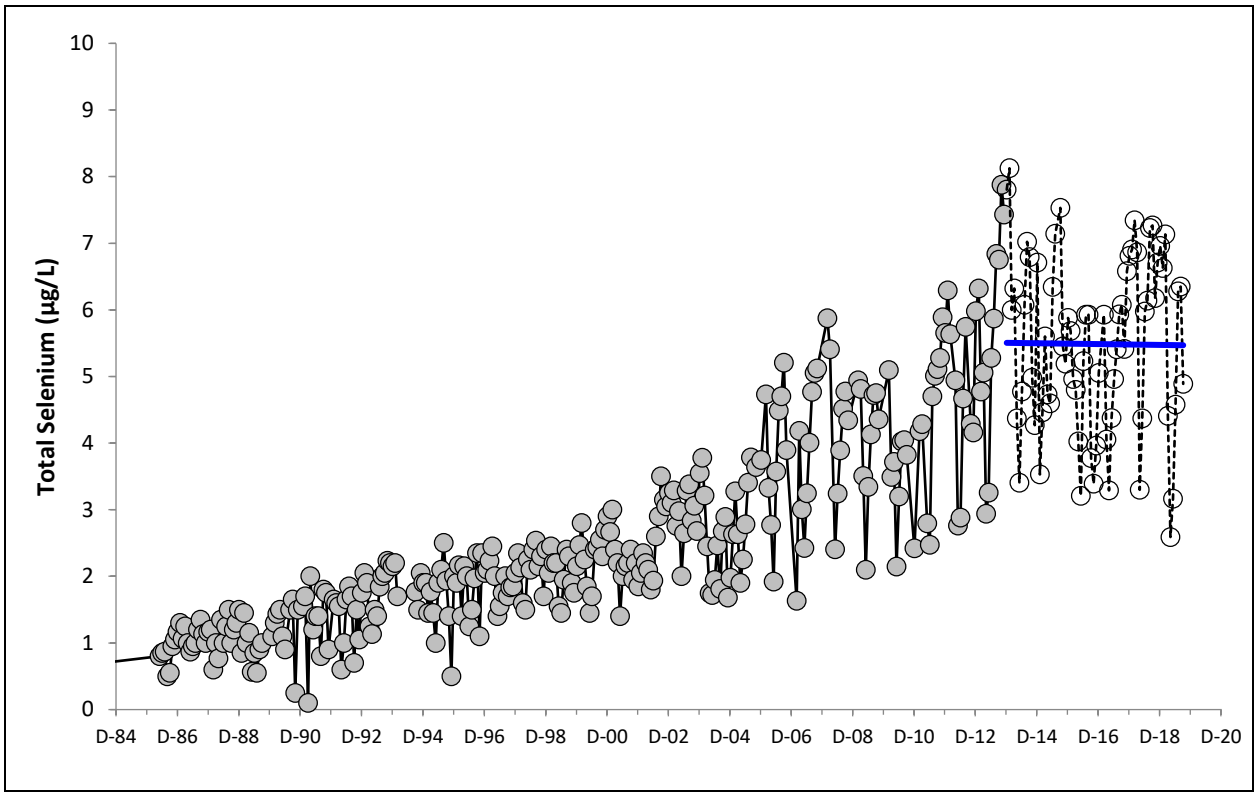


Figure 1. Monthly Average Total Selenium Concentrations as Recorded at the Mouth of the Elk River by Environment and Climate Change Canada.

Note: Data points are a monthly mean concentrations, while the blue line is a best-fit linear regression for data collected from 2014 to 2019 inclusive and has a slope of -1.7×10^{-5}.

Annual selenium loads from the Elk River into the reservoir have not been increasing and are inconsistent with gradient maps presented within the report. Presser and Naftz (2020) suggest that cumulative cross-sectional areas with selenium concentrations greater than 1 µg/L have increased three- (from 2017 to 2018) and four-fold (from 2017 to 2019) due to increasing selenium loads from the Elk River. Selenium concentrations (see Figure 1) and loads (see Figure 2) from the Elk River are inconsistent with the aforementioned suggestion.

Presser and Naftz (2020) correctly identify that the increase in proportional area of the reservoir containing selenium concentrations >1 µg/L suggests a system that is not in steady state, but fail to directly account for the significant role Libby Dam and its hydrodynamic cycles plays in this matter. Although Presser and Naftz (2020) identify Lake Koocanusa as a highly modified hydrological and ecological system, and that it experiences traditional problems associated with dam management (e.g., large elevation changes during drawdown and refilling operations), the report does not wholly consider the ramifications of such annual fluctuations particularly on the British Columbia side of the reservoir. As demonstrated by Dr. M. Sokal during the October 2016 Monitoring Research Committee meeting in Kalispell, Montana, Lake Koocanusa's hydrologic cycle significantly influences water quantity within the Canadian portion of the reservoir. During high water conditions (Figure 3, left panel) the reservoir is hydrologically like a "lake"; while under low water conditions (Figure 3, right panel) it is more like a "river" (Sokal 2016).

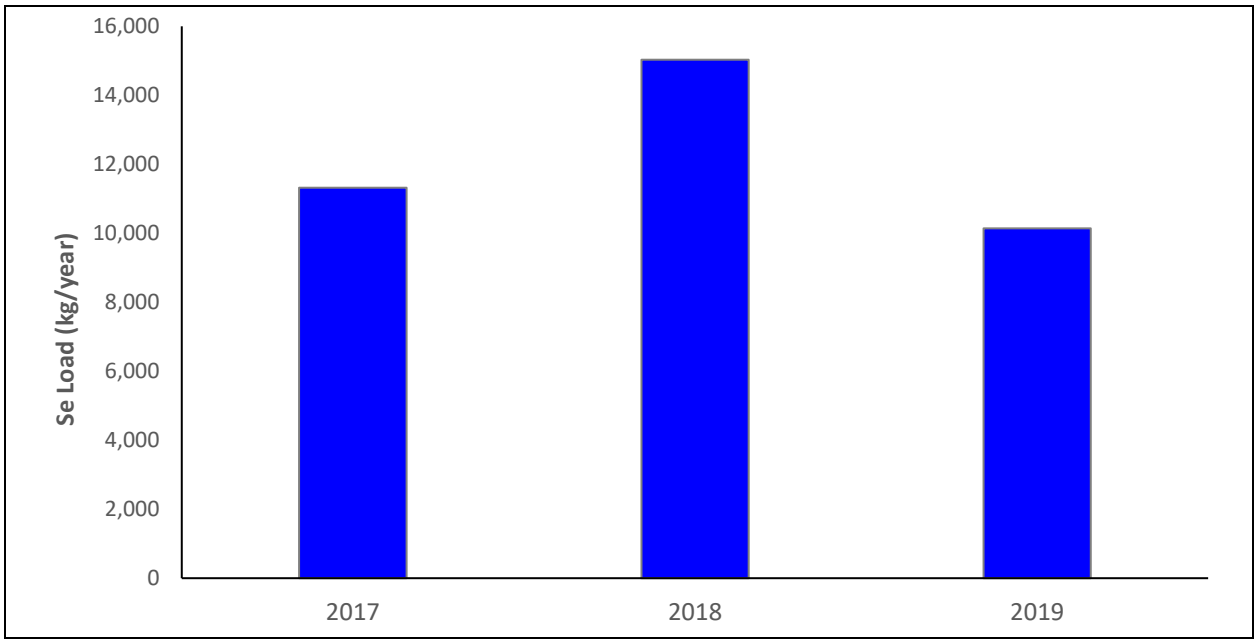


Figure 2. Selenium Loads associated with the Elk River into Lake Koocanusa.

Note: Selenium loads (2017 = 11,316 kg, 2018 = 15,035 kg, and 2019 = 10,144 kg) as determined from annual monitoring reports for the reservoir submitted to the ENV (Minnow, 2019 and 2020, and Teck 2018).

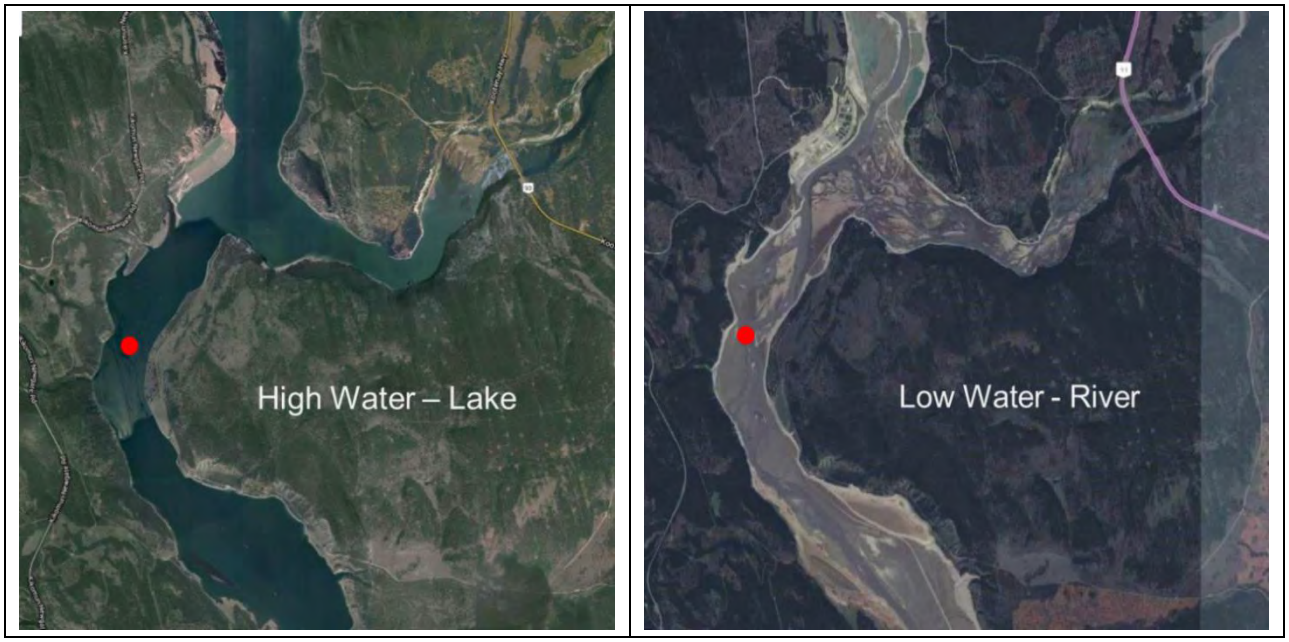


Figure 3. Aerial Imagery of Lake Koocanusa near the Confluence of the Elk River during High- (left panel) and Low-Water (right panel) Conditions.

Note: Imagery obtained from PowerPoint presentation made by Michael Sokal, PhD, Environmental Protection Division, BC ENV at the Lake Koocanusa Monitoring Research Working Group Meeting Kalispell, MT (October 17-18, 2016; Meeting No. 5). The red dot illustrated within the images reflects Order Station Environmental Monitoring System (EMS) E300230 (RG_DSELK).

Lack of model validation via fish tissue data

Model validation was limited to evaluating ranges of observed and predicted selenium concentrations in invertebrates (zooplankton and macroinvertebrates). Based on the qualitative evaluation presented within Presser and Naftz (2020), it is clear that unless an adjustment of selenium bioavailability is made, the model grossly over-predicts invertebrate concentrations. Even with a bioavailability assumption of 60 percent, the model over-estimates invertebrate concentrations. This over-estimation is carried through to the endpoint of primary concern (fish tissue).

For instance, assuming a bioavailability of 60 percent predicted whole-body fish tissue concentrations are two times greater than measured data (e.g., site-specific mean = 5.4 mg/kg dw, n = 22). Furthermore, if one considers fish muscle tissue concentrations relative to the U.S. Environmental Protection Agency's criterion which identifies a limit of 11.3 mg/kg dw for adult fish muscle this positive bias increases to a factor of three (e.g., site-specific mean = 3.8 mg/kg dw, n = 120). As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion. Therefore and consistent with recommendations made to Monitoring and Research Committee Co-Chairs on December 4, 2015, selenium criteria/objectives should focus on empirical data, specifically fish tissues, and be evaluated relative to the U.S. Environmental Protection Agency (2016) fish tissue-based selenium criteria which are protective of fish.

As a result, the question at hand for the Monitoring and Research Committee is better expressed in terms of environmental monitoring data within the reservoir, in relation to existing water quality criteria/guidelines. The presumption being that existing/pending water quality criteria/guidelines are, as intended, protective of aquatic life within the reservoir. This is different than what is currently being expressed which assumes that existing/pending water quality criteria/guidelines are not protective of aquatic life within the reservoir.

Process toward a recommended value

According to the BC-MT 2020 Work Plan, revised May 1, 2020, the Co-Chairs of the SeTSC will finalize a technical assessment report and recommendations of a draft selenium criteria for consideration by Montana Department of Environmental Quality and by the British Columbia Ministry of Environment and Climate Change. It is not clear whether the Co-Chairs will include responses to comments on the USGS report submitted by SeTSC members and observers. In the interest of transparency and in recognition of the deep technical expertise on the SeTSC, we recommend that the report include how SeTSC member and observer input was considered to inform the recommended value.

Appendix: references

Presser, T.S., and Naftz, D.L., 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020–1098, 40 p., https://doi.org/ 10.3133/ ofr20201098.

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wildsight

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LKMRC Co-chairs:
Tim Davis, Montana DEQ
Sean Moore, BC MoE

SeTSC Co-chairs:
Sheldon Reddekopp, BC MoE
Lauren Sullivan, Montana DEQ

August 28, 2020

Thank you for the opportunity to provide input on the selection of the shared water column selenium limit to protect aquatic life for Lake Koocanusa.

Wildsight is strongly supportive of conservative, protective selenium limit for Lake Koocanusa, both for the protection of aquatic life in the reservoir and downstream in the Kootenai/Kootenay River and even in Kootenay Lake. As a general principle, we believe that modelling assumptions should be conservative and err on the side of caution in order to ensure protection for all species.

We recognize that ecological modelling will never be as precise or simple as we may wish it to be, nor will we ever have all the data we'd like; however, the USGS report and the SeTSC process that came before it have been open, transparent and comprehensive, involving detailed data collection over a number of years and peer-reviewed analysis from well-regarded experts on selenium toxicology. It is time to act on this data and modelling to set a protective selenium limit for Koocanusa, rather than allowing further increases in selenium with potentially disastrous effects on aquatic life. The USGS report makes it clear that selenium levels in Koocanusa must be reduced. Work to set a protective limit and to reduce selenium concentrations below that limit must begin immediately.

Specifically, a protective standard must be based on a protective whole-body selenium limit. We support the Ktunaxa Nation, KTOI and CSKT in calling for a 5.6 mg/kg dw whole-body limit, based on BC's 11 mg/kg egg/ovary Water Quality Guideline, which includes an uncertainty/safety factor of 2, and a conversion factor of 1.9 for rainbow trout. In this case, a median K_d of 4547 would be appropriate, as BC's Water Quality Guideline already includes an uncertainty/safety factor. However, if the limit is based on the EPA's limit of 8.5 mg/kg dw, we believe a more conservative approach is needed. In the Aquatic Life Ambient Water Quality

Criterion for Selenium, the EPA uses the 20th percentile of the translations of the egg-ovary criterion concentrations to water column concentrations to provide a "high probability of protection for most aquatic systems".[1] In this case, using a 80th percentile K_d of 5566 would be appropriate and provide an equivalent degree of protection to that intended in the EPA standard. Using a median or other lower K_d would not be appropriate if the EPA standard of 8.5 mg/kg is used.

With the very steep curve relating selenium egg/ovary concentrations to mortality, and relatively widely distributed data for selenium concentrations for individuals of the same species found in Koocanusa, it is clearly necessary to take a cautious, protective approach, which favours the lower BC whole-body limit with its uncertainty/safety factor.

We also support the 5.6 mg/kg whole-body limit to allow for preferred Ktunaxa fish consumption rates without exceeding human health limits.

We share the concerns expressed by some SeTSC members about lag times for biological effects from increasing selenium concentrations in Koocanusa. Lag times for selenium moving from water to sediment and at the various trophic levels could lead to an underestimation of the risk to aquatic life from the measured selenium concentrations. This concern should further strengthen the case for a standard with a reasonable safety margin to ensure protection.

We support the use of the W6 model (TFM w TL3 100% aquatic insects) to protect burbot and mountain whitefish. Using this model with the 5.6 mg/kg limit and a median K_d leads to a 0.6µg/L water limit, while using the model with a 8.5 mg/kg limit and a 80th percentile K_d leads to a 0.8µg/L water limit.

Additionally, a conservative approach is needed to protect downstream species in the Kootenai/Kootenay River and Kootenay Lake. Of particular concern are white sturgeon, endangered in both Canada and the US, and burbot. We note that elevated selenium levels in water above 1µg/L have been found in both the US and Canadian portions of the river and recent KTOI data shows significant selenium levels in fish tissue. With the Canadian portion of the Kootenay River between the US border and Kootenay Lake, as well as the Kootenay River delta into Kootenay Lake, designated as critical habitat for white sturgeon under Canadian SARA, it is crucial that a protective standard be set for Koocanusa that ensures protection for white sturgeon in this critical habitat. While we are not aware of any current tissue data available for sturgeon in Canada, we note the sensitivity of the species in the EPA Criterion, the recent troubling KTOI data that showed selenium in fish tissue above EPA and BC standards, and BC water quality data showing selenium exceeding 1.0µg/L in the Kootenay River at Creston and exceeding 0.6µg/L in the southern portion of Kootenay Lake[2]. Clearly, more study is needed, but these pieces of evidence already available should be highly concerning. A conservative

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[1] Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf, p. 92.

[2] Data from BC EMS, Locations IDs E206587 and E216952

standard should be set immediately in Koocanusa, the only significant source of downstream selenium, while work is done to quantify the effects of selenium concentrations in the Kootenai/Kootenay River and Kootenay Lake on sturgeon, burbot and other species.

Comparing the USGS model to the EVWQP model

We have heard concerns about the validity of the USGS model and the assumptions made for inputs to the model. In this context, we believe it is constructive to compare the USGS modelling to the modelling done to support the selenium limits set under Teck's EMA discharge permit in the Elk Valley, based on the 2014 Elk Valley Water Quality Plan.

While converting from the established thresholds for egg/ovary selenium to whole body values may not be ideal, it is necessary to use the existing, available data and to accept that obtaining reliable egg/ovary data is difficult. Here we compare the USGS approach to Teck's approach for the EVWQP modelling in 2014[3]. While Teck's approach uses egg/ovary data directly, the study on which the EVWQP modelling is based gives a large range for the 95% confidence interval of 12-31µg/L for WCT reproductive EC_{10} in eggs/ovaries, with a result that the derived selenium concentrations in water for 10% effect size should be subject to large uncertainties, though no uncertainties are given. Consequently, the calculated water concentration for 10% effect size and the water limit based on this concentration should be subject to a large degree of uncertainty.

We believe the USGS approach, based on established egg-ovary criteria, is far superior to the species-specific approach taken by Teck. The conversion to whole body values may increase uncertainty to a small degree, but far less than relying on one study with eggs from a few fish to determine thresholds.

We also have heard significant concern about widely varying values used in the USGS model, especially K_d values. In Teck's EVWQP modelling, we also observe very wide ranges of values in matched samples for water to invertebrates and invertebrates to fish eggs. We believe the USGS conclusions are far better supported by the data than the conclusions in the EVWQP modelling, because the data used by the USGS is mostly in the relevant ranges of selenium concentrations, while Teck's data is largely outside the relevant ranges. The overwhelming majority of the data used by Teck is significantly below or significantly above the relevant egg/ovary and water concentrations. Teck's data is also from a mix of lentic and lotic environments.

Overall, if BC is willing to rely on the relatively weak modelling behind the EVWQP to protect fish in the Elk River Watershed, they should also be willing to immediately adopt limits based on the modelling in the stronger USGS study. In any case, uncertainty in the data or the model should

[3] This modelling work was done by Golder Associates.

lead to a more protective limit in order to keep aquatic life safe, rather than further delay while selenium in the reservoir is already above safe limits in water and in fish tissue.

Timeline for adoption

We support Montana's plan to adopt a protective limit in 2020 and urge BC to also adopt the same limit this year. Increasing selenium concentrations in recent years have already put fish and other aquatic life at risk. We cannot afford to continue to allow selenium concentrations to increase. BC must provide a regulatory framework to give strong incentives for Teck to immediately reduce selenium loadings entering Lake Koocanusa to safe levels. We cannot continue to rely on promises of future selenium reductions, while mining and selenium-leaching waste rock dumps expand daily.

Additionally, BC needs to take immediate action to characterize the full timescale of the selenium leaching problem and to evaluate proposed solutions such as active water treatment or saturated rock fills in that context. BC needs to ensure that Teck meets this Koocanusa limit not only in the immediate future, but also in the long term, after mining ends and for the centuries or longer that selenium leaching will continue. BC must not allow a situation where $100 million for operating and capital costs is required annually to maintain water treatment in perpetuity in order to meet the Koocanusa limit.[4]

With Teck's plans to nearly double the amount of selenium-leaching waste rock over the next 20 years per the 2019 Implementation Plan Adjustment, it is essential that BC provide a clear and strong regulatory signal to Teck immediately, in order to ensure the full costs of mitigating pollution from that waste rock, if such mitigation is possible, are included in Teck's plans—and selenium levels are not allowed to increase past the point where Teck is able to reduce selenium below the Koocanusa limit.

The adoption of the conservative, protective limit for Koocanusa this year would also enable effective environmental assessments over the next two years, both federally and provincially, for Teck's Castle Project and North Coal's Michel Project.

Geographic application of the limit

We urge BC to adopt the shared Koocanusa limit in concert with Montana and to apply that limit to the entire Canadian portion of Lake Koocanusa. Fish may be resident in certain areas of

[4] Teck 2019 Annual Report, p. 13 indicates an estimated long-term operating cost for water treatment of $3 per tonne and annual production of roughly 24 million tonnes, leading to an estimate of $72 million annually. Including capital costs for replacement of treatment facilities would increase this estimate significantly. However, the report states that current operating costs are $31 million ($1.30 per tonne) with only two small facilities in operation and 14 or more additional facilities are planned. This estimate appears to be highly optimistic and the true cost may be much higher.

the reservoir, especially smaller species like redside shiner and peamouth chub, which have both shown tissue concentrations well above BC and EPA limits. We oppose Teck's proposal in their 2019 EVWQP Implementation Plan Adjustment to measure selenium concentrations in Lake Koocanusa as an average of four sampling sites distributed from the Elk River to the border.

In particular, BC should adopt the Koocanusa limit at the current LK2 (South of the Elk River) permit order station. If there are concerns about full mixing at this location, BC should allow an average concentration of a reservoir transect, at various depths, to measure compliance with the limit. The limit should also apply at the border, in the Forebay, and at any other sampling locations in BC or Montana.

Sincerely,

Lars Sander-Green
Mining Lead
Wildsight

Appendix F

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 8
1595 Wynkoop Street
Denver, CO 80202-1129
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February 25, 2021

Ref: 8WP-CWQ

Steven Ruffatto
Chair, Montana Board of Environmental Review
Montana Department of Environmental Quality
Metcalf Building, 1520 East Sixth Avenue
P.O. Box 200901
Helena, Montana 59620-0901

Subject: EPA's action on Montana's Revised Selenium Criteria for Lake Koocanusa and the Kootenai River (ARM 17.30.632 & ARM 17.30.602(32))

Dear Mr. Ruffatto:

The U.S. Environmental Protection Agency (EPA) has completed its review of Montana's revised water quality standards (WQS) and is approving the Administrative Rules of Montana (ARM) 17.30.632 and 17.30.602(32) as described in the enclosure to this letter. Receipt of the submission on December 28, 2020, initiated EPA's review of the revised WQS pursuant to Section 303(c) of the Clean Water Act (CWA) and the implementing federal WQS regulation (40 C.F.R. Part 131). The submission included: (1) the revised WQS adopted by the Board of Environmental Review on December 11, 2020 now codified at ARM 17.30.632 and 17.30.602(32); (2) rulemaking documents including a Technical Support Document, public notices, public comments, and response to comments; (3) transcript of the public hearing on November 5, 2020; and (4) Special Assistant Attorney General's certification that the WQS were duly adopted pursuant to state law. Although the new and revised rules took effect under state law on December 25, 2020, the EPA's approval under CWA Section 303(c) is required before the WQS are effective for CWA purposes.

Clean Water Act Review Requirements

CWA section 303(c)(2), requires states and authorized Indian tribes[1] to submit new or revised WQS to EPA for review. EPA is required to review and approve, or disapprove, the submitted standards. Pursuant to CWA § 303(c)(3), if EPA determines that any standard is not consistent with the applicable requirements of the Act, the Agency shall, no later than the ninetieth day after the date of submission, notify the state or authorized tribe and specify the changes to meet the requirements. If such changes are not adopted by the state or authorized tribe within ninety days after the date of notification, EPA is to promptly propose and then promulgate such standard pursuant to CWA section 303(c)(4). The Region's

[1] CWA section 518(e) specifically authorizes EPA to treat eligible Indian tribes in the same manner as states for purposes of CWA section 303. *See also* 40 C.F.R. § 131.8.

goal has been, and will continue to be, to work closely with states and authorized tribes throughout the water quality standards development process to ensure that statutory and regulatory requirements are clear. Pursuant to 40 C.F.R. § 131.21(c), new or revised state standards submitted to EPA after May 30, 2000, are not effective for CWA purposes until approved by EPA.

Today's Action

Montana adopted revised selenium criteria for the protection of the Class B-1 designated uses[2] for the portions of Lake Koocanusa and the Kootenai River (summarized in Table 1) in Montana. 40 C.F.R. § 131.11 describes the regulatory requirements for water quality criteria. Today's action addresses submitted changes to ARM 17.30.602(32) and 17.30.632 that include new or revised WQS requiring EPA's review and action under CWA section 303(c). EPA is approving ARM 17.30.602(32) and 17.30.632, except for portions of ARM 17.30.632(4) and 17.30.632(6) that EPA has determined are not new or revised WQS requiring EPA action pursuant to CWA section 303(c). The rationale for EPA's decisions is in the enclosure.

Selenium criteria adopted by Montana for Lake Koocanusa and the Kootenai River

Media Type	Fish Tissue		Water Column
Criterion Element	Egg/Ovary	Whole Body or Muscle	Monthly Average Exposure
Magnitude	15.1 mg/kg dw	Whole Body 8.5 mg/kg dw Muscle 11.3 mg/kg dw	Lake Koocanusa 0.8 µg/L Kootenai River 3.1 µg/L
Duration	Instantaneous measurement	Instantaneous measurement	30 days
Frequency	Not to be exceeded	Not to be exceeded	Shall not be exceeded more than once in three years, on average

Endangered Species Act Requirements

EPA's approval of Montana's revised selenium criteria submitted on December 28, 2020 is in compliance with the Endangered Species Act (ESA), 16 U.S.C. § 1536 *et seq*. Under Section 7(a)(2) of the ESA, EPA must ensure that its approval of these modifications to Montana's WQS is not likely to jeopardize the continued existence of threatened and endangered species or result in the destruction or adverse modification of designated critical habitat of such species. EPA initiated consultation with the US Fish and Wildlife Service (USFWS) regarding the potential effects of this action on April 28, 2020 via an email sent to Jacob Martin, Assistant Field Supervisor, Montana Ecological Services Field Office. EPA kept the USFWS apprised of the state's development of the criteria throughout 2020. EPA sent a final Biological Evaluation to the USFWS on February 18, 2021. EPA received a letter from the USFWS on February 25, 2021 concurring with EPA's determination that approval of Montana's revised water quality standards for selenium "may affect, but is not likely to adversely affect" either the bull trout and its designated critical habitat or the white sturgeon within the action area.

[2] Class B-1 includes the following designated uses: drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply. See ARM 17.30.609 and ARM 17.30.623.

Indian Country

EPA's approval of Montana's submitted WQS does not extend to Indian country as defined in 18 U.S.C. §1151. Indian country generally includes (1) lands within the exterior boundaries of the following Indian reservations located within Montana: the Blackfeet Indian Reservation, the Crow Indian Reservation, the Flathead Reservation, the Fort Belknap Reservation, the Fort Peck Indian Reservation, the Northern Cheyenne Indian Reservation, and the Rocky Boy's Reservation; (2) any land held in trust by the United States for an Indian tribe; and (3) any other areas that are "Indian country" within the meaning of 18 U.S.C. §1151. Today's action is not intended as an action to approve or disapprove WQS for waters within Indian country. EPA, or eligible Indian tribes, as appropriate, retain responsibilities under CWA section 303 in Indian country.

Conclusion

EPA commends Montana for collaborating with multiple stakeholders for over five years to develop a site-specific selenium water column element for Lake Koocanusa consistent with the approaches recommended by EPA for developing site-specific selenium criteria. The adoption of fish tissue criterion elements for Lake Koocanusa as well as fish tissue elements and a water column criterion element for the Kootenai River that are the same as the current EPA recommended selenium criterion elements are also important improvements. We thank Montana for your work to protect and improve these waters and look forward to continued partnership in this watershed. If you have any questions, please contact Tonya Fish on my staff at fish.tonya@epa.gov.

Sincerely,

JUDY BLOOM

Digitally signed by JUDY BLOOM
Date: 2021.02.25 15:31:06 -07'00'

Judy Bloom
Manager, Clean Water Branch

Enclosure

Rationale for the EPA's Approval of Revised Selenium Criteria for Lake Koocanusa and the Kootenai River (ARM 17.30.632 and ARM 17.30.602(32))

Water quality standards (WQS) include: (1) designated uses; (2) water quality criteria that support the designated uses; (3) antidegradation requirements; and optional general policies. 40 C.F.R. Part 131. At issue in this action are water quality criteria for selenium adopted by Montana for the protection of the Class B-1 designated uses[3] in Lake Koocanusa and the Kootenai River (ARM 17.30.632 and ARM 17.30.602(32)).[4]

1. Clean Water Act and 40 C.F.R. Part 131 Requirements Relevant to Water Quality Criteria

Clean Water Act (CWA) section 101(a)(2) establishes as a national goal the achievement of water quality that provides for the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water. CWA section 304(a)(1) requires EPA to develop and publish and, from time to time, revise national recommended criteria for protection of water quality and human health that accurately reflect the latest scientific knowledge. Water quality criteria developed under CWA section 304(a) are based solely on data and scientific judgments on the relationship between pollutant concentrations and environmental and human health effects. CWA section 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting pollutant concentrations in ambient water.

EPA uses Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (1985) (commonly referred to as the "1985 Guidelines" or "Aquatic Life Guidelines" and hereafter referred to in this document as "Aquatic Life Guidelines") to derive 304(a) criteria recommendations to protect aquatic life from the effects of toxic pollutants. These Aquatic Life Guidelines describe an objective way to estimate the highest concentration of a substance in water that will not present a significant risk to the aquatic organisms in the water. This EPA method relies primarily on acute and chronic laboratory toxicity data for aquatic organisms from eight taxonomic groups reflecting the distribution of aquatic organisms' taxa that are intended to be protected by water quality criteria.

EPA's WQS regulation at 40 C.F.R. Part 131 interprets and implements CWA sections 101(a)(2) and 303(c). 40 C.F.R. § 131.11(a)(1) requires that water quality criteria adopted by states and authorized tribes[5] "be based on sound scientific rationale and must contain sufficient parameters or constituents to protect the designated use." For waters with multiple use designations, the criteria must support the most sensitive use. Designated uses are those uses specified in WQS for each water body or segment whether or not they are being attained (40 C.F.R. § 131.3(f)). In other words, designated uses establish the environmental objectives for each water body (*e.g.*, aquatic life, recreation, drinking water, agriculture,

[3] Class B-1includes the following designated uses: drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply. See ARM 17.30.609 and ARM 17.30.623.
[4] See www.mtrules.org/gateway/Subchapterhome.asp?scn=17%2E30.6.
[5] CWA section 518(e) specifically authorizes the EPA to treat eligible Indian tribes in the same manner as states for purposes of CWA section 303. See also 40 C.F.R. § 131.8.

etc.). Numeric criteria may be based on EPA's CWA section 304(a) guidance, CWA section 304(a) guidance modified to reflect site-specific conditions, or other scientifically defensible methods (40 C.F.R. § 131.11(b)). CWA section 510 and EPA's CWA implementing regulations allow states to adopt water quality standards that are more stringent than may be strictly necessary under federal law.[6]

2. Background

Montana's revised selenium criteria are applicable to the surface waters of Lake Koocanusa and the Kootenai River within Lincoln County, Montana. The Kootenay River (note different spelling in British Columbia) originates in southeast British Columbia and flows south into Montana near the town of Eureka. The river is impounded by Libby Dam, creating Lake Koocanusa. Downstream of Libby Dam, the Kootenai River flows west into Idaho and then north into British Columbia, forming Kootenay Lake (see Figure 1).

Selenium is an essential micronutrient and low levels of selenium in the diet are required for normal cellular function in almost all animals. However, selenium at amounts not much above the required nutritional levels can have toxic effects on aquatic life and aquatic-dependent wildlife, making it one of the most toxic of the biologically essential elements. Egg-laying vertebrates have a lower tolerance for selenium than do mammals, and the transition from levels of selenium that are biologically essential to those that are toxic for these species occurs across a relatively narrow range of exposure concentrations. Elevated selenium levels above what is nutritionally required in fish and other wildlife inhibit normal growth and reduce reproductive success through effects that lower embryo survival, most notably teratogenesis (i.e., embryo/larval deformities). The deformities associated with exposure to elevated selenium in fish may include skeletal, craniofacial, and fin deformities, and various forms of edema that result in mortality. Elevated selenium exposure in birds can reduce reproductive success including decreased fertility, reduced egg hatchability (embryo mortality), and increased incidence of deformities in embryos.

Scientific studies indicate that selenium toxicity to aquatic life and aquatic-dependent wildlife is driven by diet (i.e., the consumption of selenium contaminated prey) rather than by direct exposure to dissolved selenium in the water column. Unlike other bioaccumulative contaminants such as mercury, the single largest step in selenium accumulation in aquatic environments occurs at the base of the food web where algae and other microorganisms accumulate selenium from water. The vulnerability of a species to selenium toxicity is determined by a number of factors in addition to the amount of contaminated prey consumed. A species' sensitivity to selenium, its population status, and the duration, timing and life stage of exposure are all factors to consider. In addition, the hydrologic conditions and water chemistry of a water body affect bioaccumulation; in general, slow-moving, calm waters or lentic waters enhance

[6] *See* 40 C.F.R. 131.4(a) ("As recognized by section 510 of the Clean Water Act, States may develop water quality standards more stringent than required by this regulation."); *see also City of Albuquerque v. Browner*, 97 F.3d 415, 423 (10th Cir. 1996) (noting "states' inherent right to impose standards or limits that are more stringent than those imposed by the federal government").

the production of bioavailable forms of selenium (selenite), while faster-moving waters or lotic waters limit selenium uptake given the rapid movement and predominant form of selenium (selenate).[7]

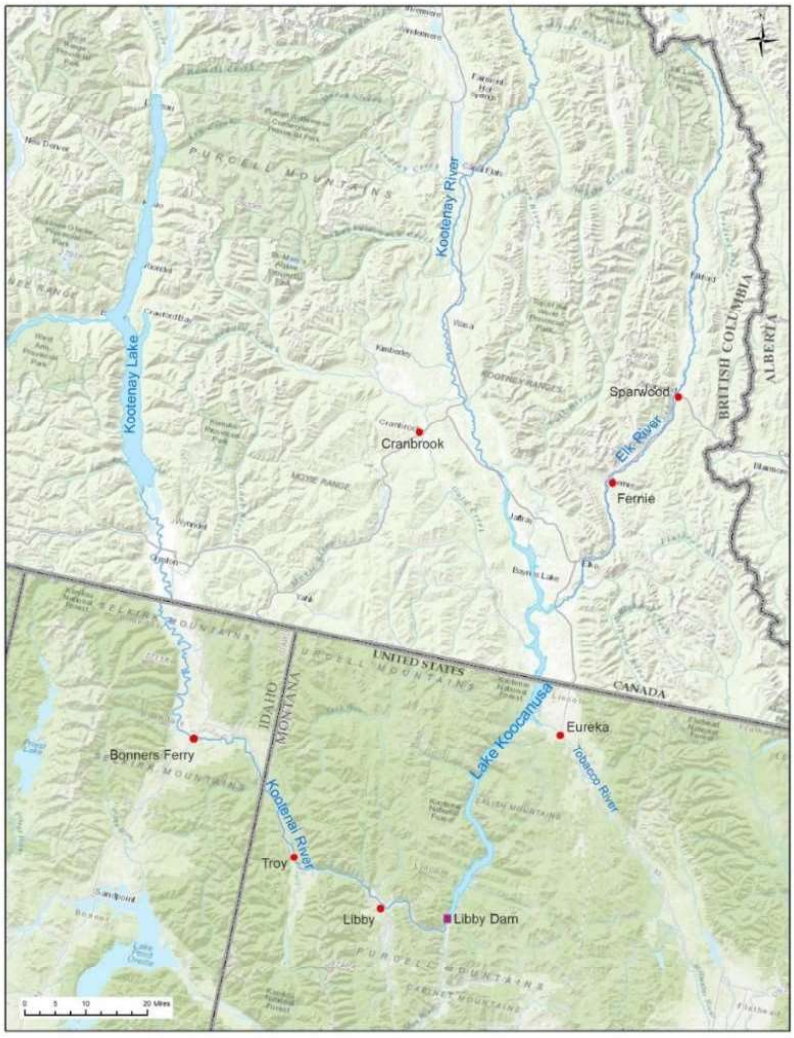


Figure. 1 Map of Lake Koocanusa and the Kootenai River

3. EPA Recommended Selenium Criterion

EPA's national recommended water quality criterion for selenium (EPA 2016),[8] developed by EPA in accordance with CWA section 304(a), provides recommendations to states and authorized tribes to establish WQS pursuant to the CWA. EPA 2016 recommends states/authorized tribes adopt one selenium criterion composed of four criterion elements: two fish tissue criterion elements (egg/ovary and whole body and/or muscle) and two water column criterion elements (30-day average and intermittent exposure). The water column criterion elements are further refined into values for lentic

[7] Excerpt from 83 Fed. Reg. 64063 (December 13, 2018).
[8] See www.epa.gov/wqc/aquatic-life-criterion-selenium.

waters (e.g., lakes/reservoirs) and lotic waters (e.g., streams/rivers) because selenium bioaccumulates differently in these two water body types. Adopting all four criterion elements ensures protection when fish tissue data are unavailable (See Table 1 below).

Table 1. Summary of EPA's Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	**Fish Tissue[1]**		**Water Column[4]**	
Criterion Element	**Egg/Ovary [2]**	**Fish Whole Body or Muscle [3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.

2. Egg/Ovary supersedes any whole body, muscle, or water column element when fish egg/ovary concentrations are measured.

3. Fish whole body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.

4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation. Water column values are the applicable criterion element in the absence of steady-state fish tissue measurements.

5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with fint assigned a value ≥0.033 (corresponding to 1 day).

6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

EPA recognizes selenium bioaccumulation potential depends on the structure of the food web, hydrology, and several biogeochemical factors that characterize a particular aquatic system. Therefore, site-specific water column criterion element values may be necessary at aquatic sites with high selenium bioaccumulation to ensure adequate protection of aquatic life. In its CWA section 304(a) criterion, EPA

provided two methods[9] for translating the recommended fish tissue criterion elements into site-specific water column criterion elements:

- Mechanistic model – uses scientific knowledge of aquatic system food webs to establish a relationship between the concentration of selenium in the water column and the concentration of selenium in fish tissue. EPA worked with the United States Geological Survey (USGS) to derive a translation equation utilizing a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature to derive recommended water column criterion elements.
- Empirical Bioaccumulation Factor (BAF) model – uses direct measurement of selenium concentrations in both the water column and fish tissue to calculate the ratio of the two concentrations. The ratio (BAF) can then be used to estimate the target concentration of selenium in the water column as related to the target fish tissue criterion element.

4. Montana's Revised Selenium Criteria for Lake Koocanusa and the Kootenai River

Montana adopted revised selenium criteria to protect Class B-1 designated uses in Lake Koocanusa and the Kootenai River that are consistent with the recommendations in EPA 2016 for fish tissue and water column criterion elements (summarized in Table 2). For the Kootenai River, Montana adopted the EPA 2016 recommended water column criterion element for lotic waters. For Lake Koocanusa, Montana used the EPA 2016 recommended mechanistic model method for translating the recommended fish tissue criterion elements into a site-specific water column criterion element. The selenium criteria in Department Circular DEQ-7 of 5 µg/L (chronic) and 20 µg/L (acute) continue to apply for CWA purposes for the rest of Montana.[10]

Table 2. Selenium criteria adopted by Montana for Lake Koocanusa and the Kootenai River

Media Type	Fish Tissue		Water Column
Criterion Element	Egg/Ovary	Whole Body or Muscle	Monthly Average Exposure
Magnitude	15.1 mg/kg dw	Whole Body 8.5 mg/kg dw Muscle 11.3 mg/kg dw	Lake Koocanusa 0.8 µg/L Kootenai River 3.1 µg/L
Duration	Instantaneous measurement	Instantaneous measurement	30 days
Frequency	Not to be exceeded	Not to be exceeded	Shall not be exceeded more than once in three years, on average

The egg/ovary criterion element supersedes the whole body or muscle criterion element. The fish tissue criterion elements supersede the water column elements only when the water bodies are in steady state (see section 5.2).

[9] Appendix K provides recommendations and examples for developing site-specific selenium criteria at www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf.
[10] See deq.mt.gov/Portals/112/Water/WQPB/Standards/PDF/DEQ7/DEQ-7.pdf.

5. EPA Analysis and Rationale for Approval

5.1 Selenium Criteria

40 C.F.R. § 131.11(a)(1) requires that water quality criteria adopted by states and authorized tribes "be based on sound scientific rationale and must contain sufficient parameters or constituents to protect the designated use."[11] For waters with multiple use designations, the criteria must support the most sensitive use. For the reasons discussed below, EPA has concluded that Montana's revised selenium criteria are both supported by a sound scientific rationale and based on EPA's 304(a) national recommended criteria as permitted by 40 C.F.R. 131.11(b)(1).

5.1.1 Protection of Designated Uses

Both Lake Koocanusa and the Kootenai River are designated Class B-1, which includes the following designated uses: drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply.[12] Montana determined in *Derivation of a Site-Specific Water Column Selenium Standard for Lake Koocanusa* (MT TSD) [13] that the most sensitive designated use for selenium is growth and propagation of salmonid fishes and associated aquatic life (see MT TSD sections 1.31, 2.3.5 and 3.6).

EPA's CWA section 304(a) recommended selenium criteria for the protection of human health are 170 µg/L (consumption of water + organism) and 4200 µg/L (consumption of organism only),[14] and are much less stringent than the CWA section 304(a) recommended water column criterion element for the protection of aquatic life in EPA 2016 of 1.5 µg/L (lentic) and 3.1 µg/L (lotic) (See Table 1). Montana adopted the Maximum Contaminant Level established by EPA under the Safe Drinking Water Act of 50 µg/L for the protection of human health[15] (see Department Circular DEQ-7), which is less stringent than the EPA 2016 water column criterion element. Therefore, selenium criteria adopted by states/authorized tribes that protect aquatic life are expected to also protect humans.

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[11] For the reasons explained herein, EPA has concluded that the state's water quality standard submission is supported by a sound scientific rationale. EPA notes that its charge under federal law is to review state water quality criteria submissions only to ensure that sound science shows they are protective of the designated use, not to determine whether the precise value selected by the state is the most scientifically rigorous number possible. EPA's regulations at 40 C.F.R. 131.4(a) expressly preserve states' right to "develop water quality standards more stringent than required." Accordingly, once EPA has determined that sound scientific rationale shows that a state submission is protective of the designated use, its role under the cooperative federalism framework of the CWA is not to second guess the state's scientific analysis. *See City of Albuquerque v. Browner*, 97 F.3d 415, 426 (10th Cir. 1996) ("If the proposed standards are more stringent than necessary to comply with the Clean Water Act's requirements, the EPA may approve the standards without reviewing the scientific support for the standards"); *Ctr. for Regulatory Reasonableness v. United States Envtl. Prot. Agency*, No. CV 16-1435, 2019 WL 1440303, at *10 (D.D.C. Mar. 31, 2019) ("States are expressly empowered to adopt criteria substantially below any hypothetical 'impairment threshold'").

[12] See ARM 17.30.609 and ARM 17.30.623.

[13] See deq.mt.gov/Portals/112/Water/WQPB/Standards/Koocanusa/TSD_Lake%20Koocanusa_Sep2020_Final.pdf.

[14] See www.epa.gov/wqc/national-recommended-water-quality-criteria-human-health-criteria-table.

[15] See www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations.

Analyses conducted for the derivation of EPA 2016 concluded that available data indicates fish are more sensitive to selenium than amphibians, aquatic invertebrates, and plants. The EPA 2016 criterion is based on reproductive effects on fish and this is expected to also protect the less sensitive taxa in the aquatic community.

In addition, EPA completed a review of scientific literature related to the toxicity of selenium to aquatic-dependent wildlife, of which aquatic-dependent birds were determined to be the most sensitive taxa. EPA concluded that since the translated water column values for aquatic-dependent wildlife are equal or extremely close to EPA's 2016 selenium water column criterion elements, the EPA's 2016 selenium water column elements would also protect aquatic-dependent wildlife.[16]

In summary, EPA agrees with DEQ's identification of growth and propagation of salmonid fishes and associated aquatic life as the most sensitive designated use for Lake Koocanusa and the Kootenai River.

5.1.2 Sound Scientific Rationale

EPA criteria recommendations consist of three components: (l) magnitude - how much of a pollutant (or pollutant parameter such as toxicity), expressed as a concentration, is allowable; (2) duration - the period of time (averaging period) over which the instream concentration is averaged for comparison with criteria magnitudes (limits the duration of concentrations above the criteria magnitudes); and (3) frequency - how often criteria can be exceeded.[17] EPA 2016 recommends states/authorized tribes adopt one selenium criterion composed of four criterion elements: two fish tissue criterion elements (egg/ovary and whole body and/or muscle) and two water column criterion elements (30-day average and intermittent exposure).

5.1.2.1 Magnitude

Fish Tissue Criterion Elements

EPA developed a chronic criterion reflective of the reproductive effects of selenium concentrations on fish species, consistent with consensus recommendations of expert panels and with peer review and public comments on draft criteria. Based on the available dietary exposure data from lab studies and field exposures, the egg/ovary criterion element concentration is 15.1 milligrams selenium per kilogram dry weight (mg Se/kg dw) based primarily on 17 reproductive studies representing 12 fish species (10 fish genera). EPA applied the sensitivity distribution concepts from the *U.S. EPA Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses*[18] to derive the national selenium criterion. The Lake Koocanusa fish assemblage is represented in the EPA 2016 selenium toxicity database by quantitative reproductive toxicity values for 3 of 10 fish

[16] See *Aquatic Life* and *Aquatic-Dependent Wildlife Selenium Water Quality Criterion* for *Freshwaters* of *California* (Part 4), at www.epa.gov/sites/production/files/2019-03/documents/ca_statewide_se_tsd_508_compliant.pdf.
[17] See *Technical Support Document for Water Quality-based Toxics Control* (Section 2.2.1) at www3.epa.gov/npdes/pubs/owm0264.pdf.
[18] See www.epa.gov/wqc/guidelines-deriving-numerical-national-water-quality-criteria-protection-aquatic-organisms-and.

genera (13 fish species) that reside in Lake Koocanusa (Dolly Varden (surrogate for bull trout), rainbow trout, and Westslope cutthroat trout), and 1 genera (that resides in the Montana portion of the Kootenai River (white sturgeon). Although white sturgeon, the most sensitive species in the EPA 2016 dataset, do not reside in Lake Koocanusa, per 40 C.F.R. § 131.10(b), the criteria for Lake Koocanusa must provide for the attainment and maintenance of the WQS in the Kootenai River. Also, qualitative species or genus surrogate level tissue values for an additional 5 species (mountain whitefish, kokanee, largescale and longnose sucker, and redside shiner), were considered in the derivation process, leaving only 4 of 13 species unrepresented in the toxicity database. One of the important principles for site-specific criteria development established by the Selenium Technical Subcommittee during that process was that all fish species without toxicity data should be considered equally sensitive to the white sturgeon. Therefore, the white sturgeon tissue values would be applicable to the burbot, northern pikeminnow, peamouth chub, and yellow perch. The fish genera present in the Kootenai River are similarly represented by EPA's 2016 dataset, with a majority of the species in the river represented by either quantitative data for the specific species or qualitative data for species or genus level surrogates, and all fish species without toxicity data considered equally sensitive to the white sturgeon.

Selenium concentrations measured either in fish whole body or muscle tissue in non-reproductive studies (typically evaluating juvenile growth and survival), were available for 8 genera. Several studies measured whole body and muscle concentrations in reproductive studies concurrent with measurements in egg or ovary tissues resulting in directly measured chronic values for 2 genera. Whole body and muscle criterion elements were derived using these directly measured tissue concentration data, or by applying conversion factors (*CF*) to egg or ovary concentrations to derive species-specific whole body or muscle tissue concentrations. Then the sensitivity distribution concept was applied to distributions of whole body and muscle tissue concentrations to derive the whole body (8.5 mg Se/kg dw) and muscle (11.3 mg Se/kg dw) criterion elements. EPA determined that the egg/ovary criterion element was most relevant to the toxic manifestations of selenium in fish resulting in a hierarchal application of the tissue criterion where the egg/ovary criterion supersedes the whole body or muscle tissue criterion when fish egg/ovary concentrations are measured at a site.

Montana's revised selenium criteria in ARM 17.30.632 include fish tissue criterion elements that are the same as the recommended magnitudes in EPA 2016 for both Lake Koocanusa and the Kootenai River: egg/ovary 15.1 mg/kg dw, muscle 11.3 mg/kg dw, and whole body 8.5 mg/kg dw. EPA 2016 provides the basis for EPA's approval of these criterion elements.

Water Column Criterion Element for the Kootenai River

The water column criterion element (30-day average) that Montana adopted for the Kootenai River is the same as the recommended water column value in EPA 2016: 3.1 µg/L total dissolved selenium for lotic waters. EPA 2016 provides the basis for EPA's approval of this criterion element

Water Column Criterion Element for Lake Koocanusa

The site-specific water column criterion element for Lake Koocanusa was developed through a five year collaboration between DEQ and British Columbia Ministry of Environment and Climate Change Strategy (BC-ENV). The Lake Koocanusa Monitoring and Research Working Group and a Selenium

Technical Subcommittee were established to coordinate this work. Presser and Naftz (2020)[19] and the companion data release[20] that includes a comprehensive set of site-specific data compiled from public databases (Federal, State, and Provincial) and reports by Teck Coal Ltd., provided the foundational selenium modeling for both DEQ and BC-ENV to use to develop a protective water column criterion element for Lake Koocanusa that both Montana and British Columbia could then adopt through their respective regulatory processes.

For Montana, the culmination of this work was the adoption of the water column criterion element (30-day average) for Lake Koocanusa (0.8 µg/L total dissolved selenium). As described in more detail below, this criterion element was derived consistent with the mechanistic model method in EPA 2016 for translating the recommended fish tissue criterion elements into site-specific water column criterion elements.

The mechanistic model approach uses scientific knowledge of the bioaccumulation dynamics and aquatic food webs of a site to establish a relationship between the concentration of selenium in the water column and the concentration of selenium in fish tissue. Selenium dissolved in surface water enters aquatic food webs by assimilating into trophic level 1 primary producer organisms (e.g., algae) or adsorption to other biotic (e.g., detritus) and abiotic (e.g., sediment) particulate material. Organic particulate material is consumed by trophic level 2 organisms (usually aquatic invertebrates, but also some fish species that are herbivores/detritivores) resulting in the accumulation of selenium in the tissues of those organisms. Trophic level 2 organisms are then consumed by trophic level 3 organisms (typically fishes) resulting in accumulation of selenium in the tissues of those fish (and so on up the food web). The transfer of selenium up the food web can be characterized by a number of parameters and modeled with an equation. An enrichment factor (*EF*) characterizes the assimilation of dissolved selenium into the base of the food web by quantifying the partitioning of selenium between the dissolved and particulate state. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer factor (*TTF)*. A conversion factor (CF), which establishes the ratio of selenium concentrations between different fish tissues, may also be used if the fish tissue being modeled is muscle or egg/ovary rather than whole body. These parameters are used in the mechanistic model with a target protective fish tissue selenium concentration (e.g., egg/ovary 15.1 mg/kg dw, muscle 11.3 mg/kg dw, or whole body 8.5 mg/kg dw), to derive a selenium water column criterion element that will ensure the protective fish tissue criterion element is met and will therefore be protective of the site-specific ecosystem.

EPA 2016 describes six steps for deriving a site-specific water column criterion element from the selenium egg/ovary criterion element using EPA's mechanistic model approach. Following is a summary of how the work of Presser and Naftz (2020) and additional work by Montana is consistent with the six steps.

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[19] Presser, T.S., and Naftz, D.L., 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020–1098, 40 p., doi.org/ 10.3133/ ofr20201098.

[20] See Presser, T.S., and Naftz, D.L., 2020, Selenium concentrations in food webs of Lake Koocanusa in the vicinity of Libby Dam (Montana) and the Elk River (British Columbia) as the basis for applying ecosystem-scale modeling, 2008–2018: U.S. Geological Survey data release, doi.org/10.5066/P9VXYSNZ.

1) Identify the appropriate target fish species.

The overall goal of Presser and Naftz (2020) was to provide an ecosystem-scale model that illustrates the site-specific range of potential selenium exposure and bioaccumulation that can inform the basis for regulatory decision-making by Montana and British Columbia. Therefore, they did not select one target fish species and instead provided generalized food webs based on fish species present that could be further refined by the respective governments. Presser and Naftz (2020) used available Lake Koocanusa data including fish species abundance and fish catches to identify fish species present. Based on recommendations from the Selenium Technical Subcommittee, twelve species of fish were considered as potential target species for the modeling: bull trout, burbot, kokanee, longnose sucker, largescale sucker, mountain whitefish, northern pikeminnow, peamouth chub, rainbow trout (wild strain), redside shiner, Westslope cutthroat trout, and yellow perch. Species-specific dietary data summarized as percentage of taxa-specific invertebrate biomass, recent selenium concentrations for invertebrate taxa in 2018, and a study of the contents of the stomachs of fish species caught in 2017 were used to assign each fish species to a generalized food-web category to reduce the number of modeling scenarios. Two generalized food-web categories were identified and modeled: an invertebrate to fish model (IFM) and a trophic fish model (TFM). The IFM is based on fish consuming only invertebrates (i.e., zooplankton and/or insects) and protects a community of rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker, peamouth chub, largescale sucker, mountain whitefish, and kokanee. The TFM is based on forage fish (trophic level 3 (TL3)) consuming invertebrates and predator fish (trophic level 4 (TL4)) consuming forage fish and protects a community of bull trout, burbot, and northern pikeminnow.

In general, EPA recommends selecting fish species in the aquatic system with the greatest selenium sensitivity and bioaccumulation potential. Presser and Naftz (2020) provided a qualitative vulnerability ranking for Lake Koocanusa fish species. The most vulnerable species include the redside shiner, peamouth chub, and northern pikeminnow based on sensitivity and burbot based on its demersal feeding and winter spawning period. Given this, Montana followed the recommendation of the Selenium Technical Subcommittee to use the more conservative TFM model food web for protection of potentially sensitive piscivorous species and species of cultural importance (see MT TSD section 5.1.3).

2) Model the food web of the targeted fish species.

Presser and Naftz (2020) used available Lake Koocanusa data including dietary metrics for fish and invertebrate taxa in fish stomachs to develop two primary food web models: IFM and TFM. Montana selected the TFM for modeling the water column value. Montana then selected the version of this model that resulted in the greatest bioaccumulation potential. This was the model that represents TL4 fish consuming 100% TL3 fish which consume 100% aquatic insects (chironomids).

3) Identify appropriate trophic transfer factor (*TTF)* values by either:
   a. selecting the appropriate *TTF* values from a list of EPA 2016-derived values, or
   b. deriving *TTF* values from other existing data, or
   c. deriving *TTF* values by conducting additional studies, or
   d. extrapolating *TTF* values from existing values.

Following option b and Presser and Naftz (2020), Montana used previously published laboratory-derived *TTFs* from Presser and Luoma (2010)[21]: 2.8 (aquatic insects), 1.5 (zooplankton), and 1.1 (fish). The mean "all insect" *TTF* (2.8) that Presser and Naftz (2020) used to model Lake Koocanusa is composed of: mayfly, caddisfly, cranefly, stonefly, damselfly, corixid (waterboatmen), and chironomid (midge). The zooplankton *TTF* reflects a zooplankton composite and the fish *TTF* is the mean of all fish species included in Presser and Luoma (2010). These *TTFs* are not identical to those that EPA used in EPA 2016 but are close in magnitude to those in EPA 2016 and scientifically defensible. Montana did not use site-specific *TTFs* due to data limitations identified in Presser and Naftz (2020).

4) Determine the appropriate value of *EF* (enrichment factor) by either:
   a. deriving a site-specific *EF* value from current field measurements, or
   b. deriving an appropriate *EF* value from older existing data, or
   c. extrapolating from *EF* values of similar waters.

Montana derived site-specific *EF* values from field measurements (option a above). Presser and Naftz (2020) and Montana used the term K_d instead of *EF* to describe the relationship between selenium concentrations in particulate and dissolved phases. EPA 2016 indicates that the K_d (or *EF*) is the most influential model parameter and therefore the most critical element for which to use site-specific data. Available data included a robust dataset of 87 matched samples for particulate and dissolved selenium collected over multiple years (2015-2019), seasons, and water depths. Rather than selecting a single representative value from the K_d dataset to use in the model, Presser and Naftz (2020) present each K_d calculation as an independent scenario (n=87), resulting in 87 predicted dissolved selenium concentrations for each model scenario. Montana used this distribution of K_d*'s* and resulting dissolved selenium concentrations to derive their water column criterion element.

5) Determine the appropriate *CF* (conversion factor) value by either:
   a. selecting the appropriate *CF* value from a list of EPA 2016-derived values, or
   b. deriving a *CF* value from other existing data, or
   c. deriving a *CF* value by conducting additional studies, or
   d. extrapolating a *CF* value from existing values.

[21] Presser, T.S., and Luoma, S.N., 2010, A methodology for ecosystem-scale modeling of selenium: Integrated Environmental Assessment and Management, v. 6, no. 4, p. 685–710, doi.org/ 10.1002/ ieam.101.

A conversion factor (*CF*) quantifies the relationship between the concentration of selenium in the eggs and/or ovaries and the concentration of selenium in the whole body or muscle tissues of fish. Montana used EPA's whole body tissue guideline (8.5 mg/kg dw) in their modeling, therefore no *CF* was needed.

6) Translate the applicable fish tissue element into a site-specific water concentration value.

To derive a site-specific water column criterion element for Lake Koocanusa that is protective of the chosen fish tissue criterion elements, Montana used the mechanistic model to translate the whole body fish tissue criterion element into a water column criterion element using the following equation:

$$C_{water\ column\ criterion\ element} = \frac{C_{whole\ body\ criterion\ element}}{TTF^{composite} \times (Kd/1000) \times \text{SPM \% bioavailability}}$$

$C_{water\ column\ criterion\ element}$	=	translated site-specific water column criterion element (µg/L),
$C_{whole\ body\ criterion\ element}$	=	whole body fish tissue criterion element (µg/g),
$TTF^{composite}$	=	product of the trophic transfer factor (TTF) values in each trophic level of the food web of the target fish model (no units of measurement),
K_d	=	environmental partitioning factor (L/g),
SPM % bioavailability	=	percent bioavailability of suspended particulate matter

Montana used the following values to populate the equation:

$C_{whole\ body\ criterion\ element}$	=	8.5 µg/g,
$TTF^{composite}$	=	$TTF^{TL4Fish}$ x $TTF^{TL3Fish}$ x $TTF^{aquatic\ insects}$ = 1.1 x 1.1 x 2.8 = 3.39
K_d	=	75th percentile of distribution
SPM % bioavailability	=	60%

The use of these values results in a water column criterion element of 0.8 µg/L. Although this criterion element is more stringent than the recommended water column criterion element for lentic aquatic systems in EPA 2016 (1.5 µg/L), based on the state's technical documentation included in its submission, summarized above, EPA concludes that it is supported by a sound scientific rationale.[22]

As Montana adopted the EPA 2016 recommended fish tissue criterion elements, the whole body criterion element that was used in this translation was the value of 8.5 µg/g dw. The $TTF^{composite}$ used in this translation was calculated using the TFM and fish and invertebrate *TTFs* from Presser and Luoma 2010. As presented in step 3 above, the use of existing *TTFs* is an approach recommend in EPA 2016.

As presented in Presser and Naftz (2020), Montana also included a bioavailability factor for suspended particulate matter in the model, which reflects the bioavailability of selenium from particulate matter to organisms in the ecosystem. In validation runs of the model, Presser and Naftz (2020) showed that a

[22] As noted above, the possibility that this criterion element may be more stringent than necessary to protect the designated use would not provide a valid legal justification under Section 303(c) of the CWA or EPA's implementing regulations for disapproval. *See* 40 C.F.R. 131.4(a).

60% bioavailability factor better represented the measured invertebrate and zooplankton selenium concentration in Lake Koocanusa than a 100% bioavailability factor.

Lastly, Montana selected the 75th percentile of the K_d distribution for the translation. This is a conservative K_d value protective of a majority of the scenarios observed in Lake Koocanusa.

Intermittent Criterion Element

In addition to the monthly exposure water column criterion element discussed above, EPA 2016 includes a recommended intermittent exposure water column criterion element. Montana did not adopt an intermittent exposure water column criterion element for either Lake Koocanusa or the Kootenai River. The state's rationale in the response to comments is "The intermittent exposure element is unnecessary because MPDES [Montana Pollutant Discharge Elimination System] rules do not differentiate between intermittent and continuous discharges for purposes of developing water quality-based effluent limits. When calculating the reasonable potential for a discharger to cause or contribute to an exceedance of a water quality standard, DEQ methods treat continuous and intermittent dischargers the same."[23] The MPDES program uses the maximum effluent concentration during the period of record to evaluate reasonable potential for a discharge to cause or contribute to an exceedance of a water quality standard.[24] EPA concludes Montana's approach will protect the applicable designated uses without the intermittent exposure water column criterion element. EPA notes that there are currently no public or private entities discharging to the Kootenai River or Lake Koocanusa with MPDES permit effluent limits for selenium.[25]

5.1.2.2. Duration

EPA's recommended duration for the water criterion elements is 30 days. EPA 2016 provides a detailed analysis for the derivation of a 30-day averaging period. This differs from typical criteria averaging periods based on EPA's 1985 Guidelines, where the basis for the criterion averaging period is a time period less than or equal to the "characteristic time," which describes the toxic speed of action due to direct waterborne toxicity of metals. The derivation of the averaging period for the selenium water column concentration was based on the kinetics of bioaccumulation and depuration rates for different trophic levels. The duration for Montana's water column criterion elements for Lake Koocanusa and the Kootenai River is specified as "30-day average" in ARM 17.30.632(7), which is consistent with EPA 2016.

EPA's recommended duration for the fish tissue criterion elements is instantaneous because fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish populations(s) at a given site. The fish reflect bioaccumulation of selenium that has already occurred and reflect the extended exposure to selenium in the water body. The duration for Montana's fish tissue criterion elements for Lake Koocanusa and the Kootenai River is specified as "instantaneous" in ARM 17.30.632(6), which is consistent with EPA 2016.

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[23] Notice of Amendment and Adoption p. 2394, response to comment #186.
[24] September 4, 2020 email from Myla Kelly to Tonya Fish.
[25] Notice of Amendment and Adoption p. 2343, response to comment #26.

5.1.2.3 Frequency

The recommended frequency in EPA 2016 of once in three years on average is based on the ability of an aquatic ecosystem to recover when pollutant impacts are associated exclusively with water column exposure.[26] The frequency for Montana's water column criterion elements for Lake Koocanusa and the Kootenai River is specified as "shall not be exceeded more than once in three years, on average" in ARM 17.30.632(7), which is consistent with EPA's recommendations in the 1985 Guidelines for water column criteria and in EPA 2016.

The recommended frequency of exceedance in EPA 2016 for the fish tissue criterion elements of the selenium criterion is "not to exceed." Selenium is a bioaccumulative pollutant; therefore, elevated levels in various ecological compartments (e.g., biota, surficial sediments) require a long period to decrease, and the associated aquatic community requires a long time to recover following reduction or removal of an elevated selenium exposure to a given system. As selenium is bioaccumulative and the pathway for exposure is through the food web, the typical criteria return frequency of once in three years on average is not appropriate for selenium in fish tissue as this could lead to sustained ecological impacts. As fish tissue has a much longer recovery time than water column concentrations, a frequency of "not to exceed" is appropriate for the tissue criterion element. The frequency for Montana's fish tissue criterion elements for Lake Koocanusa and the Kootenai River is specified as "not to exceed" in ARM 17.30.632(6), which is consistent with EPA 2016.

5.2 Definition of Steady State and Criteria Element Hierarchy

Montana adopted ARM 17.30.602(32) and added this definition:

> "Steady state" means, for the purposes of ARM 17.30.632, conditions whereby there are no activities resulting in new, increasing, or changing selenium loads to the lake or river aquatic ecosystem, and selenium concentrations in fish living in the aquatic ecosystem have stabilized.

EPA 2016 does not include a definition of "steady state," but does recommend fish tissue elements of the selenium criterion supersede water column elements under steady state conditions because the selenium concentrations in fish tissues are a more sensitive and reliable indicator of the negative effects of selenium in aquatic life. EPA 2016 also states that fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem in areas with new selenium inputs:

> "New inputs are defined as new activities resulting in selenium being released into a lentic or lotic waterbody. New inputs will likely result in increased selenium in the food web, likely resulting in increased bioaccumulation of selenium in fish over a period of time until the new or increased selenium release achieves a quasi-'steady state' balance within the food web. EPA estimates that concentrations of selenium fish tissue will not

[26] See *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (1985 Guidelines) at www.epa.gov/sites/production/files/2016-02/documents/guidelines-water-quality-criteria.pdf.

> represent a 'steady state' for several months in lotic systems, and longer time periods (e.g., two to three years) in lentic systems, depending upon the hydrodynamics of a given system such as the location of the selenium input related to the shape and internal circulation of the waterbody, particularly in reservoirs with multiple riverine inputs, hydraulic residence time, and the particular food web. Estimates of steady state under new or increased selenium input situations are expected to be site dependent, so local information should be used to better refine these estimates for a particular waterbody. Thus, EPA recommends that fish tissue concentration not override water column concentration in these situations until these periods of time have passed in lotic and lentic systems, respectively, or steady state conditions can be estimated." (EPA 2016 pp. 101-102).

Consistent with this, EPA 2016's Table 1 (also Table 1 of this enclosure) footnotes 1 and 4 specify that the fish tissue elements are expressed as steady-state and water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.

The language above from EPA 2016 was intended to address the scenario where fish tissue data are not exceeding those criterion elements, but the water column data are exceeding that element. However, another scenario DEQ raised in discussions with EPA is how to address the situation where fish tissue data are exceeding those criterion elements, but the water column data are not. EPA advised that in that scenario, EPA would still consider the water body impaired.[27] In other words, if a water body is not in steady-state, it is considered impaired if either the fish tissue or water column elements are exceeded. As a result, Montana adopted the following language in ARM 17.30.632(2): "When the aquatic ecosystem is in steady state and selenium data is available for both fish tissue and the water column, the fish tissue standards supersede the water column standard. When the aquatic ecosystem is in non-steady state, both the fish tissue and water column standards apply." ARM 17.30.632(3) specifies that Lake Koocanusa and the Kootenai River are in non-steady state and the Department will reassess the status triennially and amend the rule if necessary.

EPA concludes that the definition of "steady state" in ARM 17.30.602(32), the criteria element hierarchy in ARM 17.30.632(2), and the statement in ARM 17.30.632(3) that Lake Koocanusa and the Kootenai River are not in steady state are consistent with EPA 2016.

5.3 Protection of Downstream Waters

40 C.F.R. § 131.10(b) requires that criteria provide for the attainment and maintenance of the WQS of downstream waters. Montana addressed this in section 6.2 of the MT TSD. The Kootenai River is downstream of Lake Koocanusa. The fish tissue criterion elements are the same for both water bodies: egg/ovary 15.1 mg/kg dw, muscle 11.3 mg/kg dw, and whole body 8.5 mg/kg dw. Lake Koocanusa's water column criterion element of 0.8 µg/L is more stringent than the water column criterion element of

[27] See September 2, 2020 email from Tonya Fish to Lauren Sullivan.

3.1 µg/L in the Kootenai River. Fish tissue and water column criterion elements are the same for the Kootenai River in Montana and the downstream segment of the Kootenai River in Idaho.[28]

Based on the information above, EPA concludes Montana's revised selenium criteria will provide for the attainment and maintenance of downstream uses.

5.4 EPA's Action

Based on the information above, EPA approves the revised selenium criteria in ARM 17.30.632 because they are "based on sound scientific rationale and … contain sufficient parameters or constituents to protect the designated use" as required by 40 C.F.R. § 131.11. The selenium criteria also provide for the attainment and maintenance of the WQS of downstream waters consistent with 40 C.F.R. § 131.10(b). In addition, EPA approves the definition of "steady state" in ARM 17.30.602(32) because it informs application of the revised criteria consistent with 40 C.F.R. § 131.11. As with all WQS, these provisions are subject to state review at least every three years pursuant to 40 C.F.R. § 131.20(a).

Today's action is limited to waters under Montana's jurisdiction and Montana's revised WQS that apply to Lake Koocanusa from the US-Canada international boundary to the Libby Dam as specified in ARM 17.30.632(6) and 7(a). EPA remains committed to continued collaboration with Montana, British Columbia, the Confederated Salish and Kootenai Tribes, Kootenai Tribe of Idaho, First Nations, and other interested parties.

6.0 Provisions That EPA Has Determined Are Not WQS

EPA has determined the following provisions are not WQS:[29]

- In ARM 17.30.632(4): "Permit conditions and limits developed from the water column standards comply with the fish tissue standards." This language does not describe a desired ambient condition of a waterbody to support a particular designated use. Rather, these statements provide information related to permit conditions.
- ARM 17.30.632(5): "No person may violate the numeric water quality standards in (6) and (7)." This language does not describe a desired ambient condition of a waterbody to support a particular designated use. Rather, these statements provide information related to criteria implementation.
- In ARM 17.30.632(6): "Fish tissue sample results shall be reported as a single value representing an average of individual fish samples or a composite sample, each option requiring a minimum number of five individuals from the same species." This language does not describe a desired ambient condition of a waterbody to support a particular designated use. Rather, these statements provide information related to sampling and monitoring for compliance with the criteria. The state has flexibility in how it interprets discrete fish samples, and it is reasonable to apply the

[28] See IDAPA 58.01.02.210.01 at adminrules.idaho.gov/rules/current/58/580102.pdf.
[29] See *What is a New or Revised Water Quality Standard Under CWA 303(c)(3)? Frequently Asked Questions* at www.epa.gov/sites/production/files/2014-11/documents/cwa303faq.pdf.

instantaneous fish tissue elements to a composite sample or average of individuals of the same species, as adopted by MT.

7.0 Conclusion

EPA commends Montana for collaborating with multiple stakeholders for over five years to develop a site-specific selenium water column element for Lake Koocanusa consistent with the approaches recommended by EPA for developing site-specific selenium criteria. The adoption of fish tissue criterion elements for Lake Koocanusa as well as fish tissue elements and a water column criterion element for the Kootenai River that are the same as the current EPA recommended selenium criterion elements are also important improvements. The adopted criteria are based on sound science including robust site-specific data for Lake Koocanusa showing that they protect the applicable designated uses of Lake Koocanusa and the Kootenai River.

Appendix G

United States Environmental Protection Agency
Office of Water 4304T
EPA 822-R-21-006 August 2021

2021 Revision* to:

Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016

* This 2021 revision entails errata regarding footnotes on pages xv and 99, as described on page vii.

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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ERRATA

In 2021, EPA identified that the following text was missing from the second sentence in footnote 4 in the criterion table: "When selenium inputs are increasing," Corrected footnote 4 now states: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data." Footnotes 2 and 3 also reflect that footnote 4 was corrected.

NOTICE

This document has undergone a contractor-led external expert peer-review, as well as an EPA review process following publication and public comments received on the May 14, 2014, and July 28, 2015 criteria drafts. Final review by the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, has been completed, and the document has been approved for publication.

This document provides guidance to States and Tribes authorized to adopt water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of selenium. Under the CWA, States and Tribes are to adopt water quality criteria to protect designated uses. State and tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. While this document contains EPA's scientific recommendations regarding ambient concentrations of selenium that protect aquatic life, it does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally-binding requirements on EPA, States, Tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. EPA may change this document in the future. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document can be downloaded from: http://www.epa.gov/waterscience/criteria/aqlife.html

FOREWORD

Section 304(a)(l) of the Clean Water Act requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document presents EPA's updated chronic ambient water quality criterion (AWQC) for the protection of aquatic life based upon consideration of all available information relating to effects of selenium on aquatic organisms. EPA has incorporated revisions into this final document based on comments from the general public and an external expert peer review panel on an earlier draft published in the Federal Register in May 14, 2014, and comments from the general public on a second draft published in the Federal Register in July 28, 2015.

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(l) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criterion presented in this document is such a scientific assessment. If water quality criteria associated with specific designated uses are adopted by a state or authorized tribe as water quality standards under section 303, and approved by EPA, they become applicable Clean Water Act water quality standards in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. However, states and authorized tribes may adopt water quality criteria that reflect adjustments to EPA's recommended section 304 criteria to reflect local environmental conditions and human exposure patterns. Alternatively, states and authorized tribes may derive numeric criteria based on other scientifically defensible methods but the criteria must be protective of designated uses. It is not until their adoption as part of state or tribal water quality standards, and subsequent approval by EPA, that criteria become Clean Water Act applicable water quality standards. Guidelines to assist the states and authorized tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994, as updated), which along with additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

This document provides guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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ACRONYMS

AE	Assimilation Efficiency
AWQC	Ambient Water Quality Criteria
BAF	Bioaccumulation Factor
CF	Conversion Factor
CV	Chronic Value (expressed in this document as an EC10)
CWA	Clean Water Act
dw	Dry Weight
ECx	Effect Concentration at X Percent Effect Level
EF	Enrichment Factor
EPA	Environmental Protection Agency
EO	Egg Ovary
FCV	Final Chronic Value
GMCV	Genus Mean Chronic Value
HNOEC	Highest No Observed Effect Concentration
IR	Ingestion Rate
k_e	Rate of selenium loss
k_u	Rate of selenium uptake
LOEC	Lowest Observed Effect Concentration
M	Muscle
MATC	Maximum Acceptable Toxicant Concentration (expressed mathematically as the geometric mean of the NOEC and LOEC)
MDR	Minimum Data Recommendations or Requirements
NPDES	National Pollutant Discharge Elimination System
NOEC	No Observed Effect Concentration
SMCV	Species Mean Chronic Value
SSD	Species Sensitivity Distribution
TMDL	Total Maximum Daily Load
TRAP	EPA's Statistical Program: Toxicity Relationship Analysis Program
TTF	Trophic Transfer Factor
WB	Whole body
WQBELS	Water Quality-based Effluent Limitations
WQC	Water Quality Criteria
WQS	Water Quality Standards
ww	Wet Weight

EXECUTIVE SUMMARY

This document sets forth the basis for and derivation of the Clean Water Act, Section 304(a) water quality criterion for protecting freshwater aquatic life from harmful effects of selenium, a naturally occurring chemical element that is nutritionally essential in small amounts, but toxic at higher concentrations. This assessment provides a critical review of all data identified in EPA's literature search quantifying the toxicity of selenium to freshwater aquatic organisms, and provides a basis for a criterion that will assure protection of populations of fish, amphibians, aquatic invertebrates, and plants, based on available data.

Although selenium may cause acute toxicity at high concentrations, the most deleterious effect on aquatic organisms is due to its bioaccumulative properties; these chronic effects are found at lower concentrations than acute effects. Organisms in aquatic environments exposed to selenium accumulate it primarily through their diets, and not directly through water (Chapman et al. 2010). The best science also indicates that selenium toxicity occurs primarily through transfer to the eggs and subsequent reproductive effects. Consequently, in harmony with the recommendations of expert panels (U.S. EPA 1998; Chapman et al. 2010) and with peer review and public comments on previous U.S. EPA (2004, 2014, 2015) drafts, the Agency developed a chronic criterion reflective of the reproductive effects of selenium concentrations on fish species.

The 2016 "Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater, 2016," is a chronic criterion that is composed of four elements. All elements are protective against chronic selenium effects. Two elements are based on the concentration of selenium in fish tissue and two elements are based on the concentration of selenium in the water column. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent element to account for potential chronic effects from short-term exposures (one value for lentic and one value for lotic aquatic systems). The assessment of the available data for fish, invertebrates, and amphibians indicates that a criterion value derived from fish will protect the aquatic community. All four criterion elements applied together should protect aquatic life from the chronic effects of exposure to total selenium in waters inhabited by fish, as well as "fishless waters."

Because the factors that determine selenium bioaccumulation vary among aquatic systems, site-specific water column criterion element values may be necessary at aquatic sites with high selenium bioaccumulation to ensure adequate protection of aquatic life (Appendix K). Finally, this freshwater chronic selenium criterion applies only to aquatic life, and is not intended to address selenium toxicity to aquatic-dependent wildlife such as aquatic-dependent birds.

The toxicity studies relevant to the derivation of the fish tissue selenium criterion elements involve (a) extended duration dietary exposure, and (b) measurement of total selenium in the tissue of the target organism. Selenium either in fish whole-body or in muscle is usually measured in non-reproductive studies, and selenium in eggs or ovaries is typically measured in reproductive studies. Selenium accumulation in the eggs of the exposed adult female prior to spawning has been shown to yield the most robust relationship (statistically significant) with occurrence of deformities and reduced survival of the offspring.

The outcome of assessing both reproductive and non-reproductive studies under laboratory and field conditions led EPA to the conclusion, consistent with expert consensus (Chapman et al. 2009, 2010), that reproductive effects, linked to egg-ovary selenium concentrations, provide the most sound basis for the criterion compared to non-reproductive (e.g., survivorship, growth) endpoints. Reproductive effects have been linked to observed reductions in the populations of sensitive fish species in waterbodies having elevated concentrations of selenium (Young et al. 2010). EPA applied the sensitivity distribution concepts from the U.S. EPA *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses* (Stephan et al. 1985) to derive the national selenium criterion. Based on the available data, expressed as EC_{10} values, the egg-ovary criterion element concentration is 15.1 milligrams selenium per kilogram dry weight (mg Se/kg dw), based primarily on 17 reproductive studies representing 10 fish genera.

EPA recommends states and tribes adopt all four elements of the criterion into their water quality standards. Two elements are based on the concentration of selenium in fish tissue (eggs or ovaries, and whole-body or muscle) and two elements are based on the concentration of selenium in the water column (a 30-day chronic element and an intermittent exposure element). Both water column elements are further refined into values for lentic waters (e.g., lakes and impoundments) and lotic waters (e.g., rivers and streams). The difference between lentic and lotic water column values reflect the observed difference in selenium bioaccumulation in these

two categories of aquatic systems (ATSDR 2003; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). EPA derived the intermittent exposure element based on the chronic 30-day water column element and the fraction of any 30-day period during which elevated selenium concentrations occur. EPA recommends the intermittent element to address short-term exposures that contribute to chronic effects through selenium bioaccumulation (e.g., releases from storage ponds or other intermittent releases). EPA derived the values for the water-column criterion elements from the egg-ovary element by assessing food-chain bioaccumulation based on available data collected at lentic and lotic systems in the continental United States. Thus, all four criterion elements are based on reproductive effects in freshwater fish.

EPA primarily used field studies in freshwater systems to provide quantitative estimates of selenium bioaccumulation in particulate material (algae, detritus, and sediment) from water, and used field observations and laboratory data to quantify and model the trophic transfer of selenium from particulate material into invertebrates, and from invertebrates into fish. EPA additionally used field and laboratory observations to assess species-specific selenium partitioning between different tissues within a fish (whole-body, eggs and/or ovaries, and muscle). EPA developed food web models of fish in aquatic systems with a range of bioaccumulation potentials and used the food web models with the species-specific estimates of trophic transfer (or the most proximate taxonomic surrogate when species-specific data was not available) to develop water column criterion elements from the egg-ovary criterion element for lotic and lentic aquatic systems. EPA validated this approach using selenium measurements from aquatic systems with a range of bioaccumulation potentials. Similar approaches could be used in the derivation of selenium criteria in saltwater or estuarine systems with selenium data and food webs relevant to those systems.

While more than half the available chronic studies were fish studies, available field data and laboratory toxicity studies suggest that a criterion based on fish will protect amphibians, aquatic invertebrates, and plants since these taxa appear to be less sensitive to selenium than fish (see **Sections 3.1.4** and **6.1.4**).

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	**Fish Tissue**[1]		**Water Column**[4]	
Criterion Element	**Egg/Ovary** [2]	**Fish Whole Body or Muscle** [3]	**Monthly Average Exposure**	**Intermittent Exposure**[5]
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and fint is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

The recommended chronic selenium criterion is expected to protect the entire aquatic community, including fish, amphibians, and invertebrates, based on available data. Because fish are the most sensitive to selenium effects, EPA recommends that selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) data take precedence over the criterion elements based on water column selenium data due to the fact, noted above, that fish tissue concentrations provide a more robust and direct indication of potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium

bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) "fishless" waters, and 2) areas with new selenium inputs.

For purposes of this document, EPA defines "fishless waters" as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (e.g., extirpation) due to temporary or permanent changes in water quality (e.g., selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas. Appendix K of this criterion document discusses approaches to develop a site-specific water column criterion element in such situations.

For purposes of this document EPA defines "new inputs" as new activities resulting in the release of selenium into a lentic or lotic aquatic system. New inputs will likely result in a greater concentrations of selenium in the food web and a relatively slow increase in the selenium concentration in fish until the new selenium release achieves a quasi-"steady-state" balance in the aquatic system. EPA estimates that the concentration of selenium in fish tissue will not reach steady state for several months in lotic systems and longer time periods (e.g., 2 to 3 years) in lentic systems. Achievement of steady state in an aquatic system also depends on the hydrodynamics of the aquatic system, (particularly reservoirs with multiple riverine inputs), the location of the selenium input and the particular food web. EPA expects the time needed to achieve steady state with new or increased selenium inputs to be site specific. Thus, EPA recommends that fish tissue criterion elements not take precedence over the water column criterion elements until the aquatic system achieves steady state. In the interim, EPA recommends sampling and using site-specific data to determine steady state in the receiving water to gain a better understanding of the selenium bioaccumulation dynamics in a given system.

EPA recommends states and tribes adopt into their water quality standards a selenium criterion that expresses the four elements as a single criterion composed of multiple parts in a manner that explicitly affirms the primacy of the whole-body or muscle element over the water column elements, and the egg-ovary element over any other element. Adopting the fish whole-

body or muscle tissue element into water quality standards ensures the protection of aquatic life when measurements from fish eggs or ovary are not available, and adopting the water column element ensures protection when fish tissue measurements are not available.

EPA recommends that when states adopt a four-part criterion for selenium reflecting EPA's recommended criterion, states use the default monthly average exposure water column elements of the criterion, adopted as part of the state's water quality criterion when implementing the criterion under the National Pollutant Discharge Elimination System (NPDES) permits program and to assist with implementation of other Clean Water Act programs. Alternatively, states may want to develop adopt, and submit for EPA approval, either a site-specific water column criterion element (or set of lentic/lotic criterion element values), or a set of procedures to facilitate the translation of the fish tissue criterion concentration elements into site-specific water concentration values. A site-specific water column criterion element or set of lentic/lotic criterion element values can be developed using a mechanistic modeling approach (Presser and Luoma 2010) or using the empirical bioaccumulation factor approach, both described in Appendix K, for the specific waterbody or waterbodies. Any translation procedure must be scientifically defensible, produce repeatable, predictable outcomes, and result in criterion element values that protect the applicable designated use. Examples of such procedures include the mechanistic modeling approach and the empirical BAF approach described in Appendix K.

This recommended selenium criterion applies to freshwater lentic and lotic systems, as it is based on the toxicity of selenium to freshwater organisms. A similar approach may be appropriate for deriving criteria for selenium in estuarine and marine waters if appropriate data are available.

1 INTRODUCTION AND BACKGROUND

The objective of the Clean Water Act (CWA) is to "restore and maintain the chemical, biological and physical integrity of the Nation's waters." One of the tools that EPA uses to meet this objective is the development of recommended ambient water quality criteria (AWQC) under section 304(a)(1) of the Act. As provided for by the Clean Water Act, EPA reviews and from time to time revises 304(a)(1) AWQC to ensure the criteria are consistent with the latest scientific knowledge. Section 304(a) aquatic life criteria serve as recommendations to states and authorized tribes for defining ambient water concentrations that will protect against adverse ecological effects to aquatic life resulting from exposure to a pollutant found in water from direct contact or, ingestion of contaminated water and/or food. Aquatic life criteria address the Clean Water Act goals of providing for the protection and propagation of fish and shellfish. When adopted into state or tribal water quality standards (WQS), these criteria can become a basis for establishing National Pollutant Discharge Elimination System (NPDES) program permit limits and, the basis for listing impaired waters under Section 303(d) and establishing Total Maximum Daily Loads (TMDLs).

1.1 HISTORY OF THE EPA RECOMMENDED SELENIUM AWQC FOR AQUATIC LIFE

In 1980 EPA first published numeric aquatic life criteria for selenium in freshwater. These criteria were based on water-only exposure (no dietary exposure). In order to address the lack of consideration of bioaccumulation in the 1980 selenium criteria, in 1987 EPA published updated selenium criteria to address field-based toxicity observed in aquatic ecosystems at levels below the existing criteria values. The 1987 criteria were field-based and accounted for both the water column and dietary uptake pathways manifested at Belews Lake, North Carolina (USA), a cooling water reservoir where water quality and fish communities had been affected by selenium loads from a coal-fired power plant. At that time EPA also provided an acute criterion of 20 µg/L derived from a reverse application of an acute-chronic ratio obtained from conventional water-only exposure toxicity tests applied to the 5 µg/L chronic value based on dietary and water column exposure in Belews Lake.

In 1998-1999 EPA published a revised acute criterion, a formula that recognized that the two oxidation states, selenate and selenite, appeared to have substantially different acute

toxicities. This acute criterion assumed toxicity was based on water-only exposure. Subsequent research has demonstrated that sulfate levels influence selenate toxicity in water-only exposures.

In 1998 EPA held a peer consultation workshop (EPA-822-R-98-007) to evaluate new science available for selenium relevant to the selenium aquatic life criterion. EPA concluded, and the peer reviewers agreed, that fish-tissue values more directly represent chronic adverse effects of selenium than the conventional water concentration approach used by EPA to protect aquatic life, because chronic selenium toxicity is primarily based on the food-chain bioaccumulation route, not on a water column route of exposure.

In 2004 EPA published a draft chronic whole-body fish-tissue criterion with a water-based monitoring trigger in the summer and fall. The critical effect considered at that time was the impact on survivorship based on overwintering stress to bluegill sunfish. An acute criterion was estimated at that time that addressed concerns with the species of selenium present and adjusted for sulfate levels; however, it did not address the dietary uptake pathway.

Further refinement of the fish tissue approach occurred in 2009 based on the findings of a Pellston scientific workshop on the ecological risk assessment of selenium (Chapman et al. 2009, 2010). As presented by Chapman et al. (2009), some key findings resulting from that workshop are:

- Diet is the primary pathway of selenium exposure for both invertebrates and vertebrates.
- Traditional methods for predicting toxicity on the basis of exposure to dissolved [water column] concentrations do not work for selenium because the behavior and toxicity of selenium in aquatic systems are highly dependent upon site-specific factors, including food web structure and hydrology.
- Selenium toxicity is primarily manifested as reproductive impairment due to maternal transfer, resulting in embryotoxicity and teratogenicity in egg-laying vertebrates.

In this 2016 final recommended freshwater chronic criterion for selenium, EPA includes revisions based on the public and external expert peer reviews of the 2014 draft, public comments on the 2015 draft, data and information from additional studies provided by the public and peer reviewers, and additional scientific analyses. EPA also conducted a new literature review and reanalyzed data considered in the 2004 and 2009 draft criteria documents. This final criterion reflects the latest scientific consensus (e.g., Chapman et al. 2010) on the reproductive

effects of selenium on aquatic life and their measurement in aquatic systems and supersedes all previous national aquatic life water quality criteria for selenium.

EPA is recommending a national selenium criterion expressed as four elements. All elements are protective against chronic selenium effects, and account for both short term and longer term exposure to selenium. Two elements are based on the concentration of selenium in fish tissue (eggs and ovaries, and whole-body or muscle) and two elements are based on the concentration of selenium in the water-column (two 30-day chronic values and an intermittent value). EPA derived the 30-day chronic water column element from the egg-ovary element by modeling selenium bioaccumulation in food webs of lotic and lentic aquatic systems. EPA is recommending the intermittent value to address short-term exposures that could contribute to chronic effects through selenium bioaccumulation in either lotic or lentic systems. EPA derived the intermittent element based on the chronic 30-day water column element and the fraction of any 30-day period during which elevated selenium concentrations occur. These water column criterion elements apply to the total of all oxidation states (selenite, selenate, organic selenium, and any other forms) (See Appendix L for Analytical Methods for Measuring Selenium). Aquatic communities are expected to be protected by this chronic criterion from any potential acute effects of selenium.

2 PROBLEM FORMULATION

Problem formulation provides a strategic framework for water quality criteria development by focusing the effects assessment on the most relevant chemical properties and endpoints. The structure of this effects assessment is consistent with EPA's Guidelines for Ecological Risk Assessment (U.S. EPA 1998).

This ecological effects assessment defines a scientifically-defensible water quality criterion for selenium under section 304(a)(1) of the Clean Water Act. Clean Water Act Section 304(a)(1) requires EPA to develop criteria for water quality that accurately reflect the latest scientific knowledge. These criteria are based solely on high quality data and best professional scientific judgments on toxicological effects. Criteria are developed following overarching guidance outlined in the Agency's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985), hereafter referred to as "EPA Ambient Water Quality Criteria Guidelines." States and authorized tribes may adopt EPA's recommended criteria into their water quality standards to protect designated uses of water bodies, they may modify EPA's criteria to reflect site-specific conditions, or they may derive criteria using other scientifically-defensible methods, all subject to EPA review and approval.

2.1 OVERVIEW OF SELENIUM SOURCES AND OCCURRENCE

Selenium is a naturally occurring element present in sedimentary rocks and soils. It is also present in the aquatic environment as methyl derivatives of selenium, naturally occurring in freshwaters through methylation by bacteria (Ranjard et.al. 2003). Selenium's occurrence in surficial soils and aquatic sediments in the United States is illustrated in **Figure 2.1**. There are around 40 known selenium-containing minerals, some of which can have as much as 30% selenium, but all are rare and generally occur together with sulfides of metals such as copper, zinc and lead (Emsley 2011). Sedimentary rocks, particularly shales, have the highest naturally occurring selenium content (Burau 1985). The distribution of organic-enriched, sedimentary shales, petroleum source rocks, ore deposits, phosphorites, and coals, in which selenium typically co-occurs, is well characterized in the United States (Presser et al. 2004). Natural weathering of selenium-bearing geologic strata containing selenium can lead to selenium leaching into groundwater and surface water. Two major anthropogenic activities are known to

cause increased selenium mobilization and introduction into aquatic systems. The first is the mining of metals, minerals and refinement and use of fossil fuels; the second is irrigation of selenium-rich soils.

Mining activities bring selenium-enriched deposits to the surface, where they are exposed to physical weathering processes. The release of selenium related to resource extraction activities is most common in the phosphate deposits of southeast Idaho and adjacent areas of Wyoming, Montana, and Utah, and in coal mining areas in portions of West Virginia, Kentucky, Virginia, and Tennessee (Presser et al. 2004). Where selenium-containing minerals, rocks, and coal are mined, selenium can be mobilized when rock overburden and waste materials are crushed, increasing the surface area and exposure of material to weathering processes. Selenium contamination of surface waters can also occur when sulfide deposits of iron, uranium, copper, lead, mercury, silver, and zinc are released during the mining and smelting of these metal ores. Where coal is burned for power production, selenium can enter surface waters as drainage from fly-ash ponds and fly-ash deposits on land (Gillespie and Baumann 1986). Fly ash deposits have a high surface area to volume ratio, resulting in rates of selenium in leachate several times higher than from the parent feed coal (Fernández-Turiel et al. 1994). The refining of crude oil containing high levels of selenium can also be a major source of loading in certain water bodies (Maher et al. 2010).

Irrigation of selenium-rich soils for crop production in arid and semi-arid regions of the country can mobilize selenium and move it off-site in surface water runoff or via leaching into ground water. Where deposits of Cretaceous marine shales occur, they can weather to produce high selenium soils; such soils are present in many areas of the western U.S. (Lemly 1993c). Selenium is abundant in the alkaline soils of the Great Plains, and some ground waters in California, Colorado, Kansas, Oklahoma, South Dakota and Wyoming contain elevated concentrations of selenium due to weathering of and leaching from rocks and soils. In semi-arid areas of the West, irrigation water applied to soils containing soluble selenium can leach selenium. The excess water (in tile drains or irrigation return flow) containing selenium can be discharged into basins, ponds, or streams. For example, elevated selenium levels at the Kesterson Reservoir in California originated from agricultural irrigation return flow collected in tile drains that discharged into the reservoir (Ohlendorf et al. 1986).

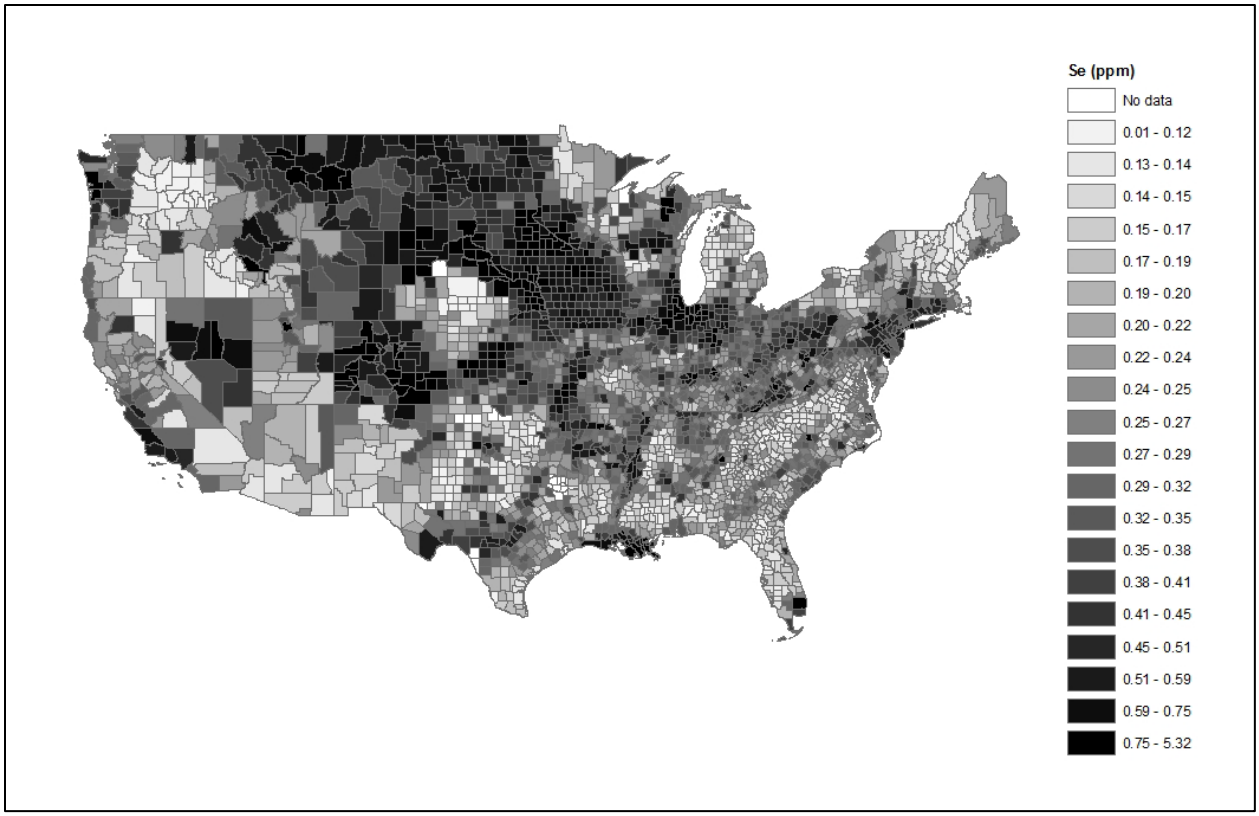


Figure 2.1. Selenium in Surficial Soils and Aquatic Sediments in counties of the Conterminous United States.
U.S. Geological Survey Open-File Report 2004-1001. URL: http://mrdata.usgs.gov/geochem/doc/averages/countydata.htm. Data are available from: http://mrdata.usgs.gov/geochem/doc/groups-cats.htm.

Atmospheric emissions of selenium can originate from several sources including power plants and other facilities that burn coal or oil, selenium refineries that provide selenium to industrial users, base metal smelters and refineries, resource extraction industries, milling operations, and end-product manufacturers (e.g., semiconductor manufacturers) (ATSDR 2003). Airborne selenium particles can settle either on surface waters or on soils from which selenium can be further transported and deposited into water bodies through ground or surface water conveyances or runoff.

The chemical form of selenium that dominates a location is usually dependent on its sources, effluent treatments, and biogeochemical processes in the receiving waters. Irrigation activities in areas with seleniferous soils typically mobilize selenate (SeO_4^{2-}, or Se[VI]) (Seiler et al. 2003). Combustion of coal for power generation creates predominantly selenite (SeO_3^{2-}, or Se[IV]) in the fly ash waste due to the temperatures, pH, and redox conditions involved with the

process (Huggins et al. 2007). Similar conditions during refinement of crude oil can also result in high concentrations of selenite relative to selenate, as was observed in the San Francisco Bay estuary in the 1980s (Cutter 1989). Although selenite is the dominant species in the discharges resulting from crude oil refining and coal burning using conventional technologies, the implementation of alternative treatment technologies can alter the relative concentrations of selenate and selenite. For example, in scrubbers with forced oxidation systems that produce strong oxidizing conditions and high temperatures, the majority of discharged selenium is in the form of selenate (Maher et al. 2010). However for flue gas desulfurization systems that are the inhibited oxidation type, the selenium chemistry is more complex, and selenite may not be the primary form emitted (Petrov et al. 2012). **Table 2.1** shows the predominant form of selenium that is associated with different activities and industries.

EPA's Office of Water and Office of Research and Development conducted the first statistically based survey of contaminants in fish fillets from U.S. rivers from 2008 through 2009. This national fish survey was conducted under the framework of EPA's National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers (Lazorchak et al. 2014). During June through October of 2008 and 2009, field teams applied consistent methods nationwide to collect samples of fish species commonly consumed by humans at 541 randomly selected river locations ($\geq 5^{th}$ order based on 1:100,000-scale Strahler order) in the lower 48 states. They collected one composite fish sample at every sampling location, with each composite consisting of five similarly sized adult fish of the same species from a list of target species. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. Samples were collected from both non-urban (379 sites) and urban locations (162 sites). Each fillet composite sample was homogenized and analyzed using an ICP-MS (Inductively Coupled Plasma- Mass Spectrometry) method for total selenium, and results were reported as wet weight. Three of the 541 samples (approximately 0.6%) exceeded the 2016 criterion for muscle tissue, 11.3 mg/kg dw. The maximum value detected was 17.75 mg Se/kg dw muscle, the median was 1.90 mg Se/kg dw, and the minimum 0.41 mg Se/kg dw.

Table 2.1. Predominant Chemical Forms of Selenium in Discharges Associated with Different Activities and Industries.

Selenium Form	Sources
Selenate	Agricultural irrigation drainage Treated oil refinery effluent Mountaintop coal mining/ valley fill leachate Copper mining discharge
Selenite	Oil refinery effluent Fly ash disposal effluent Phosphate mining overburden leachate
Organoselenium	Treated agricultural drainage (in ponds or lagoons)

Source: Presser and Ohlendorf 1987; Zhang and Moore 1996; Cutter and Diego-McGlone 1990.

2.2 Environmental Fate and Transport of Selenium in the Aquatic Environment

The fate and transport of selenium in aquatic systems is affected by the distribution of selenium species and their transformations in water, sediment, and biota. These transformations include the assimilation and conversion of inorganic selenium to organic selenium species in plants and microbes that are transferred to higher trophic level consumer species throughout the aquatic food web.

2.2.1 Selenium Species in Aquatic Systems

Aquatic organisms are exposed to a combination of predominantly organic selenium species present in the food web throughout their life history; reproductive effects integrate these exposures to transformed inorganic and organic species of selenium. The bioavailability and toxicity of selenium depend on both its concentration and speciation (Cutter and Cutter 2004; Meseck and Cutter 2006; Reidel et al. 1996). Selenium exists in four oxidation states (VI, IV, 0, -II) and in a wide range of chemical and physical species across these oxidation states (Doblin et al. 2006; Maher et al. 2010; Meseck and Cutter 2006). Therefore, in the effects assessment that follows, we have correlated the adverse effects on aquatic life with total dissolved selenium.

In oxygenated surface waters, the primary dissolved selenium species are selenate (SeO_4^{2-}, or Se[VI]) and selenite (SeO_3^{2-}, or Se[IV]), as well as dissolved organic selenides (-II) formed from fine particulate organic matter (e.g., Doblin et al. 2006; Meseck and Cutter 2006). The relative abundance of selenate and selenite depends on relative contributions from the

geologic and anthropogenic sources of selenium to the receiving waters, as there is negligible inter-conversion between the two species (e.g., Maher et al. 2010). Aqueous selenite is more abundant than selenate when the majority of selenium originates from discharges from coal fly ash tailings or oil refineries (e.g., Cutter 1989; Huggins et al. 2007). Particulate species in the water column include selenate, selenite, and elemental selenium (Se(0)) bound to resuspended sediments and organic particles, as well as particulate organic selenium species incorporated into suspended detritus (e.g., Cutter and Bruland 1984; Meseck and Cutter 2006).

In sediments, selenate and selenite can be reduced to iron selenides or elemental selenium under abiotic or biotic processes; elemental selenium and selenides can be converted to selenate under oxidizing conditions (Maher et al. 2010). For example, selenate can be reduced to elemental selenium in sediments (e.g., Oremland 1990) in the presence of iron oxides (Chen et al. 2008) and iron sulfides (Breynaert et al. 2008). Elemental selenium and organic selenides are produced by selenate-reducing microbes in sediments. Overall, the reduction of selenate and particularly selenite in sediments increases with increasing sediment organic matter (Tokunaga et al. 1997). Selenite in particular is readily bound to iron and manganese oxy-hydroxides (Maher et al. 2010), and is readily adsorbed to inorganic and organic particles, particularly at a lower pH range (e.g., McLean and Bledsoe 1992; Tokungawa et al. 1997). Microbial reduction of selenite to organic forms (via methylation) increases the solubility and bioavailability of selenium (Simmons and Wallschlägel 2005). Plants and algae produce volatile selenium species by biomethylation of excess selenium, which upon reaching the sediment can be transformed to a more bioavailable species, or deposited in the sediments and effectively removed from the system (Diaz et al. 2009). Depending on environmental conditions, the reduction processes described above are largely reversible, as elemental selenium and selenides in sediments can be oxidized to selenate through microbial or abiotic transformations (e.g., Maher et al. 2010; Tokunaga et al. 1997).

The most important transformation of selenium, with respect to its toxicity to aquatic organisms, is in the uptake of dissolved inorganic selenium into the tissues of primary producers at the base of the food web. The main route of entry of selenium into aquatic foodwebs is from the consumption of particulate selenium of primary producers, and to a lesser degree, from the consumption of sediments (Doblin et al. 2006; Luoma and Presser 2009). For algae, selenite and organic selenides are similarly bioavailable, and both dissolved species are more bioavailable

than selenate (e.g., Baines et al. 2001; Luoma et al. 1992). In vascular plants, selenate uptake is greater than for the other dissolved species, as the majority of selenium uptake occurs in the roots, and selenate is more easily transported to the shoots and leaves than selenite or organic selenides (Dumont 2006). Following uptake, selenium is metabolized into a variety of organic species that are assimilated into plant tissues. Selenium metabolism in plants is analogous to sulfur metabolism (e.g., Dumont et al. 2006; Ouerdane et al. 2013). Selenate is reduced to selenite, which is then reduced to selenide in a process involving reduced glutathione (Dumont et al. 2006). Selenide is converted to selenocysteine (SeCys), which is then converted to selenomethionine (SeMet) (Dumont et al. 2006). In addition to SeCys and SeMet, a variety of other organic selenium species can be formed; however, SeCys, and particularly SeMet are toxicologically important because these amino acids nonspecifically replace cysteine and methionine in proteins, and are more bioavailable to higher trophic level consumers (Fan et al. 2002; Freeman et al. 2006).

2.2.2 Bioaccumulation of Selenium in Aquatic Systems

Dissolved selenium uptake by animals is slow, whatever the form, such that under environmentally relevant conditions, dissolved selenium in the water column makes little or no direct contribution to bioaccumulation in animals (Lemly 1985a; Ogle and Knight 1996), but does influence the concentration of selenium in particulate matter. Selenium bioaccumulation in aquatic organisms occurs primarily through the ingestion of food (Fan et al. 2002; Luoma et al. 1992; Maher et al. 2010; Ohlendorf et al. 1986; Presser and Ohlendorf 1987; Presser et al. 1994; Saiki and Lowe 1987). However, unlike other bioaccumulative contaminants such as mercury, the single largest step in selenium accumulation in aquatic environments occurs at the base of the food web where algae and other microorganisms accumulate selenium from water by factors ranging from several hundred to tens of thousands (Luoma and Presser 2009; Orr et al. 2012; Stewart et al. 2010). Bioaccumulation and transfer through aquatic food webs are the major biogeochemical pathways of selenium in aquatic ecosystems. Dissolved selenium oxyanions (selenate, selenite) and organic selenides are assimilated into the tissues of aquatic primary producers (trophic level 1 organisms), such as periphyton, phytoplankton, and vascular macrophytes; and subsequently biotransformed into organoselenium. These organisms, together with other particle-bound selenium sources, constitute the particulate selenium fraction in the water column. Selenium from this particulate fraction is then transferred to aquatic primary

consumers such as zooplankton, insect larvae, larval fish, and bivalves (trophic level 2), and then to predators such as fish and birds (trophic level 3 and above). In addition to the water concentration of selenium, the process of selenium bioaccumulation in aquatic life residing in freshwater systems depends on several factors specific to each aquatic system. These factors include:

Water residence time. Residence time is a measure of the average time a water molecule will spend in a specified region of space. Residence time influences both the proportion of selenium found in particulate and dissolved forms and the predominant form of selenium. Organisms in waters with long residence times such as lakes, ponds, reservoirs, wetlands or estuaries will tend to bioaccumulate more selenium than those living in waters with shorter residence times such as rivers and streams (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). Several interrelated factors underlie selenium's greater bioaccumulation potential in slow moving systems, such as food web complexity and the organic content and reduction/oxidation potential of sediments. Finally, selenium toxicity in flowing waters with shorter residence times may only be apparent downstream of their selenium sources, whereas waters with longer residence times are more likely to exhibit selenium toxicity near their sources (Presser and Luoma 2006).

Distribution of selenium between particulate and dissolved forms. Selenium is found in both particulate and dissolved forms in water, but direct transfer of selenium from water to animals is only a small proportion of the total exposure. The proportion of selenium found in particulate matter (algae, detritus, and sediment) is important because it is the primary avenue for selenium entering into the aquatic food web (Luoma et al. 1992; Luoma and Rainbow 2005; Ohlendorf et al. 1986; Presser and Ohlendorf 1987; Presser et al. 1994; Presser and Luoma 2006; Saiki and Lowe 1987).

Bioaccumulation in prey. Trophic level 1 organisms such as periphyton and phytoplankton, as well as other forms of particulate material containing selenium, such as detritus and sediment, are ingested by trophic level 2 organisms such as mollusks, planktonic crustaceans, and many insects, increasing the concentration of selenium in the tissues of these higher-level organisms. Differences in the physiological characteristics of these organisms result in different levels of bioaccumulation. Also, selenium effects on invertebrates typically occur at concentrations higher than those that elicit effects on the vertebrates (e.g., fish and birds) that

prey upon them. Additionally, certain molluscan taxa such as mussels and clams can accumulate selenium to a much greater extent than planktonic crustaceans and insects (although the levels do not seem to be toxic to the mussels) due to higher ingestion rates of both particulate-bound (algae) and dissolved selenium from the water column through filter feeding, as well as the lower rate at which they eliminate selenium (Luoma and Rainbow 2005; Stewart et al. 2013). Because egg-laying (oviparous) vertebrates such as fish and birds are most sensitive to selenium effects, (Janz et al. 2010), these vertebrate consumers are also the most vulnerable groups to selenium poisoning and the focal point of most selenium environmental assessments (Ogle and Knight 1996; Stewart et al. 2010).

Trophic transfer to predators. Bioaccumulation of selenium by higher trophic level organisms, such as trophic level 3 and 4 fish, is highly influenced by the food web of the aquatic environment. For example, fish that primarily consume freshwater mollusks (e.g., redear sunfish) will exhibit greater selenium bioaccumulation than fish that consume primarily insects or crustaceans from waters with the same concentration of dissolved selenium because mollusks tend to accumulate selenium at higher concentrations than other trophic level 2 organisms, as noted above (Luoma and Presser 2009; Stewart et al. 2004).

2.3 Mode of Action and Toxicity of Selenium

Selenium is a naturally occurring chemical element that is also an essential micronutrient. Trace amounts of selenium are required for normal cellular function in almost all animals. However, excessive amounts of selenium can also have toxic effects, with selenium being one of the most toxic of the biologically essential elements (Chapman et al. 2010). Egg-laying vertebrates have a lower tolerance than do mammals, and the transition from levels of selenium that are biologically essential to those that are toxic occurs across a relatively narrow range of exposure concentrations (Luckey and Venugopal 1977; U.S. EPA 1987, 1998; Haygarth 1994; Chapman et al. 2009, 2010). Selenium consumed in the diet of adult female fish is deposited in the eggs, when selenium replaces sulfur in vitellogenin, which is transported to the ovary and incorporated into the developing ovarian follicle (Janz et al. 2010), the primary yolk precursor.

Selenium is a member of the sulfur group of nonmetallic elements, and consequently, the two chemicals share similar characteristics. Selenium can replace sulfur in two amino acids, the seleno-forms being selenomethionine and selenocysteine. It has been a long-standing hypothesis

that the cause of malformations in egg-laying vertebrates is due to the substitution of selenium for sulfur in these amino acids and their subsequent incorporation into proteins, which causes disruption of the structure and function of the protein. When present in excessive amounts, selenium is erroneously substituted for sulfur, resulting in the formation of a triselenium linkage (Se-Se-Se) or a selenotrisulfide linkage (S-Se-S), either of which was thought to prevent the formation of the normal disulfide chemical bonds (S-S). The end result was thought to be distorted, dysfunctional enzymes and protein molecules that impaired normal cellular biochemistry (Diplock and Hoekstra 1976; Reddy and Massaro 1983; Sunde 1984).

Recent research, however, suggests that selenium's role in oxidative stress plays a role in embryo toxicity, whereas selenium substitution for sulfur does not. The substitution of selenomethionine for methionine does not appear to affect either the structure or function of proteins (Yuan et al. 1998; Mechaly et al. 2000; Egerer-Sieber et al. 2006). The reason is apparently due to selenium not being distally located in selenomethionine, which insulates the protein from an effect on its tertiary structure. Although the incorporation of selenomethionine into proteins is concentration-dependent (Schrauzer 2000), selenocysteine's incorporation into proteins is not (Stadtman 1996). This suggests that neither selenomethionine nor selenocysteine affect protein structure or function. In fact, Se as an essential micronutrient is incorporated into functional and structural proteins as selenocysteine.

The role of selenium-induced oxidative stress in embryo toxicity and teratogenesis appears to be related to glutathione homeostasis. A review of bird studies by Hoffman (2002) showed exposure to selenium altered concentrations and ratios of reduced to oxidized glutathione thereby increasing measurements of oxidative cell damage. Palace et al. (2004) suggested oxidative stress due to elevated selenium levels results in pericardial and yolk sac edema in rainbow trout embryos. Evidence for the role of oxidative stress in selenium toxicity is growing, but mechanistic studies are needed to better understand its effects on egg-laying vertebrates. For a more in depth discussion on the mechanism of toxicity at the cellular level including the evidence against sulfur substitution as a cause and the role of oxidative stress, see Janz et al. (2010).

The most well-documented, overt and severe toxic symptoms in fish are reproductive teratogenesis and larval mortality. Egg-laying vertebrates appear to be the most sensitive taxa, with toxicity resulting from maternal transfer to eggs. In studies involving young organisms

exposed through transfer of selenium from adult female fish into their eggs, the most sensitive diagnostic indicators of selenium toxicity in vertebrates occur when developing embryos metabolize organic selenium that is present in egg albumen or yolk. It is then further metabolized by larval fish after hatching.

A variety of lethal and sublethal deformities can occur in the developing fish exposed to selenium, affecting both hard and soft tissues (Lemly 1993b). Developmental malformations are among the most conspicuous and diagnostic symptoms of chronic selenium poisoning in fish. Terata are permanent biomarkers of toxicity, and have been used to identify impacts of selenium on fish populations (Maier and Knight1994; Lemly 1997b). Deformities in fish that affect feeding or respiration can be lethal shortly after hatching. Terata that are not directly lethal, but distort the spine and fins, can reduce swimming ability, and overall fitness. Because the rate of survival of deformed young would be less than that for normal young, the percentage of deformed adults observed during biosurveys will likely understate the underlying percentage of deformed young, although quantitation of the difference is ordinarily not possible.

In summary, the most sensitive indicators of selenium toxicity in fish larvae are effects modulated through the reproductive process and exhibited in fish larvae as teratogenic deformities such as skeletal, craniofacial, and fin deformities, and various forms of edema that result in mortality (Lemly 2002). The toxic effect generally evaluated is the reduction in the number of normal healthy offspring compared to the starting number of eggs. In studies of young organisms exposed to selenium solely through their own diet (rather than via maternal transfer), reductions in survival and/or growth are the effects that are generally evaluated.

2.4 Narrow Margin between Sufficiency and Toxicity of Selenium

Selenium has a narrow range encompassing what is beneficial for biota and what is detrimental. Selenium is an essential nutrient that is incorporated into functional and structural proteins as selenocysteine and selenomethionine. Several of these proteins are enzymes that provide cellular antioxidant protection. Selenomethionine is readily oxidized, and its antioxidant activity arises from its ability to deplete reactive oxygen species. Selenomethionine is required as a mineral cofactor in the biosynthesis of glutathione peroxidases. All of the classic glutathione peroxidases contain selenium and are found to be involved in the catalytic reaction of these many enzymes (Allan 1999). The major function of the glutathione peroxidases involves the reduction

of hydrogen peroxide to water at the expense of the oxidation of glutathione, the enzyme's cofactor, an important antioxidant process at normal dietary levels.

Aquatic and terrestrial organisms require low levels of selenium in their diet to sustain metabolic processes, whereas excess concentrations of selenium that are only an order of magnitude greater than the required level have been shown to be toxic to fish, apparently due to generation of reactive oxidized species, resulting in oxidative stress (Palace et.al. 2004). Dietary requirements in fish have been reported to range from 0.05 to 1.0 mg Se/kg dw (Watanabe et al. 1997). Selenium requirements for optimum growth and liver glutathione peroxidase activity in channel catfish were reported as 0.25 mg Se/kg dw (Gatlin and Wilson 1984). Estimated selenium dietary requirements in hybrids of striped bass, based on selenium retention, were reported as 0.1 mg Se/kg dw (Jaramillo 2006). Selenium deficiency has been found to affect humans (U.S. EPA 1987), sheep and cattle (U.S. EPA 1987), deer (Oliver et al. 1990), fish (Thorarinsson et al. 1994; Wang and Lovell 1997; Wilson et al. 1997; U.S. EPA 1987), aquatic invertebrates (Audas et al. 1995; Caffrey 1989; Cooney et al. 1992; Cowgill 1987; Cowgill and Milazzo 1989; Elendt 1990; Elendt and Bais 1990; Harrison et al. 1988; Hyne et al. 1993; Keating and Caffrey 1989; Larsen and Bjerregaard 1995; Lim and Akiyama 1995; Lindstrom 1991; U.S. EPA 1987; Winner 1989; Winner and Whitford 1987), and algae (Doucette et al. 1987; Keller et al. 1987; Price 1987; Price et al. 1987; Thompson and Hosja 1996; U.S. EPA 1987; Wehr and Brown 1985). The predominance of research on selenium deficiency in invertebrates and algae is related to optimizing the health of test organisms cultured in the laboratory. A summary of several studies that evaluated the deficiency and/or the sufficiency of selenium in the diet of fish is provided in Appendix E.

2.5 Interactions with Mercury

The most well-known interactions with selenium occur with both inorganic and organic mercury, and are generally antagonistic (Micallef and Tyler 1987; Cuvin and Furness 1988; Paulsson and Lundbergh 1991; Siegel et al. 1991; Southworth et al. 1994; Ralston et al. 2006), with the most likely mechanism being the formation of metabolically inert mercury selenides (Ralston et al. 2006; Peterson et al. 2009). However, other studies have found interactions between mercury and selenium to be additive (Heinz and Hoffman 1998) or synergistic

(Huckabee and Griffith 1974; Birge et al. 1979). The underlying mechanism for these additive and synergistic interactions between mercury and selenium are unknown.

2.6 ASSESSMENT ENDPOINTS

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" and are defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (U.S. EPA 1998). Assessment endpoints may be identified at any level of organization (e.g., individual, population, community). In the context of the Clean Water Act, aquatic life criteria for toxic pollutants are typically determined based on the results of toxicity tests with aquatic organisms in which unacceptable effects on growth, reproduction, or survival occurred. The goal of criteria is to protect the diversity, productivity, and stability of aquatic communities. To achieve this goal, the endpoint of criteria assessment is the survival, growth, and reproduction of a high percentage of species of a diverse assemblage of freshwater aquatic animals (fish, amphibians, and invertebrates) and plants. Toxicity data are aggregated into a sensitivity distribution that indicates the impact of the toxicant under study to a variety of genera representing the broader aquatic community. Criteria are designed to be protective of the vast majority of aquatic animal species in an aquatic community (i.e., approximately 95^{th} percentile of tested aquatic animals representing the aquatic community). As a result, health of the aquatic community may be considered as an assessment endpoint indicated by survival, growth, and reproduction. Assessment endpoints are the ultimate focus in risk characterization and link the measurement endpoints to the risk management process (e.g., policy goals). When an assessment endpoint can be directly measured, the measurement and assessment endpoints are the same. In most cases, however, the assessment endpoint cannot be directly measured, so a measurement endpoint (or a suite of measurement endpoints) is selected that can be related, either qualitatively or quantitatively, to the assessment endpoint. For example, a decline in a sport fish population (the assessment endpoint) may be evaluated using laboratory studies on the mortality of surrogate species, such as the fathead minnow (the measurement endpoint) (EPA/630/R-92/001 February 1992). The assessment endpoint for selenium is the protection of freshwater aquatic life; because we know that fish are the most sensitive aquatic taxon to the toxicological effect of selenium, the criterion is expressed in terms of fish tissue using eggs and ovarian tissue as the most representative element related to selenium toxicity.

To assess potential effects on the aquatic ecosystem by a particular stressor, and develop 304(a) aquatic life criteria under the CWA, EPA typically requires the following, as outlined in the EPA Ambient Water Quality Criteria Guidelines: acute toxicity test data (mortality, immobility, loss of equilibrium) for aquatic animals from a minimum of eight diverse taxonomic groups; as well as chronic toxicity data (e.g., survival, growth and reproduction) for aquatic animals from 8 eight taxonomic groups (described in more detail below). The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. In the case of bioaccumulative compounds like selenium, these acute toxicity studies do not address risks that result from exposure to chemicals via the diet (through the food web). They also do not account for the slow accumulation kinetics of many bioaccumulative compounds such as selenium and may underestimate effects from long-term accumulation in different types of aquatic systems (SAB 2005).

Because the most sensitive adverse effects of selenium are reproductive effects (larval deformities and mortality) on the offspring of exposed fish, chronic effects from long term exposure are the focus of this selenium assessment. In addition to continuous discharges, shorter-term intermittent or pulsed exposures to elevated levels of selenium may also result in bioaccumulation through the aquatic food web and may subsequently adversely affect fish reproduction, and such measures of effect are therefore also estimated from chronic assessment endpoints. Selenium toxicity in the water body could potentially threaten fecundity and recruitment in fishes, resulting in extirpation of sensitive species in a waterbody, and potentially shifting the trophic dynamics of the system. Therefore, the assessment endpoint for selenium is the protection of fish populations. In some waters where ESA-listed fish species occur, a protection goal oriented to protection of individuals may be more appropriate. This should be reflected using site-specific data to derive an SSC for the site.

Chronic toxicity test data (longer-term survival, growth, or reproduction) for aquatic animals are needed from a minimum of eight diverse taxonomic groups (or less generically, [minimum of three taxa] if the derivation is based on an acute to chronic ratio). The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. Specific minimum data recommendations or requirements (MDRs) identified for development of criteria in the EPA Ambient Water Quality Criteria Guidelines require aquatic animal toxicity data from:

1. the family Salmonidae in the class Osteichthyes,
2. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.),
3. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.),
4. a planktonic crustacean (e.g., cladoceran, copepod, etc.),
5. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.),
6. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.),
7. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.), and
8. a family in any order of insect or any phylum not already represented.

Acceptable quantitative chronic values for selenium are available for six of the eight MDRs (requirements 1, 2, 3, 6, 7, and 8). Acceptable chronic values for selenium are not available for two of the MDRs (requirements 4 and 5: planktonic and benthic crustaceans, respectively). Following the approach of U.S. EPA (2008b), which was reviewed by the Science Advisory Board, if information is available to demonstrate that an MDR is not sensitive, then a surrogate value can be used in place of actual toxicity data to represent the missing MDR. Based on the data estimating the sensitivity of insects (*Centroptilum triangulifer*), rotifers (*Brachionus calyciflorus*), and oligochaetes (*Lumbriculus variegatus*), EPA determined that invertebrates (e.g., insects and crustaceans) are generally less sensitive to selenium than fish, based on the characteristics of selenium toxicity to aquatic life. Therefore, the available fish data were used in the genus-level sensitivity distribution to derive the chronic selenium criterion (Note: invertebrate data were included in the sensitivity distribution for the whole body criterion element to demonstrate that the derivation of the criterion element based on the fish egg-ovary to whole body translated values protected invertebrates given the sensitivity range of the available species).

The EPA Ambient Water Quality Criteria Guidelines also require at least one acceptable test with a freshwater alga or vascular plant. If plants are among the aquatic organisms most sensitive to the stressor, results of a plant in another phylum should also be available. A

relatively large number of tests from acceptable studies of aquatic plants were available for possible derivation of a Final Plant Value. However, the relative sensitivity of freshwater plants to selenium (Appendix F) is less than fish, so plant criterion elements were not developed.

The available scientific evidence indicates that for selenium, critical assessment endpoints for aquatic species are offspring mortality and severe development abnormalities that affect the ability of fish to swim, feed and successfully avoid predation, resulting in impaired recruitment of individuals into fish populations. Selenium enrichment of reservoir environments (e.g., Belews Lake, NC (Lemly 1985), Hyco Reservoir (DeForest 1999), and Kesterson Reservoir, CA (Ohlendorf 1986)) are well documented and demonstrate that adverse effects resulted from bioaccumulative processes at different levels of biological organization, resulting in population-level reductions of resident species.

2.7 Measures of Effect

Each assessment endpoint requires one or more "measures of ecological effect", which are defined as changes in the attributes of an assessment endpoint itself or changes in a surrogate entity or attribute in response to chemical exposure. Ecological effects data are used as measures of direct and indirect effects to growth, reproduction, and survival of aquatic organisms.

The toxicity testing data available for any given pollutant vary significantly, depending primarily on whether any major environmental issues are raised. An in-depth evaluation of available data for selenium has been performed by EPA to determine data acceptability and quality, based on criteria established in the EPA Ambient Water Quality Criteria Guidelines.

In traditional chronic tests used in many EPA aquatic life criteria documents, organisms are exposed to contaminated water but fed a diet grown in uncontaminated media not spiked with the toxicant prior to introduction into the exposure chambers. Such tests are not suitable for deriving a criterion for a bioaccumulative pollutant unless (1) effects are linked to concentrations measured in appropriate tissues, and (2) the route of exposure does not affect the potency of residues in tissue. For selenium, the first condition might be met, but the second condition is not, because the route of selenium exposure appears to influence the potency of a given tissue residue (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978). Consequently, toxicity tests with water-only exposures (and any tests not relying on dietary exposure) are not included in this assessment.

Selenium toxicity is primarily manifested as reproductive impairment due to maternal transfer, resulting in embryo mortality and teratogenicity. Measurements of selenium in fish tissue are most closely linked to the chronic adverse effects of selenium (Chapman et al. 2010), since chronic selenium toxicity is based on the food-chain bioaccumulation route, not a direct waterborne route. In this selenium criterion document, water column criterion element concentrations for selenium were derived from fish tissue concentrations by modeling selenium transfer through the food web. The next sections describe approaches used to establish selenium effects concentrations in fish tissue and to relate the concentrations in fish tissue to concentrations in water.

2.7.1 Fish Tissue

Chronic measures of effect concentrations are the $EC_{10,}$ $EC_{20,}$ No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), and Maximum Acceptable Toxicant Concentration (MATC). The EC_{10} is the concentration of a chemical that is estimated to result in a 10 percent effect in a measured chronic endpoint (e.g., growth, reproduction, or survival); the EC_{20} corresponds to 20 percent effect. The NOEC is the highest chemical concentration at which none of the observed effects are statistically different from the control, as determined by hypothesis testing. The LOEC is the lowest test concentration at which observed effects are found to be statistically different from the control. For selenium, in all cases the effect endpoint used in the estimation of chronic values (e.g., EC_{10}s) is an effect on offspring (with exposure via maternal transfer) from parents exposed to selenium via diet. Selenomethionine was used exclusively in dietary exposures in the lab, whereas field-exposed females would be exposed to a combination of forms of selenium as a function of the selenium in their prey items.

Whenever possible, estimates of selenium concentrations associated with a low level of effect (i.e., EC_{10}) were calculated for each study using the computer program TRAP (version 1.30a), Toxicity Relationship Analysis Program (U.S. EPA 2013). The program is based on a regression approach that models the level of adverse effects as a function of increasing concentrations of the toxic substance. With the fitted model it is possible to estimate the contaminant concentration associated with a small effect. TRAP was used when there are sufficient data for EC_{10} estimation. For studies with binary data, the analysis proceeded by tolerance distribution analyses using the log-triangular distribution, unless there was substantial

extrabinomial variability, in which case regression analysis was used. For regression analysis, the threshold sigmoidal model was used, exposure variables were log-transformed, and effects variables were weighted appropriately to address their relative uncertainties.

When there were insufficient data for TRAP to fit an effects/exposure curve (no treatments with clear effects near the EC_{10} and/or significant background variability), the EC_{10} was based on interpolation. To ensure that the interpolations were comparable to the TRAP analyses, threshold sigmoidal equation was used. This equation is fitted to two points, and constrained so that 3 equation parameters can be set. The first set-point was treated as the EC_0 with a second associated set-point being the threshold for background effects values, based on the highest NOEC (HNOEC) datum and other NOEC data. The final set-point was the LOEC. If the LOEC is a partial effect, then this point was used to estimate the equation slope. If the LOEC was a 100% effect, it was specified as the EC_{100}; with the EC_0 specified, then this relationship dictated the equation slope. It should be noted that despite the superficial resemblance of these analyses to TRAP they are also subject to the uncertainties associated with the interpolation method.

It should be noted that TRAP involves the assumption that (a) there is a single underlying relationship of the effects variable to the exposure variable which follows the specified equation and (b) the exposure variable is known with negligible error, with uncertainty being predominantly in the effects variable. Some of the reproductive data for selenium involved multiple sources of variability that led to both multiple relationships across different cohorts of offspring and uncertainty in the exposure variable, so that the resulting TRAP curves were more approximate, and TRAP error estimates were generally not useful. These issues can also affect the interpolation protocol. It should also be noted that estimating a concentration associated with a low effects level, such as an EC_{10}, is especially uncertain when treatments yielding partial effects values are lacking in the concentration response data produced by a study. These two issues prevented the use of TRAP in some datasets. When the data are insufficient to provide any meaningful EC_{10} by the first two approaches, the study should either not be used for criteria development or a chronic value should be set by other means than an estimated EC_{10} if possible.

Only studies with a reference site (field surveys) or control treatment(s) (experimental studies) were included in the analysis, because response levels at these low selenium concentrations were the most influential points for calculating the estimated response level at a

selenium concentration of zero (y_0). When considering the use of the EC_{10} versus the EC_{20}, an EC_{10} was determined to be a more appropriate endpoint for tissue-based criteria given the nature of exposure and effects for this bioaccumulative chemical. EC_{20}s have historically been used in the derivation of EPA criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually over time. Thus, where concentrations of selenium in fish tissue are used as an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that are not as bioaccumulative. Furthermore, it was found that the dose-response curves for selenium across a broad range of fish genera are very steep, such that a small change in selenium tissue concentration yielded a large increase in observed adverse effect. In many cases, the selenium data indicated a change from control effect levels to effects in excess of 50% for larval mortality or deformity over a few mg/kg dry weight increase in selenium detected in fish tissue. These issues call for use of a lower level of effect to attain sufficient protection. The EC_{10} was also preferred over the NOEC or LOEC as these measures of effect are influenced by study design, specifically the concentrations tested, the number of concentrations tested, the number of replicates for each concentration, and the number of organisms in each replicate. As noted by Campbell (2011), EC_{10}s and NOECs are generally of similar magnitude, but EC_{10}s have the advantage of being more reproducible than NOECs (Van der Hoeven et al. 1997; Warne and van Dam 2008). NOECs and MATCs are generally presented if calculated by the original investigators, but were not used where an EC_{10} could be calculated. The four lowest egg-ovary Genus Mean Chronic Values (GMCVs), whose exact values influence the calculation of the egg-ovary criterion element, are all based solely on EC_{10}s. NOECs contribute to some of the GMCVs for less sensitive species.

In this document, chronic values are presented as tissue concentrations of total selenium in units of mg/kg dry weight (dw). Studies of chronic toxicity of selenium to aquatic organisms measure concentrations in distinct tissues (e.g., whole body, ovaries, eggs, muscle, and liver) and report these values as either wet weight (ww) or dw. Studies reporting tissue concentrations only based on wet weight were converted to dry weight using tissue-specific and species-specific conversion factors. When wet to dry weight conversion factors were not available for a given species, conversion factors for a closely related taxon were used. In deriving the egg or ovary tissue criterion element, chronic values are for those tissues directly measured in the study.

Tissue-to-tissue conversions (e.g., to estimate concentrations in an unmeasured tissue from a study's measured tissue) involve some uncertainty because of variability in tissue concentration ratios (deBruyn et al. 2008; Osmudson et al. 2007). Tissue-to-tissue conversions were needed for calculating the reproductive toxicity-based whole-body and muscle chronic criterion element and water criterion concentration elements.

The overall assessment evaluates both reproductive and non-reproductive studies. Selenium concentrations measured directly in eggs or ovaries from reproductive (maternal transfer) studies are used to derive the egg/ovary criterion element, and corresponding selenium concentrations in whole body or muscle tissue resulting in reproductive effects are estimated using conversion factors. Direct measurements of selenium concentrations in whole-body or muscle from non-reproductive studies are used to examine non-reproductive, chronic effects, such as impairments to growth.

2.7.2 Water

While state monitoring programs may sample ambient waters for selenium, widespread measurements of selenium in fish tissue are relatively rare. Therefore, EPA is providing estimated chronic measures of effect for water column data. The chronic criterion element for the water column is the 30-day average concentration that corresponds to the concentration of selenium in fish tissue estimated to result in a 10 percent effect in fish for a specific water body type (lotic or lentic water bodies as described below in **Section 3.2.4**). The chronic criterion element for the water column is derived by modeling trophic transfer of selenium through the food web resulting in the fish tissue concentration that yields the chronic reproductive effects of concern.

EPA collaborated with the United States Geological Survey (USGS) to develop a model (later published in Presser and Luoma 2010) that relates the concentration of selenium in fish tissue to the water column. The approach is based on bioaccumulation and trophic transfer through aquatic system food-webs. Model parameters are calculated using both field and laboratory measurements of selenium in water, particulate material (algae, detritus and sediment), invertebrates, fish whole-body, and fish egg-ovary. Although EPA and USGS use the same model to relate the concentration of selenium in fish tissue to water, EPA starts with selenium in the egg/ovary (reproductive criterion) whereas USGS starts with selenium in the fish's whole body. The EPA approach therefore has the additional step of converting the

concentration of selenium in the egg/ovary to whole body. This model (which is a set of equations) is described in more detail in **Section 3.2.1**.

2.7.3 Summary of Assessment Endpoints and Measures of Effect

The typical assessment endpoints for aquatic life criteria are based on effects on growth, reproduction, or survival of the assessed taxa. These measures of effect on toxicological endpoints of consequence to populations are provided by results from toxicity tests with aquatic plants and animals. The toxicity values (i.e., measures of effect expressed as genus means) are used in the genus sensitivity distribution of the aquatic community to derive the aquatic life criteria. Endpoints used in this assessment are listed in **Table 2.2**.

Table 2.2. Summary of Assessment Endpoints and Measures of Effect Used in Criterion Derivation for Selenium.

Assessment Endpoints for the Aquatic Community	**Measures of Effect**
Survival, growth, and reproduction of freshwater fish, other freshwater vertebrates, and invertebrates	For effects from chronic exposure: 1. EC_{10} concentrations in egg and ovary, for offspring mortality and deformity. 2. Measured or estimated reproductive EC_{10} in whole body and muscle. 3. Estimated concentrations (µg/L) in water linked to egg-ovary EC_{10}s by food web-modeling. 4. Intermittent water concentrations yielding exposure equivalent to the above. For acutely lethal effects: Acute toxicity effects based on standard water column-only toxicity testing are not provided here for selenium, due to the dominant significance of chronic effects. Note: Chronic criterion is expected to be protective of acute effects.

2.7.4 Conceptual Model of Selenium Effects on Aquatic Life

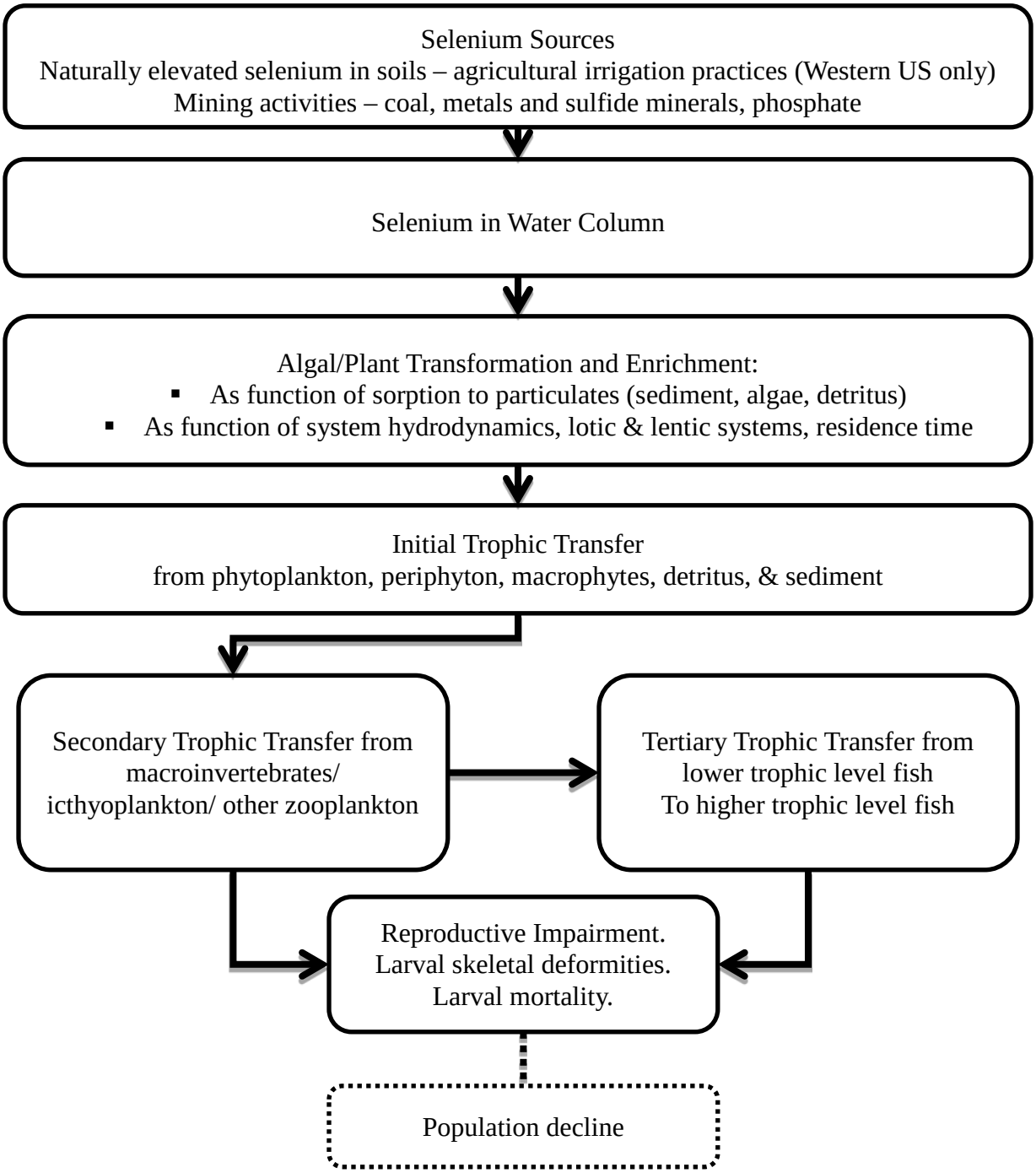


Figure 2.2. Diagram of Selenium Partitioning, Bioaccumulation, and Effects in the Aquatic Environment.

The conceptual model links sources, transformation and uptake through media phases, and consumer transfer and dynamics reflective of the movement of selenium through ecosystems (**Figure 2.2**). Diet is the dominant pathway of selenium exposure for both invertebrates and

vertebrates. Selenium moves from water to particulates, a collection of biotic and abiotic compartments that includes primary producers, detritus, and sediments, which form the base of aquatic food webs. Transfer from particulates to primary consumers (e.g., macroinvertebrates) to fish is species specific. Knowledge of the food web is one of the keys to determining which biological species or other ecological characteristics will be affected.

During the development of CWA section 304(a) criteria, EPA assembles all available test data and considers all the relevant data that meet acceptable data quality and test acceptability standards. This criterion update document is specific to selenium in fresh water. Chronic criterion elements for selenium are protective concentrations measured in fish tissue and related to protective water concentrations generated using food-web modeling. Further modeling is used to estimate short-term concentrations in water from intermittent or pulsed exposures that are protective against the chronic effect.

2.7.5 Analysis Plan for Derivation of the Chronic Fish Tissue-Based Criterion Elements

Data for possible inclusion in the selenium dataset were obtained primarily by search of published literature using EPA's public ECOTOX database (up to July 2013). These studies were screened for data quality as described in the EPA Ambient Water Quality Criteria Guidelines, and adjusted for factors related to dietary lab or field exposure, which were not considered at the time the Guidelines were written. Additional data were considered and reviewed for inclusion in this criterion based on the public and peer review comments on the 2014 "External Peer Review Draft" criterion document, and public comments on the 2015 draft.

Chronic toxicity studies (both laboratory and field studies) were further screened to ensure they contained the relevant chronic exposure conditions of selenium to aquatic organisms (i.e., dietary, or dietary and waterborne selenium exposure), measurement of chronic effects, and measurement of selenium in tissue(s). The criterion derivation uses only those studies in which test organisms were exposed to selenium in their diet, because such studies most closely replicate real-world exposures (diet and/or diet plus water). This approach accords with findings and recommendations of the 2009 SETAC Pellston Workshop (Chapman et al. 2009, 2010).

EPA grouped studies based on whether the effects were chronic reproductive (e.g., effects on offspring survival or morphology) or chronic non-reproductive (e.g., juvenile growth and survival). At the 2009 Pellston workshop (Chapman et al. 2009, 2010), a group of 46 experts in the area of ecological assessment of selenium in the aquatic environment agreed that the most

important toxicological effects of selenium in fish arise following maternal transfer of selenium to eggs during vitellogenesis, resulting in selenium exposure when hatched larvae undergo yolk absorption. Such effects include larval mortality or permanent developmental malformations, such as skeletal and craniofacial deformities. Therefore, the chronic fish-tissue-based criterion elements are based on reproductive effects only.

The egg-ovary Species Mean Chronic Values (SMCVs) were calculated from the chronic values (EC_{10}s and occasionally NOECs) obtained from the relevant toxicity tests. Genus Mean Chronic Values (GMCVs) were calculated from the SMCVs and then rank-ordered from least to most sensitive. The four lowest egg-ovary Genus Mean Chronic Values (GMCVs), whose exact values influence the calculation of the egg-ovary criterion element, are all based solely on EC_{10}s. The egg-ovary Final Chronic Value (FCV) was calculated from regression analysis of the four most sensitive GMCVs, in this case extrapolating to the 5^{th} percentile of the distribution represented by the tested genera. The FCV directly serves as the fish tissue egg-ovary criterion concentration element without further adjustment because the underlying EC_{10}s represent a low level of effect (per the EPA Ambient Water Quality Criteria Guidelines).

For the whole-body and muscle criterion element concentrations, CVs were either measured directly using the relevant tissue or the egg-ovary CVs were converted to estimated equivalent whole-body or muscle CVs. The criterion concentration element expressed as whole-body or as muscle concentration was calculated in a manner similar to the egg-ovary criterion element using a combination of directly calculated CVs or CVs that used conversion factors described below.

2.7.6 Analysis Plan for Derivation of the Fish Tissue Criterion Elements Duration

Duration of the averaging periods in national criteria restrict allowable fluctuations in the concentration of the pollutant in the receiving water and restrict the length of time that the concentration in the receiving water can be continuously above a criterion concentration, in order to protect aquatic life. A numerical value for the fish tissue criterion elements averaging period, or duration, is specified as instantaneous, because fish tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of selenium over time and space in the fish population(s) at a given site. Selenium concentrations in fish tissue are generally expected to change only gradually over time (**Section 3.2.6** and Appendix J) in response to environmental fluctuations; thus, there would be relatively little difference in tissue concentrations with

different averaging period durations if the average selenium concentrations in water are relatively stable over time. Generally fish collected to calculate average tissue concentrations for a site are collected in one sampling event, or over a short time interval due to logistical constraints and costs for obtaining samples incurred by state monitoring programs.

2.7.7 Analysis Plan for Derivation of the Fish Tissue Criterion Elements Return Frequency

Frequency is the number of times an excursion can occur over time without impairing the aquatic community or other use. The current recommendation (1985 Guidelines – EPA PB85-227049) for return frequency of once in 3 years on average is based on the ability of an aquatic ecosystem to recover from a toxic insult when pollutant impacts are associated exclusively with a water column exposure. This recommendation is based on the variability of water concentrations that aquatic life will be exposed to, and is set at a low level such that the water concentrations would mostly be below the criteria concentration. Selenium, however, is a bioaccumulative pollutant, and elevated levels in various ecological compartments (e.g., biota, surficial sediments) require a long period to decrease and the associated aquatic community requires a long time to recover following reduction or removal of an elevated selenium exposure to a given system (e.g., Belews Lake, NC, and Hyco Lake, NC).

Cumbie and Van Horn (1978) first reported young of the year losses in Belews Lake quickly followed by dramatic decreases in standing stocks of many species, and particularly game species like bluegill and largemouth bass. Fish communities were reduced to selenium-tolerant species including cyprinids (e.g., fathead minnow) and green sunfish in both lakes. Selenium reduction in Belews Lake (1985) and Hyco Lake (1990), resulted in rapid decreases in [Se] in the water column, but reductions in fish tissue took much longer. Finley and Garrett (2007) show that concentrations in bluegill and largemouth decreased from 19 and 17 mg/kg dw, respectively in 1992-1994 to ~8.0 mg/kg dw in both species sampled between 2003-2005. In Belews Lake, where Se contamination was higher, [Se] in crappie and redear sunfish averaged 18 and 17 mg/kg dw respectively in 1994-1996, and decreased to ~9-10 mg/kg dw in both species based on sampling in 2004-2006, twenty years later.

Chapman et al. (2010) also reported a similar scenario for Hyco Lake where “fish recruitment failure and the a massive fish kill in 1980 led to a decimated fishery. Selenium concentrations in the reservoir were reduced beginning in 1990 and gradual reductions in Se exposure via the food web led to the reestablishment of a diverse Hyco Lake fish community

similar to the period prior to Se impact." The Belews and Hyco Lake examples indicate that a protracted period of time (in excess of 10 years) would be necessary for fish communities to recover once a selenium in fish tissue reached concentrations associated with reproductive impacts. Thus, the typical "once-in-three years on average" criteria return frequency is not appropriate for selenium, as this could lead to sustained ecological impacts.

2.7.8 Analysis Plan for Derivation of Chronic Water-based Criterion Element

The relationship between the ambient concentration of selenium in water and the concentration of selenium in the eggs or ovaries of fish is primarily through trophic transfer of selenium, which is greatly affected by site-specific conditions. EPA used a peer-reviewed model to derive water concentrations from the egg-ovary criterion element that explicitly recognizes partitioning of selenium in water and particulate material (algae, detritus, and sediment), and trophic transfer from particulate material to aquatic invertebrates, from invertebrates to fish, and partitioning in fish whole-body and fish eggs and ovaries. The method is composed of five main steps:

1. Formulate a mathematical equation relating the concentration of selenium in the eggs and ovaries of fish to the ambient concentration of selenium in the water column.
2. Develop parameters needed to use the mathematical equation formulated in step 1 from available empirical or laboratory data related to selenium bioaccumulation in aquatic systems and aquatic organisms.
3. Classify categories of aquatic systems where a single water column concentration would be adequately protective by evaluating the bioaccumulation potential at the base of the aquatic food web.
4. Translate the egg-ovary criterion element to an equivalent water column concentration at each aquatic site.
5. Apply a statistical threshold to the distribution of translated water column concentrations for each aquatic system category to yield a water column concentration value that would be protective of each aquatic system category.

EPA worked with USGS to derive a translation equation to estimate the site-specific concentration of selenium in the water column corresponding to the egg-ovary criterion element concentration. This equation utilizes a mechanistic model of bioaccumulation previously

published in peer-reviewed scientific literature (Luoma et. al. 1992; Wang et. al. 1996; Luoma and Fisher 1997; Wang 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006, 2010; Presser 2013). The equation uses site-specific food web models, species-specific Trophic Transfer Factor (*TTF*) values, egg-ovary to whole-body conversion factor (*CF*) values, and a site-specific enrichment factor (*EF*) values to calculate a site-specific water column concentration element from the egg-ovary criterion element.

Empirical or laboratory data related to selenium bioaccumulation in aquatic organisms are needed to calculate species-specific *TTF* and *CF* parameters and a site-specific *EF* parameter. EPA obtained these data by reviewing its extensive selenium library of published papers and reports, by searching published literature using EPA's public ECOTOX database and other publically available data received through solicitation of public comments on the 2014 "External Peer Review" draft, through the external peer reviewers of the 2014 draft, and through public comments on the 2015 draft criterion document. Studies were screened using the same data quality guidelines described above. Relevant studies contained selenium measurements from field studies (water, particulate material, and aquatic organisms) or contained laboratory data on physiological parameters of selenium bioaccumulation in aquatic organisms. Literature searches for information on selenium associated with particulate matter included searches for data on all forms of algae, detritus, inorganic suspended material, and sediment.

EPA compiled a collection of selenium concentration measurements from acceptable field studies. Measurements were accepted if the study indicated the samples were collected in the field, and the study identified the unit of measure, the media from which the measurement was made, the location from where the sample was taken, and the date the sample was collected. EPA only used data from studies with adequately described field collection protocols and where concentrations were within the bounds of concentrations found using modern, rigorous protocols in similar systems (Sañudo-Wilhelmy et al. 2004). The spatial precision of field data sample collection locations were generally at the site level, although aggregate measurements were also included if exposure conditions were considered similar (e.g., averages of single or composite measurements from several locations in the same aquatic system). The temporal precision of sample collection times were usually at the level of the day they were collected, although some studies only provided enough information to determine the week, month, or year. If the day a series of samples were collected was not reported but the study provided information that

indicated the samples were taken concurrently, EPA noted sample precision, but assigned a single effective collection date to all the samples.

EPA also compiled a collection of physiological coefficients for food ingestion rate (*IR*), selenium assimilation efficiency (*AE*), and rate of selenium loss (k_e) from published literature. Coefficients were accepted if the studies provided either the actual measurements or sufficient information to derive them, and were reported in standard units (k_e: /d; *AE*: %; *IR*: g/g-d) or could be converted to standard units. Even though IR can be highly variable (Whitledge and Hayward 2000), IR values of surrogate species were occasionally used.

EPA accounted for bioaccumulation variability across aquatic sites by evaluating the parameter *EF* (representing the partitioning of selenium between the dissolved and particulate state) from representative aquatic systems. The parameter *EF* is a measure of bioaccumulation potential because it quantifies the transfer of selenium from the water column to particulate material, which is the single most influential step in selenium bioaccumulation (Chapman et al. 2010). EPA calculated *EF* values for a set of aquatic systems using data from published literature and applied statistical methods to distinguish categories with similar bioaccumulation characteristics. On this basis, a single water column concentration is deemed adequately protective when it is derived using data from aquatic sites in the same category. EPA translated the egg-ovary criterion element into a set of water concentration values and derived a water column criterion element for each aquatic system category using a percentile of the water column concentrations for each category. To ensure adequate protection, EPA selected the 20th percentile of the distribution of median water column values as the statistical cut-off (see **Section 3.2.5**). **Figure 2.3** diagrams the conceptual framework EPA used to derive water column criterion element values from the egg-ovary criterion element.

2.7.9 Analysis Plan for Derivation of the Water Criterion Elements Duration

A numerical value for the lentic and lotic water criterion elements averaging period, or duration, is specified as a 30-day average, because the presence of selenium in water is the initial step in the process of bioaccumulation from the water column to fish tissue. The bioaccumulation process for selenium takes place over a longer term than typically observed for acute and chronic effects on aquatic life based on water concentrations. The derivation of a protective water averaging period from kinetic modeling considerations is described in **Section 3.2.6** and in Appendix J. Because the intermittent criterion element values are based on the water

criteria chronic magnitudes and duration, the kinetic analysis of Appendix J also controls the intermittent criterion element values.

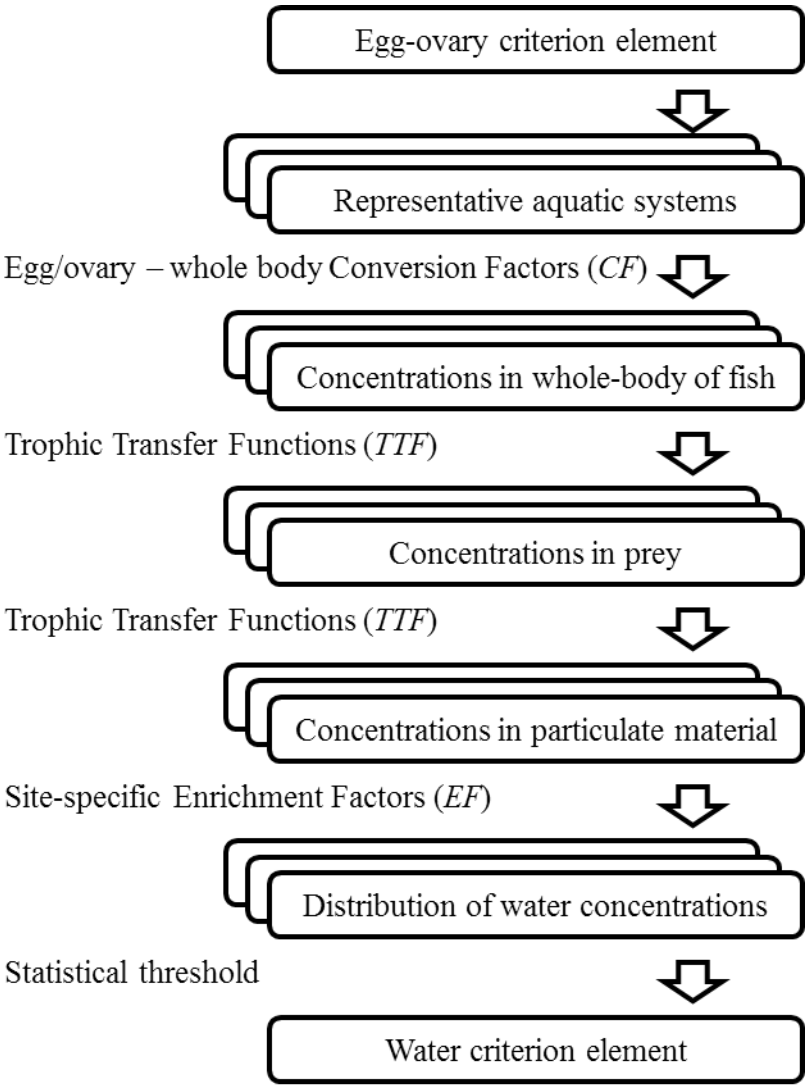


Figure 2.3. Conceptual Model for Translating the Selenium Egg-Ovary Concentration to a Water Column Concentration.

2.7.10 Analysis Plan for Intermittent-Exposure Water-based Criterion Element Derivation

Like the chronic water criterion element, the intermittent-exposure criterion element protects against cumulative exposure of selenium from multiple short-term discharges that may cause an excursion of the fish tissue criterion element. EPA derived the intermittent exposure criterion element directly from the chronic water criterion element by algebraically rearranging

the chronic water criterion element to establish a limit on an intermittent elevated concentration occurring over a specified percentage of time, while simultaneously accounting for background concentrations (see **Section 3.3**).

3 EFFECTS ANALYSIS FOR FRESHWATER AQUATIC ORGANISMS

3.1 CHRONIC TISSUE-BASED SELENIUM CRITERION ELEMENT CONCENTRATION

Data were obtained primarily by search of published literature using EPA's public ECOTOX database. The most recent ECOTOX database search extended to July 2013; this document also reflects data either gathered or received by EPA based on information from the 2014 public comment period and 2014 external expert peer review of the "External Peer Review Draft" published in May 2014, as well as information gathered based on public comments on the 2015 draft criterion. All available, relevant, and reliable chronic toxicity values were incorporated into the appropriate selenium AWQC tables and used to recalculate the FCV, as outlined in detail in the EPA Ambient Water Quality Criteria Guidelines.

The chronic values determined from acceptable chronic toxicity studies were separated into reproductive endpoint and non-reproductive endpoint categories. Although both sets of endpoints assess effects due to selenium on embryo/larval or juvenile development and survival and growth, the fundamental difference is exposure route (inherent in test design). That is, the fundamental difference is whether the aquatic organisms (e.g., fish) were directly exposed to selenium in the diet and water column or exposed via maternal transfer of selenium to the eggs/ovaries prior to reproduction. In studies with reproductive endpoints, parental females are exposed to selenium and the contaminant is transferred from the female to her eggs. In the selenium-exposed females, selenium replaces sulfur in vitellogenin, the primary yolk precursor, which is transported to the ovary and incorporated into the developing ovarian follicle (Janz et al. 2010). In most but not all of these studies, progeny from these females were not additionally exposed to aqueous selenium. The chronic values derived for the reproductive effects (survival, deformities, and edema) are based on the concentration of selenium in the eggs or ovary, the tissues most directly associated with the observed effects. In contrast, in studies grouped under non-reproductive effects (usually larval and/or juvenile survival or growth), the tested fish had no maternal pre-exposure to selenium. Chronic values for non-reproductive effects are based on the concentration of selenium in tissues measured in the study: muscle, liver and/or whole body.

The reproductive endpoint studies applied to the derivation of the chronic criterion elements are described below. Less definitive reproductive studies that are not directly applied to

the criterion derivation are described in **Section 6.1.2** and in Appendix C. Nonreproductive studies are described in **Section 6.1.9**.

3.1.1 Acceptable Studies of Fish Reproductive Effects for the Four Most Sensitive Genera

Below is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the four sensitive genera that drive the calculation of each specific chronic value. The studies in this section involve effects on the offspring of exposed female fish. Data are summarized in **Table 3.1**. Details of these studies and other chronic studies considered for criterion derivation are contained in Appendix C.

3.1.1.1 Acipenseridae

Acipenser transmontanus (white sturgeon)

Linville (2006) evaluated the effect of elevated dietary selenium on the health and reproduction of white sturgeon. Adult female white sturgeon (approximately 5 years old) were fed either a control diet (no added selenium, 1.4 mg/kg Se) or a diet spiked with selenized yeast (34 mg/kg Se) for six months in a freshwater flow through system. At the end of the dietary exposure, females were induced to spawn and fertilized with non-exposed male milt. Large cohorts of fertilized eggs from individual females (two from control and three from the treatment) were collected and separately hatched. After hatching (stage 36), n=500 sets of larvae were randomly distributed into each of six flowthrough chambers, three for stage 40 assessment and three for stage 45 assessment. Length, weight, mortality, abnormalities (edema, skeletal deformities) and selenium were measured at stages 36, 40 and 45. The mortality and abnormality observations from oldest stage (45) were used for effects analysis because these measurements showed the greatest response.

No selenium effects were observed for length or weight of larvae but effects were observed for both abnormalities (edema and skeletal deformities) and survival. Selenium concentrations in eggs from the control fish were 1.61 and 2.68 mg/kg dry weight (dw), and were 7.61, 11 and 20.5 mg/kg dw in eggs from the treatment fish. As stated above larval survival and abnormality frequency was evaluated at stage 45. Because the mortalities for each cohort were recorded up to the sample collection time for abnormalities, a combined effects variable was derived based on the total proportion of hatched larvae which were both alive and without any abnormalities at stage 45. This can be calculated as PS*(1-PA), where PS is the proportion

survival in the test chambers and PA is the proportion of the sample of surviving larvae with abnormalities. The larvae hatched from the batches of eggs with selenium concentrations of 1.61, 2.68, 7.61, 11 and 20.5 mg Se/kg dw had 0.3, 0.3, 13.6, 0.3 and 33.8% combined survival and abnormal (edema and deformities) effects, respectively.

Estimation of the EC_{10} was conducted using weighted nonlinear regression analysis with the threshold sigmoid model equation (TRAP version 1.30a). The binary data (i.e., survival and abnormalities) available in this study would normally be analyzed using the tolerance distribution analysis in TRAP; however, the combined survival/abnormalities effects variable in this study precludes its use because of the different sample sizes for survival and abnormality evaluation. When there are insufficient data for TRAP to fit an effects/exposure curve, an interpolation is conducted with the same TRAP equation, but constrained to provide interpolation between two points.

Since the study yielded only one definite partial effect, TRAP cannot be used to estimate a concentration-response curve. Instead, TRAP was used to interpolate between the last two points to estimate the EC_{10} (see Linville summary in Appendix C for detail). The resultant TRAP slope is 2.96 and the interpolated EC_{10} is 15.6 mg/kg.

The white sturgeon EC_{10} of 15.6 mg/kg egg dw is important to include in the criterion analyses because this species a commercially and recreationally important fish species in the Pacific Northwest, and also serves as a surrogate for other sturgeon species in the United States (see **Section 6.4**, Protection of Threatened or Endangered Species), and has a population listed as endangered in the Kootenai River in Idaho and Montana.

3.1.1.2 Salmonidae

Acceptable studies were available for three salmonid genera, *Oncorhynchus*, *Salvelinus* and *Salmo*. All of these studies evaluated the effects of selenium on salmonid embryo/larval survival and deformity and used wild-caught adults taken from selenium contaminated streams and spawned for effects determination. Exposure for all studies was therefore through the parents. Summaries of the studies with *Salvelinus* are discussed in **Section 6.1.2.3**; *Oncorhynchus* and brown trout (*Salmo trutta*) are discussed below.

Oncorhynchus mykiss (rainbow trout) Holm (2002) and Holm et al. (2005) obtained eggs and milt from ripe rainbow trout collected from reference streams and streams containing elevated selenium from an active coal mine in Alberta, Canada. In 2000, 2001 and 2002 eggs

were fertilized and monitored in the laboratory until swim-up stage, for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. No significant differences among sites were observed for percent fertilization and mortality. Percentages of embryonic deformities and edema were significantly different among streams, but rates of deformities at Wampus Creek, one of the reference streams, were often similar to or higher than deformities at streams with elevated concentrations of selenium (see Holm summary in Appendix C). The measurement of selenium in the otolith layers of rainbow trout collected in this watershed showed low selenium exposure in the fish's early life and a higher exposure to selenium during the fish's adult years (Palace et al. 2007), suggesting that individuals that reach adulthood tend not to start their lives in streams with elevated selenium concentrations, even though they may reside there later.

Craniofacial deformities, skeletal deformities and edema in rainbow trout embryo, as a function of selenium in egg wet weight (ww), were fitted to a curve using a weighted regression and a threshold sigmoidal equation (TRAP) from which EC_{10} values were calculated (see Appendix C for tables and figures). EC estimates for finfold deformities, length and weight of rainbow trout embryos could not be made because of inadequate dose-response data. The most sensitive endpoint was edema with an EC_{10} value of 9.5 mg Se/kg egg ww or 24.5 mg Se/kg egg dw. The conversion of ww to dw used the average percent moisture of 61.2% for rainbow trout eggs reported by Seilor and Skorupa (2001).

Oncorhynchus clarkii lewisi (Westslope cutthroat trout)

In a field study similar to those conducted by Holm et al. (2005), Rudolph et al. (2008) collected eggs from Westslope cutthroat trout from Clode Pond (exposed site) and O'Rourke Lake (reference site). Clode Pond is on the property of Fording River Coal Operations in Southeast British Columbia with reported selenium concentrations of 93 µg/L. O'Rourke Lake is an isolated water body into which Westslope cutthroat trout were stocked in 1985, 1989 and 1992 and has selenium levels reported as <1 µg/L. Eggs with the four highest Se concentrations (86.3 to 140 mg/kg dw) collected from Clode Pond fish died before reaching the laboratory. Of those eggs from both ponds that survived, there was no correlation between egg selenium concentration and frequency of deformity or edema in the fry. The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs; the EC_{10} estimate for alevin survival based on the concentration of selenium in the eggs is 24.7 mg Se/kg

dw. See Appendix C for details on the statistical analysis and how it differed from the previous draft(s).

As a follow-up to the study by Rudolph et al. (2008), Nautilus Environmental (2011) conducted a more extensive study with Westslope cutthroat trout at the same site. Adult Westslope cutthroat trout were collected from lentic and lotic environments from locations near the mining operations. The lentic fish were primarily captured in Clode Pond, a settling area used to improve water quality of the mining discharge. Lotic fish were collected from the Fording River and its tributaries near the mining operation. Reference females were obtained from Connor Lake which is located within the watershed but not exposed to mining discharges. The researchers reared fertilized eggs from the caught females in the laboratory until they reached swim-up fry stage. A subset of fry surviving at swim-up were reared for an additional 28 days. The most sensitive endpoint was larval survival at swim-up with an EC_{10} of 27.7 mg/kg egg dw determined by interpolation between the one partial effect (20.8% survival at 34.2 mg Se/kg and average NOEC of 87.2% survival at 24.8 mg Se/kg; see Appendix C for detail and how this statistical analysis differed from the previous draft(s)). This result is very similar to the EC_{10} of 24.7 mg/kg egg dw determined for the data generated by Rudolph et al. (2008). See Appendix C for more details on the Nautilus Environmental (2011) study.

The GMCV for *Oncorhynchus* reproductive endpoints is 25.3 mg Se/kg EO. This GMCV is the geometric mean of the *O. mykiss* EC_{10} of 24.5 mg (Holm 2002 and Holm et al. 2005) and the SMCV of 26.2 mg Se/kg EO dw for *O. clarkii.* The *O. clarkii* SMCV was based on the EC_{10} values of 24.7 mg Se/kg EO from Rudolph et al (2008) and 27.7 mg Se/kg EO dw from Nautilus Environmental (2011).

Salmo trutta (brown trout)

Formation Environmental (2011) collected adult female and male brown trout from sites with low and high selenium exposure in the vicinity of a phosphate mine located in Southeastern Idaho in November 2007. Eggs were collected from 26 gravid females across three sampling locations, fertilized with milt collected from several males from the same site and taken to the laboratory for hatching and observation of larval malformations and survival. In addition to the field collected fish, fertilized eggs of 14 females from two separate hatcheries were used in the study.

The study had two phases, hatch-to-swim up, and swim up-to-15 days post swim-up. There are two experimental complications that affect the interpretation of these data: (a) elevated deformity rates among the offspring that were to serve as hatchery-originated method controls (very low selenium exposure) and among some of the low exposure field-collected organisms, and (b) the accidental loss of a number of individuals from several treatments during the 15-day post swim up portion of the test due to overflow of the tank water. The current criterion document's analysis is still based on the revised count data from AECOM (2012), which built upon and superseded EPA's 2012 analysis (Taulbee et al. 2012), peer reviewed by ERG (2012).

Approximately 600 eggs/female were obtained from the majority of the field and hatchery-collected females; however, the numbers of eggs per female ranged from 20 to 609. Selenium concentrations were measured for a subsample of eggs taken from each field and hatchery-collected female. EPA's primary evaluation in this document is of the survival of larvae from hatch to swim-up. Hatching success and larval survival were monitored to swim-up, at which time the fry were thinned to a maximum of 100 individuals for monitoring survival for 15 days post swim-up. Larvae from 24 field collected and 14 hatchery collected females were assessed for survival, as no larvae hatched from the eggs of two of the 26 field collected females.

Because of uncertainties regarding how best to address the loss of fish during the overflow event during the second phase of the test, and also because of the preferential selection of healthy fish during the thinning process prior to the post-swim-up portion of the test, where only those individuals presumed to be healthy were retained for assessment of deformities, EPA used survival during only the first portion of the test (hatch to swim-up), as it provides a more reliable chronic value.

The dataset of percent survival from hatch to swim-up versus the selenium concentration in eggs is an excellent dataset and provides a good foundation for setting numeric effect concentrations for selenium. There is a narrow range between the NOEC (20.5 mg/kg) and a LOEC with severe effects (26.8 mg/kg, 61% mortality) that leaves little uncertainty in what an appropriately protective effects concentration should be. There are sufficient data for TRAP to estimate a curve, using weighted least-squares nonlinear regression with the threshold sigmoidal model. The weighting factor for the 33 no-effect points is their standard deviation, and the weighting factor for the 5 effect points is their residual standard deviation. The TRAP parameter values are 96.2% for background survival, 1.45 for the logEC_{50} (EC_{50}=28.2 mg/kg), and 4.28 for

the slope. The EC_{10} is estimated to be 21.0 mg/kg, slightly higher than the NOEC of 20.5 mg/kg. The weighted TRAP model curve fits the 5 higher effects data well, which forces the EC_0 estimate down to 16.4 mg/kg, below two of the points in the background range. In particular, the fitted curve goes through the NOEC data point at 20.5 mg/kg, so that this point is considered to be an EC_8. This is reasonable because the response is so steep at concentrations above this point that some effect at this point is plausible, and also provides further support of an EC_{10} at 21.0 mg/kg. **Section 6.1.6** provides a summary of the analysis that led to the final selection of the EC_{10} for larval survival during the first portion of the test. Appendix C presents details of the study and analysis.

3.1.1.3 Centrarchidae

Lepomis macrochirus (bluegill sunfish)

In a laboratory study, Doroshov et al. (1992a) exposed adult bluegill for 140 days to three dietary treatments of seleno-L-methionine (nominal dietary concentrations of 8, 18 and 28 mg Se/kg) added to trout chow. Near the end of the exposure, ripe females were induced to ovulate and ova were fertilized *in vitro* with milt stripped from males. Fertilized eggs were sampled for fertilization success and selenium content. They were also used in two tests, (a) a larval development study during the first 5 days after hatching, and (b) a 30-day embryo-larval test. In the 30-day larval survival test, statistical difference from the control was only found in the highest test treatment for survival and growth (length and weight) measurements. In the 5-day larval test, the average proportion of larvae with edema was 0% at an egg concentration of 8.33 mg Se/kg (8 mg/kg dietary treatment), 5% at an egg concentration of 19.46 mg Se/kg dw (18 mg/kg dietary treatment), and 95% at an egg concentration of 38.39 mg Se/kg dw (28 mg/kg dietary treatment). The latter two were statistically different from the control (0% edema). All edematous larvae died in the high treatment.

This analysis focuses on the available data for the individual replicates for the 4-day data (5-day were not used because of almost complete mortality at the highest treatment). Of the 33 edema measurements, only 15 could be used because not all the replicate egg concentrations were reported. Table 4 in the Doroshov et al. (1992a) summary in Appendix C shows both individual replicates and the treatment averages, which are only slightly different than the 5-day data (averages) previously used in the selenium document. Individual replicates rather than

treatment means were used because the exposure concentrations vary substantially and effects are correlated with exposure within the treatment (illustrated by nominal dietary treatments of 18 mg/kg (with corresponding Se concentrations in eggs at that nominal treatment level ranging from 8.55 to 30.20 mg/kg) and 28 mg/kg (with corresponding Se concentrations in eggs ranging from 25.21 to 52.18 mg/kg; see Appendix C for details).

TRAP was fitted to the available data based on the individual replicates and the treatment means using the tolerance distribution option with the log-triangular distribution. In both cases, the TRAP program indicates that the dataset contains inadequate partial responses because the partial responses are less than 10% or greater than 90%, and there are no data (responses) between 10 and 90%. However, for this dataset, these partial responses at both ends of the concentration response curve are sufficiently informative based on multiple lines of evidence (e.g., same response on both days 4 and 5, other endpoints that show effects at dietary treatment of 18 mg/kg, and several instances of edema at dietary treatment 18 mg/kg in contrast to absolutely none for many observations at any lower concentration). Also, because dietary treatment 18 mg/kg does have an effect of several percent or so, estimating the EC_{10} near these points is defensible. Therefore, the EC_{10} estimated using separate replicates is 22.6 mg/kg dw.

A similar study with similar results was done by Coyle et al. (1993) in which two year old pond-reared bluegill sunfish were exposed in the laboratory and fed (twice daily *ad libitum*) Oregon moist™ pellets containing increasing concentrations of seleno-L-methionine. Water concentrations were nominal 10 µg Se/L. The fish were grown under these test conditions for 140 days. Spawning frequency, fecundity, and percentage hatch were monitored for 60 days from the initiation of spawning. There was no effect from the highest dietary selenium concentration (33.3 mg Se/kg dw) on adult growth, condition factor, gonadal somatic index, or other endpoints (Appendix C). The effect of interest in this study was 30-d larval survival after hatch (deformities weren't examined and other reproductive endpoints showed no effect at the highest exposure). For this survival endpoint, there was complete mortality after one week at the highest exposure and no significant differences in survival at lower concentrations.

Previously, the day 5 data were used in the analysis described in Appendix C because this was the only day in which control survival was greater than 90%, with the control and all the treatments showing substantial and increasing toxicity over the next 4 days. However, upon closer analysis, EPA asserts that this is not sufficient cause to base the assessment, because from

day 6 through day 30, survival at the fifth treatment was greater than survival in the first and third treatments, indicating this is not an effect level. These later data (day 6-30) establish that the highest treatment is best considered an EC_{100} and the fifth treatment an EC_0. Therefore, the TRAP interpolation was redone using 42 mg/kg as an EC_{100} rather than an EC_{93}, resulting in a slope of 7.6 and an EC_{10} of 26.3 mg Se/kg dw in eggs.

Hermanutz et al. (1992), and Hermanutz et al. (1996) exposed bluegill sunfish to sodium selenite spiked into artificial streams (nominal test concentrations: 0, 2.5, 10, and 30 µg Se/L) which entered the food web, thus providing a simulated field exposure (waterborne and dietary selenium exposure). In an effort originally intended to improve the rigor of the statistical analysis of the Hermanutz et al. (1996) data, Tao et al. (1999) re-examined the raw data records and made corrections to the counts. This criterion document considers the Hermanutz et al. (1992) data and the Tao et al. (1999) re-examination of Hermanutz et al. (1996).

These data come from a series of three studies lasting from 8 to 11 months, conducted over a 3-year period. All three studies began with exposure of adult bluegill sunfish in the fall, and with respective studies ending in the summer of the following year. Temperatures averaged 4.6, 4.1 and 4.5°C during the winter months, and averaged 26.4, 23.9 and 22.4°C during the spawning months (June-July) for Studies I, II and III, respectively. Spawning activity was monitored in the stream, and embryo and larval observations were made *in situ* and from fertilized eggs taken from the streams and incubated within egg cups in the laboratory. None of the adult bluegill exposed to the highest concentration of selenium in the water (Study I, mean measured concentration equal to 29.4 µg/L) survived the entire exposure period (although a few did survive to spawn). Reduced survival and increased deformities on offspring were observed in the selenium-dosed streams in both Study I and Study II, but were not found during Study III (recovering from contamination, no active selenium input/treatment). The incidence of edema, lordosis, hemorrhage and larval survival in the one stream concentration common to both Study I and II, 10 µg/L, ranged from 80 to 100 percent, 5 to 18 percent, 27 to 56 percent, and 29 to 58 percent, respectively over the three years (combined egg cup and nest observations). Edema, lordosis, and hemorrhage in the lowest stream concentration in Study II, 2.5 µg/L, ranged from 0 to 4 percent, 0 to 25 percent, and 3.6 to 75 percent, respectively (combined egg cup and nest observations); larval survival was 71.6 percent (72 and 75 percent in the control streams). (See Hermanutz 1996 and 1992 in Appendix C for more detail). The effects were not observed in

larvae from fish that were not exposed to elevated concentrations of selenium (control treatment). The mean concentrations of selenium in bluegill ovaries, measured at the end of each study, ranged from 2.2 to 5.0 mg/kg dw in controls, 7.6 to 14 mg/kg dw in the 2.5 µg/L treatments, 34 to 52 mg/kg dw in the 10 µg Se/L treatments, and 16 to 55 mg/kg dw in recovering 30 µg/L treatments. Muscle and whole-body measurements were also available. For all three tissue types, concentrations measured during the spring of each exposure period were not used because they were not sufficiently co-occurrent with the observation of effects. It should also be noted that in contrast to more recent field studies, the tissue concentrations cannot be linked from particular adult females to effects on her offspring, but only from an aliquot of females in the treatment to all offspring observed in the treatment.

The egg-cup data for all streams of Studies I, II, and III of this experiment were combined and analyzed in response to measured selenium concentration in the maternal ovaries (mg/kg dw) using TRAP. That is, data for streams receiving water-borne selenium were combined with data for streams recovering from the previous year's contamination. The absence of effects at high tissue levels (55 mg Se/kg ovary dw) in the recovering stream of Study II did not affect the EC_{10} estimate because it was outweighed by three other points showing severe effects at concentrations as low as 16.7 mg Se/kg ovary dw. However, this one observation is suggestive but not definitive corroboration for the field observations of biological recovery in Belews Lake and Hyco Reservoir after selenium loads were reduced, but while tissue concentrations remained relatively high (Lemly 1997a; Crutchfield 2000; Finley and Garrett 2007).

Several egg-cup endpoints were analyzed by TRAP independently (% edema, % lordosis, and % hemorrhage) and in combination (% normal and surviving). The best fit and most sensitive was the combined percent normal and surviving larvae. Due to inadequate partial effects for the ovary analysis, a threshold sigmoidal model was used to interpolate an EC_{10} estimate between the first interpolation point set to the highest no observed effect concentration (HNOEC) of 14.0 mg/kg and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.7 mg/kg and a survival/normal of 5.76%. The resulting EC_{10} is 14.7 mg/kg ovary dw. Chronic values for muscle and whole body based on percentage surviving and free of deformities are 13.4 mg Se/kg muscle dw and 10.6 mg Se/kg whole body dw. (See Appendix C for more discussion of this study).

The SMCV for bluegill reproductive endpoints based on EC_{10} values is 20.6 mg Se/kg dw in egg/ovary, based on the EC_{10} values of 22.6 mg/kg dw in the Doroshov et al. (1992a) study, 26.3 mg/kg dw in the Coyle et al. (1993) study, and 14.7 mg/kg dw for Hermanutz et al. (1992, and 1996 as corrected by Tao et al. 1999).

3.1.2 Summary of Acceptable Studies of Fish Reproductive Effects

Table 3.1 summarizes the effect concentrations obtained from all acceptable reproductive studies with fish. Summaries of the remainder of the reproductive studies (beyond the four most sensitive genera described above) can be found in **Section 6.1.2** below.

Table 3.1. Maternal Transfer Reproductive Toxicity Studies.

Species	Reference	Exposure Route	Toxicological Endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Salvelinus malma Dolly Varden	Golder 2009	dietary and waterborne (field: Kemess Mine NW British Columbia)	EC_{10} for total deformities	56.2 E	56.2 E	56.2 E
Esox lucius northern pike	Muscatello et al. 2006	dietary and waterborne (field: Saskatoon, Sask.)	EC_{24} larval deformities	34.0 E	34.0 E	34.0 E
Cyprinodon macularius desert pupfish	Besser et al. 2012	dietary and waterborne (lab)	Estimated EC_{10} for offspring survival	27 E	27 E	27 E
Micropterus salmoides largemouth bass	Carolina Power & Light 1997	dietary (lab)	EC_{10} for larval mortality & deformity	26.3 O	26.3 O	26.3 O
Pimephales promelas fathead minnow	Schultz and Hermanutz 1990	dietary and waterborne (mesocosm: Monticello)	LOEC for larval edema and lordosis	<25.6 E[b]	NA[c]	NA
Oncorhynchus mykiss rainbow trout	Holm 2002; Holm et al. 2003, 2005	dietary and waterborne (field: Luscar River, Alberta)	EC_{10} for skeletal deformities	24.5 E[b]	24.5 E	25.3 E
Oncorhynchus clarkii lewisi Westslope cutthroat trout	Rudolph et al. 2008	dietary and waterborne (field: Clode Pond, BC)	EC_{10} for alevin mortality	24.7 E	26.2 E	
Oncorhynchus clarkii lewisi Westslope cutthroat trout	Nautilus Environmental 2011	dietary and waterborne (field: Clode Pond & Fording River, BC)	EC_{10} for survival at swim-up	27.7 E		
Salmo trutta brown trout	Formation Environmental 2011; AECOM 2012	dietary and waterborne (field: Lower Sage Creek & Crow Creek, ID)	EC_{10} for larval survival	21.0 E	21.0 E	21.0 E

Species	Reference	Exposure Route	Toxicological Endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Lepomis macrochirus bluegill	Doroshov et al. 1992a	dietary (lab)	EC_{10} larval edema	22.6 E	20.6 E	20.6 E
Lepomis macrochirus bluegill	Coyle et al. 1993	dietary and waterborne (lab)	EC_{10} for larval survival	26.3 E		
Lepomis macrochirus bluegill	Hermanutz et al. 1992, 1996	dietary and waterborne (mesocosm: Monticello)	EC_{10} for larval edema	14.7 O[b]		
Acipenser transmontanus white sturgeon	Linville 2006	dietary (lab)	EC_{10} for combined edema and deformities	15.6 E	15.6 E	15.6 E

E–Concentration reported in egg; O– concentration reported in ovary

[a] All chronic values reported in this table are based on the measured concentration of selenium in egg/ovary tissues.

[b] Tissue value converted from ww to dw. See Appendix C for conversion factors.

[c] SMCV not calculated due to variability in the observations among replicates in Schultz and Hermanutz (1990). The chronic value is presented in this table to show it is in the range of selenium effect concentrations. See Appendix C for detail. Also, see Appendix E for an additional study with fathead minnow.

In order of their sensitivity to selenium, **Table 3.2** presents the Genus Mean Chronic Values from acceptable fish reproductive-effect studies that have been measured in terms of egg-ovary concentrations.

Table 3.2. Ranked Genus Mean Chronic Values for Fish Reproductive Effects Measured as Egg or Ovary Concentrations.

Rank	GMCV* (mg Se/kg dw EO)	Species	SMCV (mg Se/kg dw EO)
8	56.2	Dolly Varden, *Salvelinus malma*	56.2
7	34**	Northern pike, *Esox lucius*	34
6	27	Desert pupfish, *Cyprinodon macularius*	27
5	26.3	Largemouth bass, *Micropterus salmoides*	26.3
4	25.3	Cutthroat trout, *Oncorhynchus clarkii*	26.2
		Rainbow trout, *Oncorhynchus mykiss*	24.5
3	21.0	Brown trout, *Salmo trutta*	21.0
2	20.6	Bluegill sunfish, *Lepomis macrochirus*	20.6
1	15.6	White sturgeon, *Acipenser transmontanus*	15.6

* This table excludes *Gambusia*, which has a reproductive chronic value expressed as adult whole-body rather than egg-ovary, because it is a live bearer.
** The Northern Pike SMCV is an EC_{24} based on larval deformities (**Table 3.1**). The EC_{10} is less than 34 mg/kg.
This table excludes GMCV for *Pimephales* due to uncertainty in the chronic value for the Schultz and Hermanutz (1990) study; the estimate of <25.6 mg/kg egg dw in **Table 3.1** shows it is in the range of reproductive effect levels for selenium (See Appendix C for study details).

3.1.3 Derivation of Tissue Criterion Element Concentrations

Data used to derive the final chronic value were differentiated based on the effect (reproductive and non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for 10 fish genera. Acceptable chronic toxicity data on non-reproductive effects are available for 7 fish genera and 3 invertebrate genera. The fish non-

reproductive effects data were not used to calculate tissue criterion elements because they were more variable and less reproducible than the data on reproductive effects. The genus sensitivity distribution is predominantly populated with data on fish species because field evidence demonstrated that fish communities were affected in situations having no observable change in the accompanying diverse array of invertebrate communities. As a result, decades of aquatic toxicity research have focused primarily on fish. The studies that have been done with invertebrates (**Table 3.8**, **Section 3.1.4**) have shown them to be more tolerant than most of the tested fish species.

Also, while amphibians are potentially sensitive due to physiologic similarities to fish, effects clearly attributable to selenium are largely unknown (Unrine et al. 2007; Hopkins et al. 2000; Janz et al. 2010). Hopkins et al. (2000) reported that amphibian larvae at sites receiving coal combustion wastes appear to efficiently accumulate selenium in their tissues and possibly due to selenium have exhibited axial malformations. In a recent laboratory exposure, Massé et al. (2015) determined an EC_{10} of 44.9 mg/kg Se for the African clawed frog (*Xenopus laevis*) suggesting that amphibians are similar to the less sensitive fish species (see **Section 6.1.4**).

3.1.3.1 Fish Egg-Ovary Concentration

The lowest four GMCVs from **Table 3.2** are shown below in **Table 3.3**.

Table 3.3. Four Lowest Genus Mean Chronic Values for Fish Reproductive Effects.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw egg-ovary)
4	*Oncorhynchus*	25.3
3	*Salmo*	21.0
2	*Lepomis*	20.6
1	*Acipenser*	15.6

With N=15 GMCVs (see **Section 3.1.6**), the 5th percentile projection yields an egg/ovary criterion element concentration of 15.1 mg Se/kg dw egg/ovary, lower than the most sensitive fish species tested, white sturgeon (*A. transmontanus*). The egg/ovary criterion element concentration is compared to the distribution of egg/ovary chronic values in **Figure 3.1**.

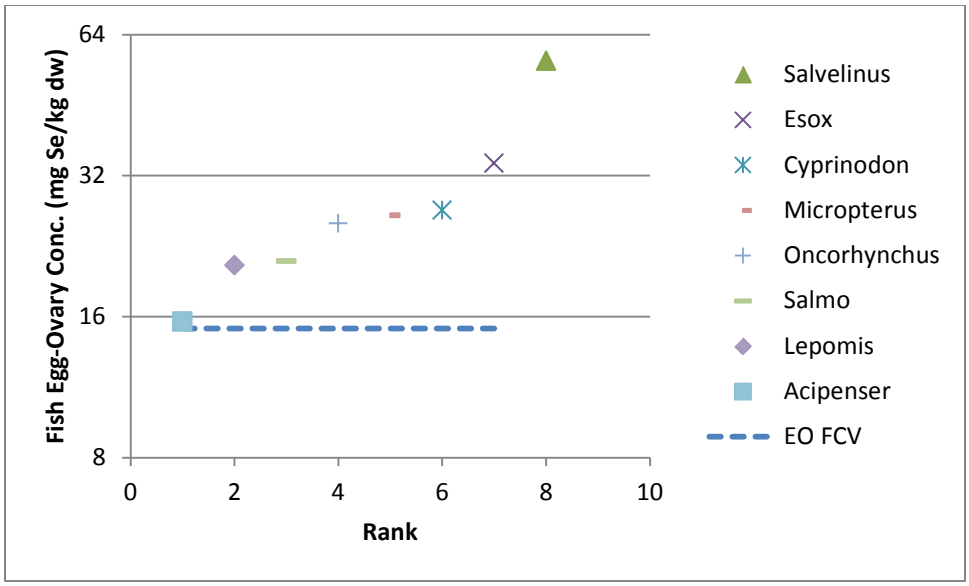


Figure 3.1. Distribution of Reproductive-Effect GMCVs for Fish Measured as Egg-Ovary Concentrations.

3.1.3.2 Fish Whole-Body Criterion Element Concentration

Whole body reproductive chronic values were calculated directly from whole body tissue concentrations measured in the study or by applying an egg-ovary (EO) to whole-body (WB) conversion factor (CF) presented subsequently in **Section 3.2.2.2**. Direct calculations were done when whole body measurements were available in the study and the data were amenable to an effect level determination. **Table 3.4** provides the chronic values for each fish genus and whether it was calculated directly or converted from the reproductive-effect egg-ovary concentrations to whole-body concentrations using a CF. The final EO/WB CF applied to each taxon was determined using a hierarchical approach based on taxonomic relatedness, and is described in **Section 3.2.2**, and in greater detail in Appendix B. The four most sensitive reproductive-effect fish whole-body GMCVs are shown in **Table 3.5**.

Table 3.4. Tested Reproductive-Effect Whole Body (WB) Concentrations Measured Directly or Converted to WB Concentrations from Egg-Ovary (EO) Concentrations.

Taxon*	EO Chronic Value	EO/WB CF	Direct or Calculated WB Repro Chronic Value	Direct Calculation or Basis for EO/WB CF (from Appendix B)
Salvelinus	56.2	1.61	34.9	Dolly Varden EO/M (1.26) x all fish M/WB (1.27)
Esox	34.0	2.39	14.2	Northern pike EO/M (1.88) x all fish M/WB (1.27)
Cyprinodon	27.0	1.20	22.6	Desert pupfish EO/WB
O. mykiss	24.5	2.44	10.0	Rainbow trout EO/M (1.92) x all fish M/WB (1.27)
Rudolph et al. 2008	24.7	1.96	12.6	*Oncorhynchus* EO/WB
Nautilus 2011	27.7	1.96	14.1	*Oncorhynchus* EO/WB
O. clarkii	26.2	NA	13.3	Geometric mean of two studies
Oncorhynchus	25.3	NA	11.6	Geometric mean of *O. mykiss* and *O. clarkii* WB SMCVs
Micropterus	26.3	1.42	18.5	*Micropterus* EO/WB
Salmo	21.0	NA	13.2	Directly calculated EC_{10}
Coyle et al. 1993	26.3	NA	8.6	Directly calculated EC_{10}
Doroshov et al. 1992a	22.6	2.13	10.6	Bluegill sunfish EO/WB
Hermanutz et al. 1992, 1996	14.7	NA	10.6	Directly calculated EC_{10}
Lepomis	20.6	NA	9.9	Geometric mean of three studies
Acipenser	15.6	1.69	9.2	White sturgeon EO/M (1.33) x all fish M/WB (1.27)

* The GMCV for *Gambusia*, a live bearer, not included in the conversion table, was originally measured as adult WB, not EO, and is >13.38 mg Se/kg dw WB. The "greater than" sign signifies that no effects were found at the highest observed concentrations. This table also excludes *Pimephales* due to uncertainty in the chronic value for the Schultz and Hermanutz (1990) study (See Appendix C for details).

Table 3.5. The Lowest Four Reproductive-Effect Whole-Body GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw whole-body)
4	*Salmo*	13.2
3	*Oncorhynchus*	11.6
2	*Lepomis*	9.9
1	*Acipenser*	9.2

Because the factors used to convert egg-ovary to whole-body concentrations vary across species, the whole-body rankings differ from the egg-ovary rankings. With N=15 GMCVs, the 5th percentile projection yields a whole body criterion element concentration of 8.5 mg Se/kg dw whole-body, slightly lower than the most sensitive fish species tested, white sturgeon (*Acipenser transmontanus*). The fish whole body criterion element is compared to the distribution of fish whole-body reproductive chronic values in **Figure 3.2**.

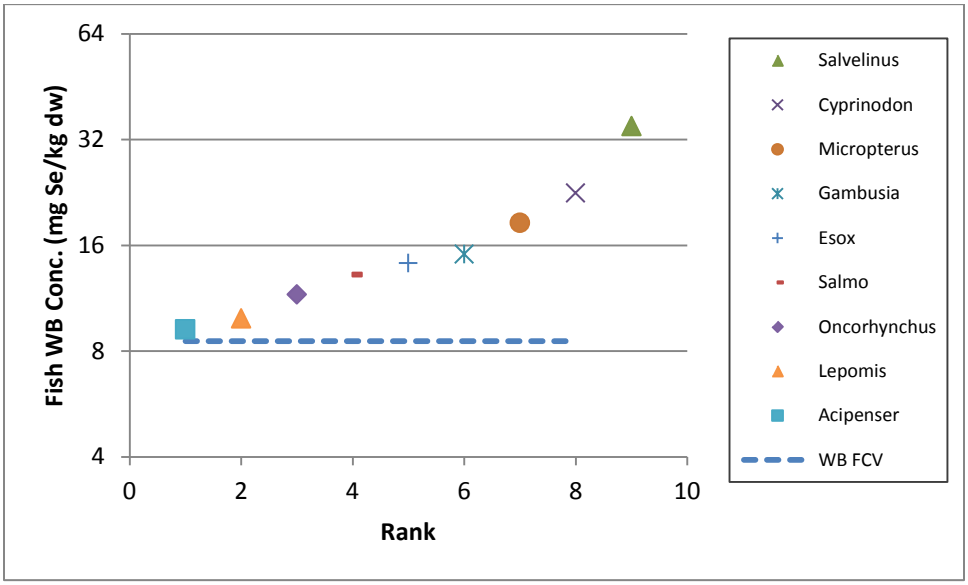


Figure 3.2. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Whole-Body Concentrations in the Original Tests, or Measured as Egg-Ovary Concentrations but Converted to Whole-Body.
(As shown in **Table 3.4**).

3.1.3.3 Fish Muscle Criterion Element Concentration

Reproductive chronic values for muscle tissue were calculated directly from muscle tissue concentrations measured in the study or from the egg-ovary to muscle conversion factors of the bioaccumulation modeling approach (presented in **Section 3.2**). Direct calculations were made when muscle measurements were available in the study and the data were amenable to an effect level determination. The final EO/M CF applied to each taxon was determined using a hierarchical approach based on taxonomic relatedness, consistent with the approach used to calculate EO/WB CFs described in **Section 3.2.2**.

Table 3.6 provides the chronic values for each fish taxa and whether it was calculated directly or converted from reproductive-effect egg-ovary concentrations to muscle concentrations. The four most sensitive reproductive-effect fish muscle GMCVs are shown in **Table 3.7**.

Table 3.6. Tested Reproductive-Effect Muscle (M) Concentrations Measured Directly or Converted to M Concentrations from Egg-Ovary (EO) Concentrations.

Taxon	EO Chronic Value	EO/M CF	Direct or Calculated Muscle Repro Chronic Value	Direct Calculation or Basis for EO/M CF (from Appendix B)
Salvelinus	56.2	1.26	44.5	Dolly Varden EO/M
Esox	34.0	NA	21.7	Directly determined EC_{24}
Cyprinodon	27.0	0.94	28.7	Desert pupfish EO/WB (1.20) / all fish M/WB (1.27)
O. mykiss	24.5	1.92	12.8	Rainbow trout EO/M
Rudolph et al. 2008	24.7	NA	16.6	Directly calculated EC_{10}
Nautilus 2011	27.7	1.81	15.3	Cutthroat trout EO/M
O. clarkii	26.2	NA	16.0	Geometric mean of two studies
Oncorhynchus	25.3	NA	14.3	Geometric mean of two SMCVs
Micropterus	26.3	1.19	22.2	*Micropterus* EO/M
Salmo	21.0	1.14	18.5	Brown trout EO/WB (1.45) / all fish M/WB (1.27)

Taxon	EO Chronic Value	EO/M CF	Direct or Calculated Muscle Repro Chronic Value	Direct Calculation or Basis for EO/M CF (from Appendix B)
Coyle et al. 1993	26.3	1.38	19.1	Bluegill sunfish EO/M
Doroshov et al. 1992a	22.6	NA	15.7	Directly calculated EC_{10}
Hermanutz et al. 1992, 1996	14.7	NA	13.4	Directly calculated EC_{10}
Lepomis	20.6	NA	15.9	Geometric mean of three studies
Acipenser	15.6	NA	11.9	Directly calculated EC_{10}

Table 3.7. The Lowest Four Reproductive-Effect Fish Muscle GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw muscle)
4	*Salmo*	18.5
3	*Lepomis*	15.9
2	*Oncorhynchus*	14.3
1	*Acipenser*	11.9

Because the factors used to convert egg-ovary to muscle concentrations vary across species based on empirical data, the whole-body rankings differ from both from the egg-ovary rankings and the muscle rankings. With N=15 GMCVs, the 5th percentile projection yields a muscle criterion element concentration of 11.3 mg Se/kg dw muscle, lower than the muscle value for the most sensitive fish genus tested, *Acipenser*. **Figure 3.3** compares the fish muscle criterion element concentration to the distribution of fish muscle reproductive chronic values in **Table 3.6**.

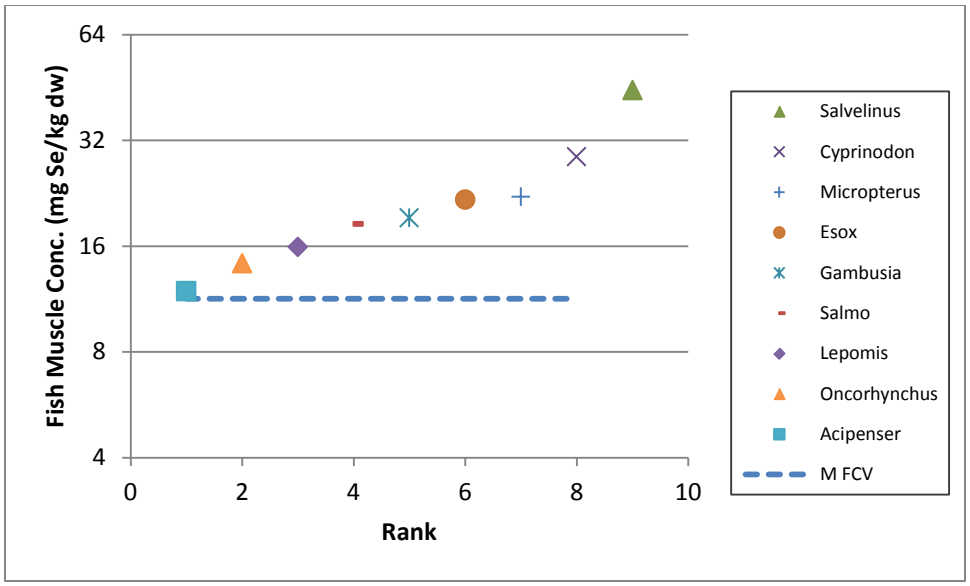


Figure 3.3. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Muscle in the Original tests, or Measured as Egg-Ovary Concentrations but Converted to Muscle Concentrations.
(As shown in **Table 3.6**). (Live-bearer *Gambusia* was converted from WB to muscle).

3.1.4 Invertebrate Chronic Effects

Below is a brief synopsis of the experimental design of the available invertebrate chronic toxicity tests, and the resulting chronic values.

Brachionus calyciflorus (rotifer)

Dobbs et al. (1996) exposed *Brachionus calyciflorus* to selenate in natural creek water for 25 days in a three-trophic level food chain test system. This is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In the Dobbs et al. (1996) study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but consumed selenium bioaccumulated in the next lower trophic level. Rotifers did not grow well at concentrations exceeding 108.1 µg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 µg Se/L in the water (40 µg Se/g dw in the algae). Regression analysis of untransformed growth data (dry weight), determined 4 day post-test initiation, resulted in a calculated EC_{10} of 37.84 µg Se/g dw tissue.

Lumbriculus variegatus (oligochaete, blackworm)

Although not intended to be a definitive toxicity study for blackworms, Besser et al. (2006) evaluated the bioaccumulation and toxicity of selenized yeast to the oligochaete, *Lumbriculus variegatus,* which was intended to be used for dietary exposure in subsequent studies with the endangered desert pupfish, *Cyprinidon macularius*. Oligochaetes fed selenized-yeast diets diluted with nutritional yeast (54 to 210 mg Se/kg) had stable or increasing biomass and accumulated Se concentrations as high as 140 mg/kg dw. The oligochaetes fed the undiluted selenized-yeast (826 µg/g Se dry wt.) showed reduced biomass. The effect level is considered >140 mg Se/kg dw.

Centroptilum triangulifer (mayfly)

Mayfly larvae (*Centroptilum triangulifer*) were exposed to dietary selenium contained in natural periphyton biofilms to eclosion (emergence) (Conley et al. 2009; Conley et al. 2011; Conley et al. 2013). In Conley et al. (2009), the periphyton fed to the mayfly larvae were exposed to dissolved selenite (radiolabeled ^{75}Se) in November 2008 (12.6 and 13.9 µg/L) and in January 2009 (2.4, 2.4, 4.9, 10.3, and 10.7 µg/L). Periphyton bioconcentrated selenium an average of 1113-fold over the different aqueous selenium concentrations (see Table E-22 in Appendix E). Twenty 4 to 6-day old mayfly larvae were exposed for 4.5 to 6 weeks to each of the periphyton diets until the larvae eclosed to subimagos (final pre-adult winged stage). The subimagos were allowed to emerge to the adult imago stage which deposited their egg masses in Petri dishes. Selenium concentrations were measured in postpartum adults along with their dry weights and clutch size. Selenium increased in concentration from periphyton to the adult mayflies (trophic transfer factor) an average of 2.2-fold. The authors observed a reduction in fecundity with diets containing more than 11 mg Se/kg dw, which is considered the dietary threshold for this study. Using the trophic transfer factor of 2.2, the periphyton selenium concentration of 11 mg/kg dw translates to an adult mayfly selenium concentration of 24.2 mg/kg dw.

Conley et al. (2011) exposed larval *C. triangulifer* larvae similar to Conley et al. (2009) to two different rations of periphyton (1x and 2x) containing low, medium and high selenium levels to evaluate the effect of feeding ration on the bioaccumulation of selenium and life cycle performance of the mayfly. Mayfly larvae were fed either a 1x or 2x ration of periphyton loaded with the three different selenium levels until the larvae eclosed to subimagos after 25-29 days.

Average periphyton Se concentrations for the three treatments in the 1x ration study were 4.2, 11.9, and 27.2 mg/kg dw, respectively. In the 2x ration study, average periphyton concentrations for the three treatments were 9.5, 19.9, and 40.9 mg/kg dw, respectively (Conley et al. 2011). Subimagos were induced to emerge to adults in petri dishes and their clutch size measured through digital imaging. Mayflies fed the 1x ration had 54% and 72% reductions in survival relative to controls in the medium and high Se treatment levels, respectively, both of which were significant ($p<0.05$). The mayflies fed the 1x ration also had significant reductions in fecundity in the low (44% reduction), medium (63% reduction) and high (77% reduction) Se treatment levels. However, for the mayflies that were fed the 2x ration, there were no significant differences between the controls and any of the three Se treatment levels for any of the endpoints measured including survival and fecundity. The 2x ration mayflies had 60% more biomass than the 1x ration mayflies. This growth difference explains why the 1x ration mayflies had higher concentrations of Se in their tissues (see Table E-23 in Appendix E). The two different rations resulted in vastly different effect levels for Se, <12.8 mg/kg dw in the 1x ration test and >37.3 mg/kg dw in the 2x ration. It is apparent from this study that if the mayflies do not obtain sufficient nutrition, they are more sensitive to selenium. Although reduced feeding levels occur in nature, it is a confounding variable in this study that cannot be used to set a chronic effect level for selenium.

Conley et al. (2013) evaluated the accumulation of selenite and selenate into periphyton with a subsequent feeding exposure to mayfly larvae. As in the previous studies, *C. triangulifer* larvae were fed periphyton previously exposed to different concentrations of selenium. In this study, periphyton plates were first exposed to low (10 µg/L) and high (30 µg/L) concentrations of either selenite or selenate and then fed to mayfly larvae to eclosion and to subimagos. The mean concentrations of selenium in the periphyton fed to the mayflies were 2.2, 12.8 and 37 mg/kg Se dw in the control, low and high treatments, respectively. Mayfly tissue (subimago) concentrations (extrapolated from Figure 4a in Conley et al. 2013) were approximately 4-7, 20-35, and 45-75 mg/kg Se dw, in the control, low and high treatments, respectively. The authors reported significant reductions in survival from the control in the high Se treatment (both pooled data and individual selenite and selenate treatments), but no significant differences were observed in the low Se treatments. Secondary production (mayfly biomass) was significantly reduced relative to the control in the high Se treatment for both selenium species. For the low Se

exposure treatments, secondary production was not significantly different than the control for the selenite treated periphyton exposure, but was for the selenate and pooled data suggesting an effect level between 20 and 35 mg/kg Se dw. These results as well as those observed in 2x ration exposures in Conley et al. (2011) where no effects were observed at 37.3 mg/kg Se dw generally support the chronic value determined for Conley et al. (2009) of 24.2 mg/kg Se dw. This information included tabulated data from these studies presented in Appendix E.

3.1.5 Summary of Relevant Invertebrate Tests

The available measured invertebrate whole-body effect concentrations are shown in **Table 3.8**. Because the intent of this assessment is to derive a concentration expressed in terms of fish tissue, **Table 3.8** also provides information on how concentrations in invertebrate tissue are translated (in **Section 3.2**) across media to predicted WB fish tissue concentrations (Trophic Level 3, TL3) in a system having invertebrates and fish. That is, consistent with the bioaccumulation modeling approach of **Section 3.2**, the second column of **Table 3.8** uses the median trophic transfer factor of 1.21 from **Table 3.11** to yield expected WB fish tissue concentrations in a system having invertebrates and fish. Whether comparing TL2 (invertebrate) whole-body GMCVs directly to **Table 3.4** TL3 (fish) whole-body GMCVs, or via the trophic transfer adjustment in the second column of **Table 3.8**, it is apparent that invertebrates are not among the most sensitive species.

The relative insensitivity of invertebrates overall when compared with the fish whole-body concentrations demonstrates that invertebrates are expected to be generally protected by selenium criterion values derived from fish. It should be noted that mayflies appear to be the most sensitive invertebrate group tested; their whole-body effects levels just below the *least* sensitive fish genus (*Salvelinus*, Dolly Varden) on whole-body basis. However these mayfly results have some uncertainty due to the indicated effect of feeding ration on selenium toxicity to mayflies that has not been fully defined. The rotifer and lumbriculus tests indicate that these organisms are less sensitive than any tested fish genus on a whole-body basis. Therefore, the invertebrates are considered implicitly in the species sensitivity distribution, and are counted toward the number of values available to calculate the fish tissue criterion elements (as egg-ovary, whole-body, and muscle), and the missing invertebrate MDRs (4 and 5) are considered satisfied by the available invertebrate data.

Table 3.8. Ranked Invertebrate Whole-Body Chronic Values with Translation to Expected Accompanying Fish Whole-Body Concentrations

SMCV & GMCV as measured (Trophic Level 2) (mg Se/kg dw WB)	Accompanying Trophic Level 3 Median Whole-Body Concentration Predicted by Bioaccumulation Model (Section 3.2) (mg Se/kg dw WB TL3)	Species
> 140	> 169.4	Oligochaete, black, *Lumbriculus variegatus*
37.84	45.8	Rotifer, *Brachionus calyciflorus*
24.2	29.3	Mayfly, *Centroptilum triangulifer*

3.1.6 Selenium Fish Tissue Toxicity Data Fulfilling Minimum Data Needs

The toxicity data currently available for genera and species fulfilling the EPA Ambient Water Quality Criteria Guidelines recommendations for calculation of the freshwater chronic criterion are described in **Sections 3.1.1, 3.1.4, 6.1.2** and Appendix C, and are summarized in **Table 3.9**.

Table 3.9. Minimum Data Requirements Summary Table Reflecting the Number of Species and Genus Level Mean Values Represented in the Chronic Toxicity Dataset for Selenium in Freshwater.

Freshwater Minimum Data Requirement	Genus Mean Chronic Value (GMCV)	Species Mean Chronic Value (SMCV)
1. Family Salmonidae in the class Osteichthyes	3	4
2. Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	2	2
3. Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	5	5
4. Planktonic Crustacean	See text	See text
5. Benthic Crustacean	See text	See text
6. Insect	1	1
7. Family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida, or Mollusca)	1	1
8. Family in any order of insect or any phylum not already represented	1	1
Total	**15**	**16**

The first three of these MDRs in **Table 3.9** are easily fulfilled by the fish species represented in **Sections 3.1.1, 6.1.2** and Appendix C. Because the field observations of contaminated sites have found effects on fish and birds in the absence of changes in invertebrate assemblages, scientific studies on the chronic toxicity of dietary selenium for invertebrates has been very limited. The few dietary chronic toxicity studies that are available for invertebrate species (arthropods **,** rotifers, and worms) indicate that they are generally less sensitive than fish, with available data indicating invertebrate whole body mean chronic values ranging from approximately 3 to 12 times higher than the fish whole body criterion element value recommended in this document (**Section 3.1.4**). The above invertebrate data address MDRs 6-8, leaving only MDRs 4 and 5, for the planktonic and benthic crustaceans, to be addressed. Because the 5^{th} percentile calculation methods for the FCV use actual numeric values for the GMCVs of the four most sensitive (fish) genera in the selenium dataset, it is only necessary to know that the more tolerant genera have GMCVs that are greater than those of the lowest four. A recommendation in the draft white paper on Aquatic Life Criteria for Contaminants of Emerging Concern Part I (U.S. EPA 2008b), which was supported by the Science Advisory Board, states "because only the four most sensitive genus mean chronic values (GMCVs) are used in the criterion calculations, chronic testing requirements for a taxon needed to meet an MDR should be waived if there is sufficient information to conclude that this taxon is more tolerant than the four most sensitive genera."

Currently, there are no available data on the chronic toxicity to crustaceans via dietary exposure to selenium. Since there are data available for insects (*Centroptilum spp.* mayfly), EPA used the taxonomic association at the level of phylum (Arthropoda) to allow insects to be a surrogate for crustaceans. There is also associative evidence that macroinvertebrates in general are less sensitive than fish. At sites where there have been documented effects to fish and aquatic-dependent birds from selenium exposure (e.g., Kesterson Reservoir, Belews Lake, Hyco Reservoir), field observations and data indicate that there has been no evidence of effects to macroinvertebrates including crustaceans (Janz et al. 2010). In addition, Janz et al. (2010) notes that the key vector for selenium toxicity via maternal transfer is selenium loading in the egg via vitellogenesis. Crustaceans, and other arthropods are not known to deposit a significant amount of vitellogenin in the egg compared with oviparous vertebrates like fish, therefore, less selenium is likely transferred to the egg via deposition of vitellogenin. These mechanistic considerations

are thus consistent with the absence of observed field effects on aquatic macroinvertebrates, including crustaceans and other arthropods, and with the Chapman et al. (2009, 2010) expert consensus that it is the egg-laying vertebrates that are most at risk.

Applying this concept to the selenium criterion 5th percentile calculations, GMCVs for MDRs 4 and 5 (the two crustacean MDRs) should be waived and counted in the total number of GMCVs in the dataset, based on (a) the difference in the measured effect values discussed above, and (b) the lack of observed invertebrate field effects linked to selenium (for example, as concluded by Lemly 2002, pages 21-23, and Janz et al. 2010). Thus data are adequate to fulfill the data needs for developing a chronic selenium criterion.

The total number of GMCVs available to derive the chronic criterion is 15. These include ten fish genera from **Sections 3.1.1 and 6.1.2** (*Acipenser*, *Salmo*, *Lepomis*, *Micropterus*, *Oncorhynchus*, *Pimephales*, *Gambusia*, *Esox*, *Cyprinodon*, and *Salvelinus*) [Added to these are the tested invertebrate genera *Centroptilum*, *Brachionus*, and *Lumbriculus* from **Section 3.1.4**, and lastly the two waived genera for MDRs 4 and 5 (crustaceans).

3.2 Chronic Water Column-based Selenium Criterion Element

3.2.1 Translation from Fish Tissue Concentration to Water Column Concentration

EPA derived the chronic water column selenium criterion element by translating the egg-ovary concentration to an equivalent water concentration. EPA worked with USGS to derive a translation equation that utilizes a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al. 1992; Wang et. al. 1996; Luoma and Fisher 1997; Wang 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006, 2010; Presser 2013). This model quantifies bioaccumulation in animal tissues by assuming that net bioaccumulation is a balance between assimilation efficiency from diet, ingestion rate, rate of direct uptake in dissolved forms, loss rate, and growth rate. The basic model is given as:

$$C_{tissue} = \frac{\left[\left(k_u \times C_{water}\right) + \left(AE \times IR \times C_{food}\right)\right]}{\left(k_e + g\right)} \qquad \text{(Equation 1)}$$

Where:

C_{water}	=	Concentration of chemical in water (µg/L)
C_{tissue}	=	Average concentration of chemical in all tissues at steady-state (µg/g)
k_e	=	Efflux rate (/d)
g	=	Growth rate (/d)
k_u	=	Uptake rate (L/g-d)
AE	=	Assimilation efficiency (%)
IR	=	Ingestion rate (g/g-d)
C_{food}	=	Concentration in food (µg/g)

3.2.1.1 Simplifying the Bioaccumulation Model

Specific application to selenium bioaccumulation permits the simplification of Equation 1 in two ways. One simplification is removing the parameter representing growth rate (*g*), and the other simplification is removing the parameter representing direct aqueous uptake (k_u).

Growth Rate

The growth rate constant *g* is included in Equation 1 because the addition of body tissue has the potential to dilute the concentration of bioaccumulative chemicals when expressed as chemical mass per tissue mass. For very hydrophobic chemicals with low excretion rates such as polychlorinated biphenyls, growth can be an important factor in bioaccumulation estimates (Connolly and Pedersen 1988). However, Luoma and Rainbow (2005) suggest that for selenium, growth rate is a relatively inconsequential parameter under most circumstances. Food consumption is typically high during periods of high growth rate. Because food consumption is the primary route of selenium uptake in aquatic organisms (Ohlendorf et al. 1986a, b; Saiki and Lowe 1987; Presser and Ohlendorf 1987; Lemly 1985a; Luoma et al. 1992; Presser et al. 1994; Chapman et al. 2010), high consumption rates of selenium-contaminated food may counteract the selenium dilution that occurs with the addition of body tissue during periods of fast growth.

EPA evaluated the effect of removing the parameter *g* in the Equation 1 by performing a sensitivity analysis. EPA analyzed a series of hypothetical tissue concentration estimates using Equation 1 with *g* ranging between 0 (no growth) and 0.2/day (a relatively high rate of growth).

In one analysis, tissue concentrations of selenium were estimated using static values of *IR*. In a second analysis, tissue concentrations of selenium were estimated using values of *IR* that were adjusted for growth rate using a method similar to the approach used in a model of organic chemical accumulation in aquatic food webs (Thomann et al. 1992). As expected, estimates of selenium tissue concentrations were significantly reduced at progressively higher growth rates when *IR* remained constant. However, selenium concentrations remained fairly steady or slightly increased with progressively higher growth rates when *IR* was adjusted for the bioenergetics of growth. This analysis supports the hypothesis that a higher *IR* (and consequently greater rate of selenium ingestion) associated with the higher bioenergetic requirements of rapidly growing young fish tends to oppose the dilution of selenium in their tissues due to growth, whereas a lower *IR* (and consequently lower rate of selenium ingestion) associated with the lower bioenergetic requirements of slower growing older fish tends to oppose the bioconcentration of selenium in their tissues. EPA concludes from this analysis that omitting the growth rate parameter *g* is an appropriate simplification of Equation 1. A more detailed description of this sensitivity analysis is provided in Appendix J.

Uptake Rate

The uptake rate constant k_u is included in Equation 1 to account for direct absorption of bioaccumulative chemicals in the dissolved phase. However, dietary intake of selenium is the dominant source of exposure, suggesting that k_u may also be relatively inconsequential for selenium accumulation (Luoma and Rainbow 2005). Because aqueous uptake of selenium makes up a small percentage of bioaccumulated selenium (Fowler and Benayoun 1976; Luoma et. al. 1992; Roditi and Fisher 1999; Wang and Fisher 1999; Wang 2002; Schlekat et. al. 2004; Lee et. al. 2006), Presser and Luoma (2010a, b, 2013) deemed removal of k_u from Equation 1 as an acceptable simplification.

EPA evaluated the effect of removing the parameter k_u in the Equation 1 by performing a sensitivity analysis. EPA analyzed a series of tissue concentration estimates using Equation 1 and a realistic range of k_u values for trophic level 2 and trophic level 3 organisms. The analysis suggests that approximately 75% of selenium exposure in trophic level 2 organisms (invertebrates) and over 90% of selenium exposure in trophic level 3 organisms occurs through consumption of selenium-contaminated food. EPA concluded that omitting the aqueous uptake

rate constant k_u is an appropriate simplification of Equation 1. A more detailed description of this sensitivity analysis is provided in Appendix J.

Derivation of the Translation Equation

Disregarding growth (g) and uptake of selenium dissolved in water ($k_u \times C_{water}$), Equation 1 becomes Equation 2 (Reinfelder et al. 1998):

$$C_{tissue} = \frac{AE \times IR \times C_{food}}{k_e}$$

or:

$$C_{tissue} = \frac{AE \times IR}{k_e} \times C_{food} \qquad \text{(Equation 2)}$$

Because application of the bioaccumulation model applies to a single species, the combination of species-specific physiological parameters expressed as $\frac{AE \times IR}{k_e}$ remains constant for the species. Thus the EPA defines the expression $\frac{AE \times IR}{k_e}$ as a single species-specific Trophic Transfer Factor (*TTF*) given as Equation 3 (Reinfelder et al. 1998):

$$TTF = \frac{AE \times IR}{k_e} \qquad \text{(Equation 3)}$$

Substituting *TTF* for $\frac{AE \times IR}{k_e}$ in Equation 2 yields:

$$C_{tissue} = TTF \times C_{food} \qquad \text{(Equation 4)}$$

The trophic level of the organisms considered can be denoted by superscripts given as:

$$C_{tissue}^{TL2} = TTF^{TL2} \times C_{food}^{TL2} \qquad \text{(Equation 5)}$$

C_{tissue}^{TL2} as defined here represents the steady-state proportional concentration of selenium in the tissue of trophic level 2 organisms relative to the concentration of selenium in their food source.

Using the same rationale, the average concentration of selenium in the tissues of trophic level 3 organisms can be expressed as the concentration of selenium in its food multiplied by a *TTF* which is given as:

$$C_{tissue}^{TL3} = TTF^{TL3} \times C_{food}^{TL3} \qquad \text{(Equation 6)}$$

For trophic level 3 organisms that consume trophic level 2 organisms, $C_{food}^{TL3} = C_{tissue}^{TL2}$. Thus:

$$C_{tissue}^{TL3} = TTF^{TL3} \times C_{tissue}^{TL2} \qquad \text{(Equation 7)}$$

Substituting C_{tissue}^{TL2} in Equation 7 with $TTF^{TL2} \times C_{food}$ in Equation 5 yields:

$$C_{tissue}^{TL3} = TTF^{TL3} \times TTF^{TL2} \times C_{food}^{TL2} \qquad \text{(Equation 8)}$$

Defining the term C_{tissue}^{TL3} as the concentration of selenium in fish tissue, defining the term C_{tissue}^{TL2} as the concentration of selenium in living and nonliving particulate material ingested by invertebrates, and expressing the product of all *TTF* values as a single term results in the equation:

$$C_{whole-body} = TTF^{composite} \times C_{particulate} \qquad \text{(Equation 9)}$$

where:

$C_{particulate}$	=	the concentration of selenium in particulate material
$C_{whole\text{-}body}$	=	the concentration of selenium in the whole body of fish
$TTF^{composite}$	=	the product of all trophic transfer factor values

Equation 9 quantitatively expresses selenium bioaccumulation in fish ($C_{whole\text{-}body}$) as the product of the concentration of selenium at the base of the food web ($C_{particulate}$) and a parameter representing the trophic transfer of selenium through all dietary pathways ($TTF^{composite}$). This model of bioaccumulation is conceptually similar to the model of bioaccumulation utilizing a bioaccumulation factor (BAF). A BAF is the ratio of the concentration of a chemical in the tissue

of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). Similar to the term $TTF^{composite}$, a BAF quantitatively represents the relationship between the chemical concentrations in multiple environmental compartments. However, a BAF is empirically derived from site-specific measurements, whereas $TTF^{composite}$ is derived from knowledge of the ecological system. Because each *TTF* is associated with a particular taxon, $TTF^{composite}$ can be inferred for an aquatic system using existing knowledge and reasonable assumptions, without the considerable time and cost of collecting and analyzing tissue and water samples.

Equation 9 characterizes the bioaccumulation of selenium as a combination of *TTF* parameters from all steps in the dietary pathway of the predator species of interest. Thus it is possible to differentiate bioaccumulative potential for different predator species and food webs by modeling different exposure scenarios. For example, where the fish species of interest is a trophic level 4 predator that primarily consumes trophic level 3 fish, the term $TTF^{composite}$ can be represented as the product of all *TTF* parameters that includes the additional trophic level given as:

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2} \qquad \text{(Equation 10)}$$

where:

TTF^{TL2} = the trophic transfer factor of trophic level 2 species

TTF^{TL3} = the trophic transfer factor of the trophic level 3 species

TTF^{TL4} = the trophic transfer factor of the trophic level 4 species

$TTF^{composite}$ = the product of all trophic transfer factors

Similarly, the consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* at a particular trophic level as the weighted average of the *TTF*s of all species consumed given as:

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \text{(Equation 11)}$$

where:

TTF_i^{TLx} = the trophic transfer factor of the i^{th} species at a particular trophic level

w_i = the proportion of the i^{th} species consumed

These concepts can be used to formulate an expression of $TTF^{composite}$ to model selenium bioaccumulation in ecosystems with different consumer species and food webs. **Figure 3.4** describes four example food web scenarios and the formulation of $TTF^{composite}$ to model selenium bioaccumulation in each of them.

The parameter $TTF^{composite}$ quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system, w_i, the proportion of species consumed. See text for further explanation.

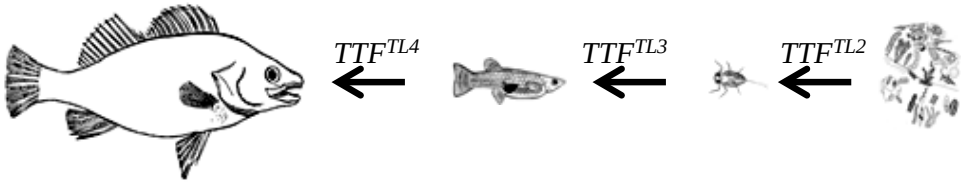
A) Three trophic levels (simple):

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$



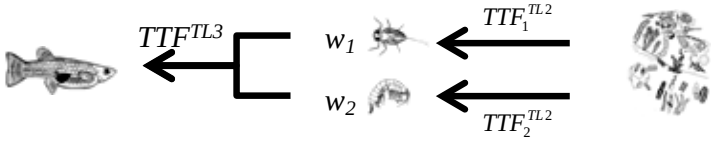
B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$



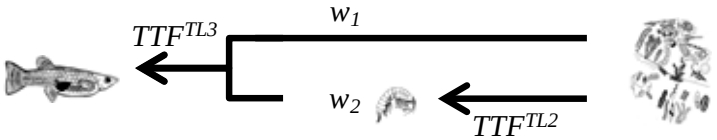
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

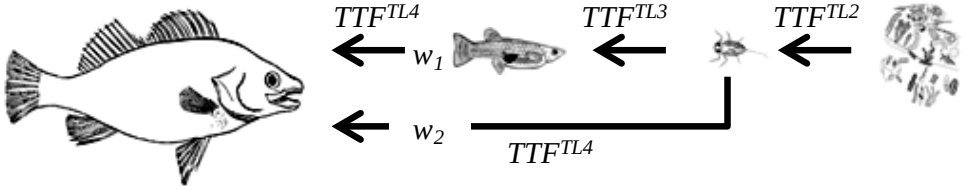


Figure 3.4. Example Aquatic System Scenarios and the Derivation of the Equation Parameter $TTF^{composite}$.

Because EPA's objective is to derive an equation that translates a fish tissue concentration of selenium to a water column concentration, the term C_{water} is reintroduced into

Equation 9 by defining the enrichment function EF representing the steady state proportional bioconcentration of dissolved selenium at the base of the aquatic food web given as:

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \text{(Equation 12)}$$

Where:

$C_{particulate}$ = Selenium concentration in particulate material (µg/g)
C_{water} = Concentration of selenium dissolved in water (µg/L)
EF = Enrichment function (L/g)

Rearranging the terms of Equation 12:

$$C_{particulate} = EF \times C_{water} \qquad \text{(Equation 13)}$$

Substituting $EF \times C_{water}$ for $C_{particulate}$ in Equation 9 results in:

$$C_{whole-body} = TTF^{composite} \times EF \times C_{water} \qquad \text{(Equation 14)}$$

Solving for the concentration of selenium in water in Equation 14 results in:

$$C_{water} = \frac{C_{whole-body}}{TTF^{composite} \times EF} \qquad \text{(Equation 15)}$$

Because Equation 15 relates a concentration of selenium in water to the concentration of selenium throughout all tissues of the body, $C_{whole\text{-}body}$ must be converted to an equivalent concentration in eggs or ovaries. EPA achieved this conversion by incorporating a species-specific conversion factor (*CF*) into Equation 15. *CF* represents the species-specific proportion of selenium in egg or ovary tissue relative to the concentration of selenium in all body tissues and is given as:

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \text{(Equation 16)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (µg/g).

Rearranging the terms of Equation 16 yields:

$$C_{whole-body} = \frac{C_{egg-ovary}}{CF} \qquad \text{(Equation 17)}$$

Substituting $C_{whole\text{-}body}$ in Equation 15 with $\frac{C_{egg-ovary}}{CF}$ yields the translation equation:

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \text{(Equation 18)}$$

where $TTF^{composite}$ equals the product of all trophic transfer factors from trophic level 2 through the target fish species.

Equation 18 describes an ecosystem-dependent relationship between the concentration of selenium in the eggs and ovaries of fish with the concentration of selenium in the water column. This approach explicitly recognizes the sequential transfer of selenium between environmental compartments (water, particulate material, invertebrate tissue, fish tissue, and eggs and/or ovary tissue) by incorporating quantitative expressions of selenium transfer from one compartment to the other. Because this approach uses food web modeling along with species-specific *TTF* and *CF* parameters to quantify most of the transfer between compartments, the only field measurements needed to relate selenium in egg-ovary and water are measurements from the water column and particulate material sufficient to calculate *EF*.

3.2.2 Equation Parameters

Empirical or laboratory data related to selenium bioaccumulation in aquatic organisms are needed to derive the equation parameters *EF*, *TTF*, and *CF*. EPA obtained data from published literature as described above The search resulted in the retrieval of 63 acceptable studies containing a total of 8,707 selenium measurements at 768 aquatic sites (2,927 from water, 373 from algae, 29 from detritus, 821 from sediment, 1,324 from various species of

invertebrates, and 3,233 from various species of fish) and 34 acceptable studies yielding 139 physiological constants (48 values of k_e, 81 values of *AE*, and 10 values of *IR*). EPA used this collection of selenium measurements to calculate site-specific *EF* values and develop species-specific *TTF* and *CF* values in an unbiased and systematic manner. A more detailed description of how EPA calculated *EF* is described below. How EPA calculated *TTF* and *CF* is described in detail in Appendix B.

3.2.2.1 Derivation of Trophic Transfer Factor (*TTF*) Values

EPA derived *TTF* values for taxonomic groups of invertebrates and fish by either using physiological coefficients found in the literature, or by evaluating the empirical relationship between matched pairs of selenium measurements in organisms and the food they consumed. When physiological coefficients were available, EPA calculated a *TTF* value using Equation 3 (**Section 3.2.1**):

$$TTF = \frac{AE \times IR}{k_e} \qquad \text{(Equation 3)}$$

Where:

k_e = Elimination rate constant (/d)

AE = Assimilation efficiency (%)

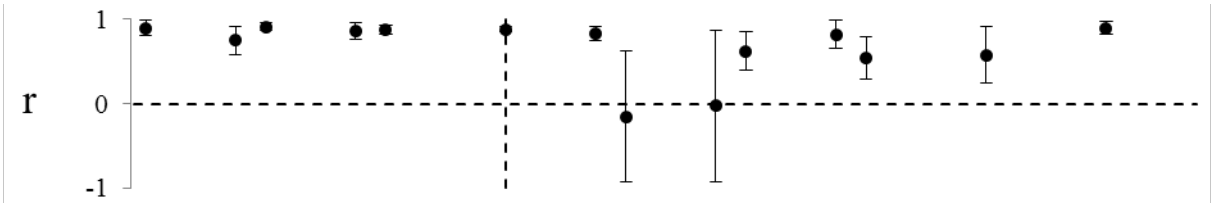
IR = Ingestion rate (g/g-d)

EPA also derived *TTF* values using empirical measurements of selenium from field studies. EPA searched its collection of available selenium measurements and identified measurements taken from aquatic organisms. For each measurement from an aquatic organism, EPA searched for additional measurements from other aquatic organisms or particulate material that was collected from the same aquatic site and of a type deemed likely to be ingested as a food source or in conjunction with feeding activity (i.e., a lower trophic level). If multiple lower trophic level measurements were matched to an aquatic organism measurement, the median of the lower trophic level measurements was calculated. Each pair of measurements, one taken from an aquatic organism and the other taken from lower trophic level organisms or particulate material, was designated as a matched pair. EPA limited particulate data used to calculate

invertebrate *TTF*s from field data to those aquatic sites with at least two particulate selenium measurements paired with invertebrate selenium measurements, and only used sediment measurements if there was at least one measurement from algae or detritus. If selenium concentrations from more than category of particulate material (algae, detritus, or sediment) were available, EPA used the median Se concentration of the available categories as the particulate concentration for that site.

Because selenium is transferred to aquatic animals primarily through aquatic food webs, the observable concentration of selenium in different environmental compartments may vary over time. To establish an appropriate time period with which to define matched pairs of selenium measurements, the effect of sample collection time on the relationship between selenium concentrations in different media was analyzed. EPA defined matched pairs of selenium measurements as described above using different relative collection time ranges and estimated the strength of the relationship between the two measurements by calculating the Pearson product-moment correlation coefficient (r). **Figure 3.5** shows the correlation coefficients for selenium measurements taken from the same aquatic sites when the measurement collection times were systematically shifted relative to one another. Each correlation coefficient was calculated from a set of data within a specified range of relative collection times with respect to the higher trophic level. For example, the correlation coefficient calculated from invertebrate and fish measurements with a relative sample collection time of 30 to 60 days were from invertebrate and fish samples collected at the same site, with the fish samples collected 30 to 60 days after the invertebrate samples. Similarly, the correlation coefficient calculated from invertebrate and fish measurements with a relative collection time of -60 to -30 days were from invertebrate and fish samples that were collected at the same site, with the fish samples collected 30 to 60 days before the invertebrate samples.

Particulate versus invertebrate



Invertebrate versus fish

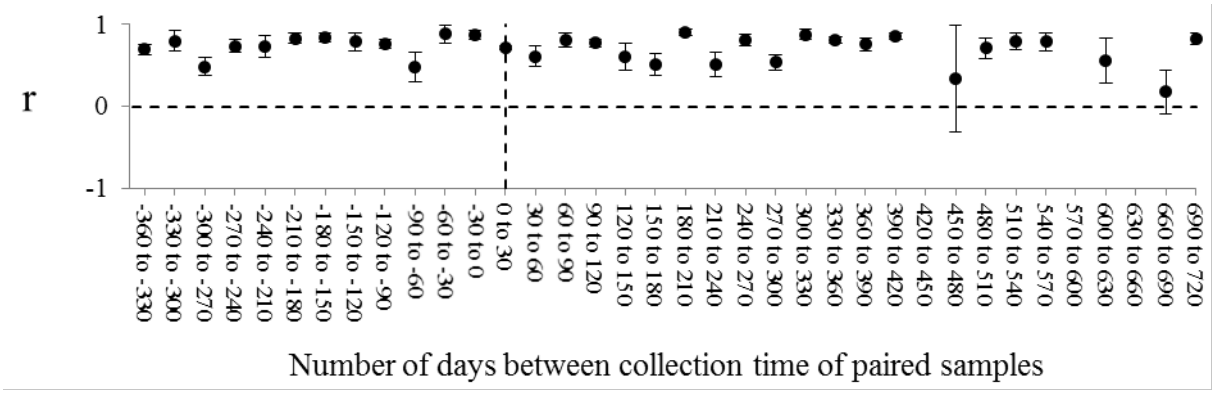


Figure 3.5. Effect of Relative Sample Collection Time on Correlation Coefficients of Selenium Measurements in Particulate Material, and Invertebrate and Fish Tissue. Error bars indicate the 95% confidence interval of r calculated using Fisher's r to z transformation. Horizontal dashed line indicates r = 0; vertical dashed line indicates relative collection time expected to have the highest correlation. The absence of a correlation coefficient indicates an insufficient quantity of data at the specified relative collection time range.

The results of this analysis suggest that the relationship between selenium concentrations in particulate material and invertebrate tissue and between invertebrate tissue and fish tissue is insensitive to relative collection time within a one year time period. These results also suggest that selenium becomes relatively persistent in the aquatic ecosystem once dissolved selenium transforms to particulate selenium and becomes bioavailable. On the basis of these analyses, EPA concludes that selenium measurements from samples collected at the same aquatic site within one year of each other are acceptable to use as matched pairs of measurements from the aquatic sites. Note that EPA chose a relative collection time period of one year on the basis of data taken from many different aquatic sites. Individual aquatic sites may have selenium loads and/or bioaccumulation characteristics that require different relative collection time criteria to accurately characterize selenium relationships.

After matched pairs of selenium measurements from samples collected in the field were identified, EPA evaluated two different analytical approaches to derive species-specific *TTF*

values from them. *TTF* was previously defined above as the steady state proportion relating the concentration of selenium in the tissue of aquatic organisms to the concentration of selenium in the food they ingest such that:

$$C_{tissue} = TTF \times C_{food} \qquad \text{(Equation 4)}$$

Rearranging the terms of Equation 4 yields Equation 19:

$$TTF = \frac{C_{tissue}}{C_{food}} \qquad \text{(Equation 19)}$$

Because *TTF* can be defined as the ratio of the concentration of selenium observed in the tissue of an aquatic organism to the concentration of selenium observed in the tissue or material the organism ingests, one approach for deriving *TTF* values from field data is to simply use the ratio of the two values. EPA evaluated this approach by calculating the ratios for all matched pairs of selenium measurements, and for each species or taxonomic group, used a statistic of central tendency of the distribution of ratios as the *TTF* value. An advantage of quantifying the relationship between selenium in two environmental compartments using ratios is that it is a simple and straightforward method that is conceptually similar to a bioaccumulation factor (BAF). A disadvantage of this approach is that it presumes that the quality and quantity of data used to derive the ratios adequately represent the relationship being characterized. Furthermore, many aquatic organisms tend to bioaccumulate more metals at low environmental concentrations (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007; U.S. EPA 2007). Thus a distribution of ratios could be biased toward larger values if the data are obtained from aquatic systems with low selenium concentrations.

Another analytical approach for deriving *TTF* values from matched pairs of selenium measurements is to model the species-specific relationships using linear regression. One possibility is to regress the concentration of selenium in the food of a particular species or taxonomic group with the concentration of selenium in the organism's tissue, and use the regression coefficient as the *TTF*. EPA evaluated this approach by applying ordinary least

squares (OLS) linear regression on the matched pairs of data. The regression coefficient (slope of the fitted line) was then taken as the *TTF* value for that species or taxonomic group. An advantage of this regression approach is that it estimates the quantitative relationship of selenium across a range of environmental concentrations in a manner that allows statistical assessment. Disadvantages of this regression approach include the assumption that the underlying data are normally distributed; one or a few very high values can have a disproportionate influence on the slope of the fitted line; and the bioaccumulation model does not account for a non-zero y-intercept. Constraining the y-intercept to zero (also known as regression through the origin or RTO) eliminates the added complexity of a non-zero y-intercept. However, RTO further increases the disproportionate influence of one or a few high values on the slope of the fitted line. Furthermore, RTO does not provide a straightforward way of evaluating goodness of fit (Gordon 1981).

After evaluating both approaches, EPA decided to use a hybrid approach by designating the median of the ratio of matched pairs of selenium measurements as the *TTF* value, but only if OLS linear regression of those data resulted in a significant ($P \leq 0.05$) fit and positive regression coefficient. Requiring a significant positive OLS linear regression coefficient confirms the relationship between selenium in organisms and the food they ingest is adequately represented by the available data. Using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias from measurements taken from aquatic systems with very high or very low selenium concentrations. Some aquatic organisms exhibit selenium bioaccumulation inversely related to water concentration (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). This inverse relationship is likely due to saturation uptake kinetics of specific transport mechanisms that regulate metals bioaccumulation within certain ranges (U.S. EPA 2007). EPA evaluated the effect of very high and very low selenium concentrations on the calculation of *TTF* values using the hybrid approach described above by calculating TTF values excluding selenium measurements above 10, 25, 50, and 100 μg/g and below 0.1, 0.5, 1.0, and 2.0 μ/g. EPA found that excluding very high or very low selenium measurements had minor effects on *TTF* values. EPA concludes that using the median ratio effectively attenuates effects of selenium concentration on the calculation of *TTF* values using the hybrid approach described above and allows the use of all available data without the need to introduce additional arbitrary exclusion criteria.

EPA calculated *TTF* values for 13 invertebrate species and 32 fish species that live in freshwater aquatic environments in North America. The data used to derive these *TTF* values are provided in Appendix B. The final *TTF* values are listed in **Table 3.10** and **Table 3.11**. The presence of physiological coefficients for a taxon in **Table 3.10** and **Table 3.11** indicates that the *TTF* values were calculated using those parameters. The absence of physiological coefficients for a taxon indicates that EPA derived the *TTF* value using field data. If a *TTF* value could be calculated from both physiological coefficients and field data, EPA used the *TTF* value calculated from the substantially larger number of field measurements to minimize statistical uncertainty.

Table 3.10. EPA-Derived Trophic Transfer Factor (*TTF*) Values for Freshwater Aquatic Invertebrates.

Common name	Scientific name	AE	IR	k_e	TTF
Crustaceans					
amphipod	*Hyalella azteca*	-	-	-	1.22
copepod	copepods	0.520	0.420	0.155	1.41
crayfish	*Astacidae*	-	-	-	1.46
water flea	*Daphnia magna*	0.406	0.210	0.116	0.74
Insects					
dragonfly	*Anisoptera*	-	-	-	1.97
damselfly	*Coenagrionidae*	-	-	-	2.88
mayfly	*Centroptilum triangulifer*	-	-	-	2.38
midge	*Chironimidae*	-	-	-	1.90
water boatman	*Corixidae*	-	-	-	1.48
Mollusks					
asian clam[a]	*Corbicula fluminea*	0.550	0.050	0.006	4.58
zebra mussel	*Dreissena polymorpha*	0.260	0.400	0.026	4.00
Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	zooplankton	-	-	-	1.89

[a] Not to be confused with *Potamocorbula amurensis*

Table 3.11. EPA-Derived Trophic Transfer Factor (*TTF*) Values for Freshwater Fish.

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

For fish species without sufficient data to directly calculate a *TTF* value, EPA estimated the *TTF* value by sequentially considering higher taxonomic classifications until one or more taxa for which a calculated *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species. For example, although data to directly calculate *TTF* for *Chrosomus eos* (northern redbelly dace) were not available, this species is in the family Cyprinidae, which also includes *Rhinichthys atratulus* (blacknose dace), *Cyprinus carpio* (common carp), *Semotilus atromaculatus* (creek chub), *Pimephales promelas* (fathead minnow), *Cyprinella lutrensis* (red shiner), *Richardsonius balteatus* (redside shiner), and *Notropis stramineus* (sand shiner). Because Cyprinidae is the lowest taxonomic classification where *Chrosomus eos* matches a species with an available *TTF* value, the median of the blacknose dace, common carp, creek chub, fathead minnow, red shiner, redside shiner, and sand shiner *TTF* values was used as the *TTF* value for northern redbelly dace. The data and analyses used to calculate all *TTF* values including those estimated by taxonomic classification are provided in Table B-8 of Appendix B.

3.2.2.2 Derivation of Whole-Body to Egg-Ovary Conversion Factor (*CF*) Values

The parameter *CF* (conversion factor) in Equation 18 (**Section 3.2.1**) represents the species-specific partitioning of selenium as measured in the whole-body and in egg-ovary tissue. EPA derived species-specific *CF* values by applying the same method used to derive species-specific *TTF* values using empirical measurements of selenium concentrations in different tissues of the same fish. To derive whole-body to egg-ovary *CF* values, EPA defined matched pairs of selenium measurements from the whole-body and from the eggs or ovaries measured from the same individual fish or from matched composite samples. Egg-ovary concentration was defined as a measurement from either the eggs or the ovaries. If multiple measurements from both eggs and ovaries of the same individual or matched composite sample were available, the average value was used. For example, both egg and ovary measurements were available for five of the 27 egg-ovary concentrations used to calculate the bluegill egg-ovary to whole body *CF* (Coyle et al. 1993), and 16 of the 69 measurements used to calculate the cutthroat trout egg-ovary to muscle *CF* (Kennedy et al. 2000).

Similar to the procedure used to derive *TTF* values, EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using OLS linear

regression of the matched pairs of measurements. If the regression resulted in a significant fit ($P \leq 0.05$) with a positive regression coefficient, EPA calculated the ratio of the egg-ovary to whole body selenium concentration of each matched pair and used the median ratio as the *CF* value for the species**.** A detailed comparison of the advantages and disadvantages of the median ratio and least squares regression approaches to calculating *CF*s, along with a comparison of *CF*s calculated from median ratios, OLS regression following log transformation, and total least squares (TLS) regression following log transformation is included in Appendix N.

EPA had sufficient egg-ovary and whole-body selenium measurements to directly derive egg-ovary to whole body *CF* values for 13 species of fish. However, matched pairs of selenium measurements in eggs and/or ovaries and muscle tissue, and matched pairs of selenium measurements in muscle and whole body were also available. To derive *CF* values for additional fish species, EPA used either the additional data or a taxonomic classification approach to estimate *CF*.

For those species of fish with neither sufficient data to directly calculate an egg-ovary to whole body *CF*, nor data to calculate a conversion factor for egg-ovary to muscle or whole body to muscle, EPA first estimated *CF* following the approach described above for the estimation of *TTF* values. In this first approach, EPA sequentially considered higher taxonomic classifications until one or more taxa for which a calculated *CF* value was available matched the taxon being considered, and if the lowest matching taxon was common to more than one species with a *CF* value available, EPA used the median *CF* from the matching species. For example, *CF* data are not available to directly calculate *CF* for *Lepomis microlophus* (redear sunfish); however, genus-level *CF*s for *Lepomis cyanellus* (green sunfish) and *Lepomis macrochirus* (bluegill) are available. Thus, EPA used the median *CF* values of *Lepomis cyanellus* and *Lepomis macrochirus* for redear sunfish.

For fish species without sufficient data to directly calculate an egg-ovary to whole body *CF*, but which had sufficient data to calculate a conversion factor for either egg-ovary to muscle or whole body to muscle, EPA followed a two stage approach based on taxonomic similarity. If a fish species had a species specific egg-ovary to muscle conversion factor, but no whole body data with which to calculate an egg to whole body *CF*, available data for other species would be used to estimate a muscle to whole body conversion factor for that species based on taxonomic relatedness. The estimated muscle to whole body factor would be multiplied by the directly

measured egg-ovary to muscle factor to estimate an egg-ovary to whole body *CF* for that species. For example, rainbow trout has a species specific egg-ovary to muscle conversion factor of 1.92, but does not have a species specific egg-ovary to whole body *CF*. Using the taxonomic approach described above, the most closely related taxa to rainbow trout with muscle to whole body conversion factors are in the class Actinopterygii. The median conversion factor for the eight species within that class is 1.27. The final egg-ovary to whole body *CF* for rainbow trout is 2.44 (**Table 3.12**), or 1.92 x 1.27.

EPA derived 13 *CF* values directly from matched pairs of egg-ovary and whole-body selenium measurements and an additional seven *CF* values by multiplying EO/M and M/WB conversion factors. Variability in the *CF* values for 19 of the 20 fish species was low (**Table 3.12**). Excluding mountain whitefish, *CF*s ranged from 1.20 to 3.11, a 2.6-fold difference. *CF* variability within each species was also low for 7 of 13 species for which egg-ovary to whole-body *CF*s were calculated. The two species with relatively high standard deviations for their *CF* values (brown trout and cutthroat trout) contained potentially anomalous hatchery data that contributed to the variability (see **Table 3.12** footnote). These species specific *CF* values are listed below in **Table 3.12** and in Table B-5 of Appendix B. All *CF* values including those estimated using the taxonomic classification approach are provided in Table B-6 in Appendix B.

Table 3.12. EPA-Derived Egg-Ovary to Whole-Body Conversion Factor (*CF*) Values.

Common name	Scientific name	CF	Std. Dev.[a]
Acipenseriformes			
white sturgeon	*Acipenser transmontanus*	1.69	
Cypriniformes			
bluehead sucker	*Catostomus discobolus*	1.82	0.19
flannelmouth sucker	*Catostomus latipinnis*	1.41	0.20
white sucker	*Catostomus commersonii*	1.38	0.36
desert pupfish	*Cyprinodon macularius*	1.20	0.10
common carp	*Cyprinus carpio*	1.92	0.49
roundtail chub	*Gila robusta*	2.07	0.29
fathead minnow	*Pimephales promelas*	1.40	0.75
creek chub	*Semotilus atromaculatus*	1.99	1.00
razorback sucker	*Xyrauchen texanus*	3.11	
Esociformes			
northern pike	*Esox lucius*	2.39	
Perciformes			
bluegill	*Lepomis macrochirus*	2.13	0.68

Common name	Scientific name	CF	Std. Dev.[a]
green sunfish	*Lepomis cyanellus*	1.45	0.23
smallmouth bass	*Micropterus dolomieu*	1.42	0.19
Salmoniformes			
brook trout	*Salvelinus fontinalis*	1.38	
Dolly Varden	*Salvelinus malma*	1.61	
brown trout	*Salmo trutta*	1.45	1.81[b]
rainbow trout	*Oncorhynchus mykiss*	2.44	
cutthroat trout	*Oncorhynchus clarkii*	1.96	2.03[b]
mountain whitefish	*Prosopium williamsoni*	7.39	

[a] Standard deviation for *CF* values for those species that had egg-ovary and whole body selenium concentrations.

[b] The brown trout and cutthroat trout standard deviations for *CF* values of 1.81 and 2.03 are considerably higher than the other standard deviations in this table. The brown trout data were taken from two studies, NewFields (2009) and Osmundson et al. (2007). *CF* values for three of the four fish samples from Osmundson et al. were four to six times greater than the median. Also, the NewFields data consisted of samples collected from natural streams and samples collected from a fish hatchery. The *CF* values for the fish hatchery samples were four to seven times lower than the median value. Although collectively, the data set meets the criteria for including the brown trout *CF*, the *CF* values for Osmundson et al. and NewFields hatchery samples may be anomalously high and low, respectively. Excluding these potentially anomalous data reduces the brown trout standard deviation to 0.47. The cutthroat trout *CF* values are from two sources (Formation 2012 and Hardy 2005). The reason for the higher variability in the cutthroat trout *CF* values is due to the relatively higher *CF* values in the hatchery fish from the Formation study. The standard deviation for cutthroat trout drops to 0.62 if the hatchery fish are excluded. See Appendix B for a presentation of the data for both of these species.

3.2.2.3 Calculation of Site-Specific Enrichment Factor (EF) Values

The most influential step in selenium bioaccumulation occurs at the base of aquatic food webs (Chapman et al. 2010). The parameter *EF* characterizes this step by quantifying the partitioning of selenium between the dissolved and particulate state. *EF* can vary by at least two orders of magnitude across aquatic systems (Presser and Luoma 2010). The greatest reduction in uncertainty when translating a fish tissue concentration of selenium to a water column concentration using Equation 18 is achieved when spatially and temporally coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity are used to accurately characterize *EF*. Thus, EPA only used aquatic sites with sufficient data to calculate a reasonably reliable *EF* value.

To calculate the *EF* of aquatic systems, EPA searched its collection of selenium concentration measurements from field studies (see **Section 2.7.8** for a description of data

sources and acceptability criteria) and identified aquatic sites with measurements from both particulate material and the water column. EPA first identified all selenium measurements from algae, detritus, or sediment, and then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water measurement was available for any given particulate measurement, the median was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median ratio. The geometric mean of the algae, detritus, and sediment ratios was used as the site *EF*. Because there were at most only three possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric mean in order to reduce the potential for one of the values to have excessive influence on the final site *EF* value.

The availability of selenium measurements from particulate material was limited. In addition, the majority of particulate measurements were from sediment samples with a significantly lower correlation to selenium in water ($r = 0.34$) compared to algae ($r = 0.68$; Fisher r-to-z transformation, $P < 0.001$) and detritus ($r = 0.94$; Fisher r-to-z transformation, $P < 0.001$). Therefore, to reduce uncertainty in estimating site-specific *EF* values, EPA limited its analysis to those aquatic sites with at least two particulate selenium measurements with corresponding water column measurements, and only used sediment measurements if there was at least one other measurement from either algae or detritus. On the basis of these requirements, *EF* values were calculated for 96 individual aquatic sites.

3.2.3 Food-Web Models

For the aquatic sites with a calculated *EF* value, EPA modeled the food webs for the fish species the studies indicated were present. Some of those studies provided information about the species and proportions of organisms ingested by fish, either through direct analysis of stomach contents, or examination of the presence and prevalence of invertebrate species. For those studies, that site-specific information in the food web models was used. Most studies, however, did not provide site-specific food web information. In those cases, the food webs of fish species present were modeled using information about their typical diet and/or eating habits obtained from the NatureServe database (http://www.natureserve.org).

After EPA developed food web models, EPA identified the appropriate species-specific *TTF* values for each model and calculated $TTF^{composite}$. Although individual *TTF* values were derived for several different taxa of invertebrates and fish (**Table 3.10** and **Table 3.11**), some of the food web models included one or more taxa for which no *TTF* value was available. EPA estimated *TTF* values for these taxa using the same taxonomic approach used to estimate egg-ovary to whole body, egg-ovary to muscle, and muscle to whole body conversion factors for taxa without sufficient data. In brief, for taxa with insufficient data to calculate a *TTF* value, EPA sequentially considered higher taxonomic classifications until one or more taxa for which a *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species. EPA used site-specific food-web models to translate the egg-ovary criterion element to a set of water column concentrations in order to derive the water column concentration element of the selenium criterion. Details of these food web models are shown in Table B-8 of Appendix B.

3.2.4 Classifying Categories of Aquatic Systems

Transformation reactions that convert dissolved selenium to particulate forms are the primary route of entry into aquatic food webs, and are critical steps in selenium bioaccumulation and toxicity (Chapman et al. 2010). Site-specific characteristics can result in substantial bioaccumulation variability and consequently different risks of selenium toxicity for a given dissolved selenium concentration. Freshwater systems fall into two distinct categories: lotic systems such as rivers and streams, characterized by flowing water, and lentic systems, such as lakes and ponds, characterized by largely standing water (e.g., Jones 1997). Water residence time is generally shorter in lotic systems than in lentic systems, and subsequently, aquatic organisms living in lentic systems tend to bioaccumulate more selenium than organisms living in lotic systems for a given dissolved selenium concentration (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005).

Although the distinction between lotic and lentic aquatic systems is often straightforward, some aquatic systems possess both lotic and lentic characteristics. For example, flow rate can vary greatly among lotic systems, with slow flowing low gradient systems (such as sloughs) having longer residence times relative to fast flowing high gradient systems. Lotic systems can also become more lentic during dry periods as hydrologic connectivity between deeper pools

decrease or cease with decreasing flow (Buffagni et al. 2009). Downstream reaches of some low gradient coastal rivers can also be influenced by tides (Riedel and Sanders 1998). Some lentic systems can exhibit some degree of flow, such as lakes fed and drained by one or more streams; however, the magnitude of flow is generally small compared to a lotic system. Even after accounting for flow, the majority of water movement in a lentic system is driven typically by wind or convection rather than gravity (e.g., Jones 1997). Finally, human-made reservoirs have some features that are intermediate between typical lotic and lentic systems. For example, reservoirs tend to be longer and narrower than natural lakes, and they have somewhat shorter water retention time than a natural lake of comparable volume (Thornton et al. 1990). Overall, however, reservoirs as a general class are considered more lentic than lotic, and have historically been classified as a type of lake (Thornton et al. 1990).

To verify the suitability of lentic and lotic aquatic system categories as the basis for independent water column criterion element values, EPA evaluated the aquatic systems that provided data for the 96 *EF* values. EPA utilized the description provided by the study authors to categorize each aquatic system as either lotic or lentic. Of the 39 lentic sites, the authors identified them as ponds (n = 18), lakes (n = 13), reservoirs (n = 4), or marshes (n = 4). Of the 57 lotic sites, the authors identified them as creeks (n = 31), rivers (n = 16), artificial channels (drains and wasteways, n = 3), springs (n = 2), sloughs (n = 2), or ephemeral systems (draws and washes, n = 3). The three ephemeral aquatic sites (two washes and one draw) were categorized as lotic because there was flowing water at the time they were sampled (Butler et al. 1995; Presser and Luoma 2009). *EF* values for these aquatic systems are shown in **Figure 3.6**.

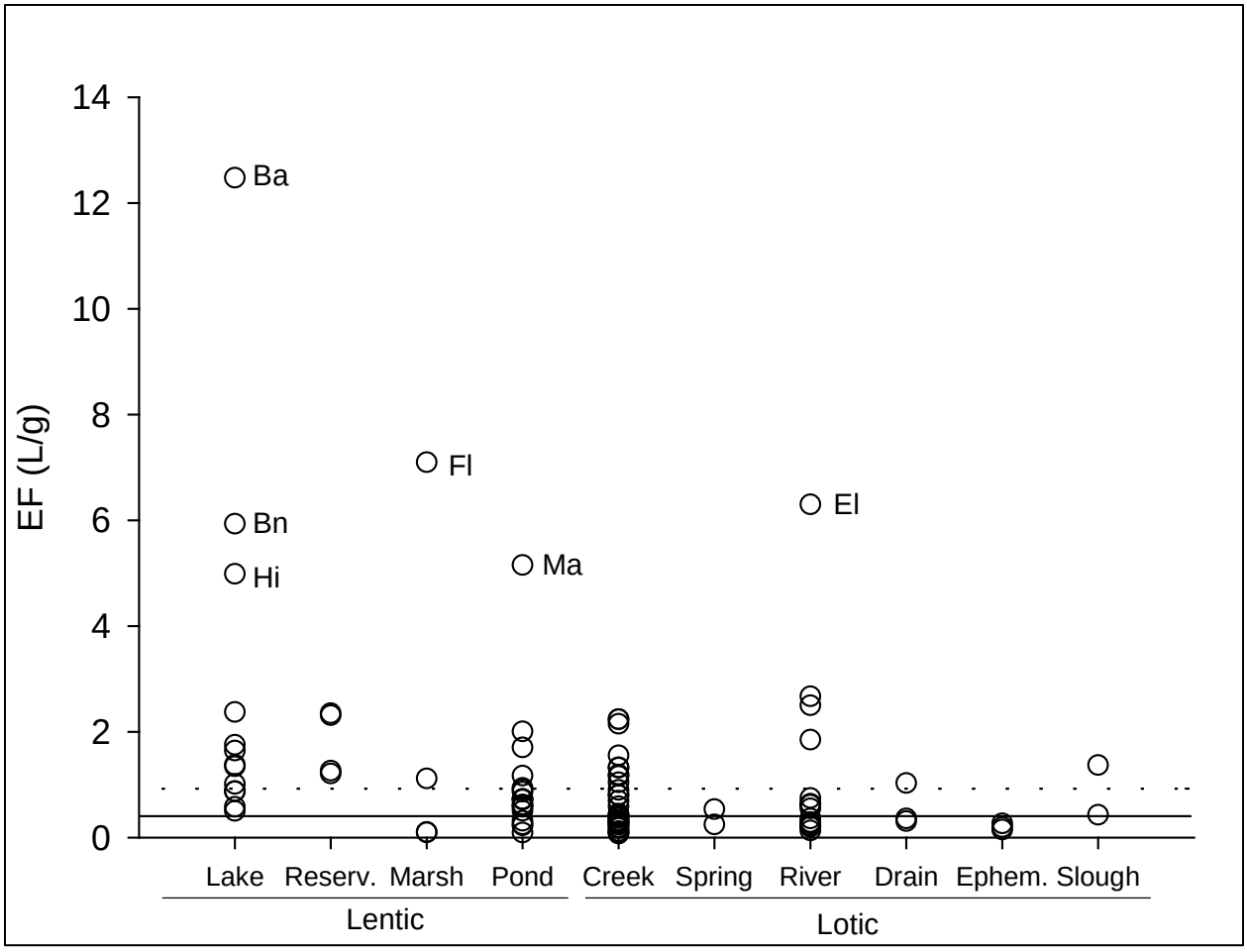


Figure 3.6. Enrichment Factors (*EF*) for 96 Aquatic Sites Derived from Published Studies and Organized by Waterbody Type.
The dashed line represents the median *EF* for the 39 lentic sites (0.9 L/g), and the solid line represents the median *EF* for the 57 lotic sites (0.4 L/g). See text for information on labeled data points.

Because the six labeled aquatic sites in **Figure 3.6** (Ma, Ba, Bn, Hi, El and Fl) appear as outliers, EPA selected them for further scrutiny. Data from site "Ma" result in an *EF* value of 5.2 L/g. Site "Ma" was a small irrigation pond within the Mancos River Valley watershed in southwestern Colorado (Butler et al. 1997). This watershed drains the Mancos Shale, a region that is naturally high in selenium. Data from sites "Hi," "Bn," and "Ba" resulted in *EF* values of 5.0, 5.9, and 12.5 L/g, respectively. Data from site "Hi" were from High Rock Lake, NC, data from site "Bn" were from Barnes Lake, British Columbia (Orr et al. 2006), and data from site "Ba" was from Badin Lake, NC (Lemly 1985). The high *EF* values at these three lakes were the result of a relatively high selenium concentration in particulate matter coupled with low aqueous

selenium concentrations. Data from site “El” result in an *EF* value of 6.3 L/g. Site “El” is an upstream site in the Elk River watershed in southeastern British Columbia, and the relatively large *EF* is the primarily the result of a low aqueous selenium concentration (McDonald and Strosher 1998). Data from “Fl” result in an *EF* value of 7.1 L/g. Site “Fl” is within Flathead wetland in southeastern British Columbia, and the relatively large *EF* is primarily the result of a low aqueous selenium concentration (Orr et al. 2012).

Figure 3.7 illustrates the variability in *EF* values across aquatic systems and substantial overlap between lotic and lentic categories. Some of this variability can be attributed to differences in the ambient concentration of selenium in the water column at these aquatic sites. *EF* is the ratio of selenium in particulate material ($C_{particulate}$) to selenium in the water column (C_{water}). As expected, the selenium concentrations in particulate material are positively correlated with the selenium concentrations in the water column (**Figure 3.7A**). The plot of $C_{particulate}$ versus C_{water} shows a significant ($P<0.001$) positive relationship for both lentic (slope = 0.65, 95% confidence interval = [0.50, 0.80]) and lotic (slope = 0.55, 95% confidence interval = [0.43, 0.68]) aquatic systems. However, selenium accrual in particulate matter is lower at aquatic sites with a higher water concentration of selenium compared to aquatic sites with a lower water concentration of selenium (**Figure 3.7B**). The plot of C_{water} versus *EF* shows a significant ($P<0.001$) negative relationship for both lentic (slope = -0.36, 95% confidence interval = [-0.51, -0.22]) and lotic (slope = -0.42, 95% confidence interval = [-0.55, -0.30]) aquatic systems. Consistent with other studies (e.g., Hamilton and Palace 2001; Brix et al. 2005; Orr et al. 2006), these results illustrate that the overall longer residence times of lentic systems result in increased bioaccumulation of selenium compared to lotic systems.

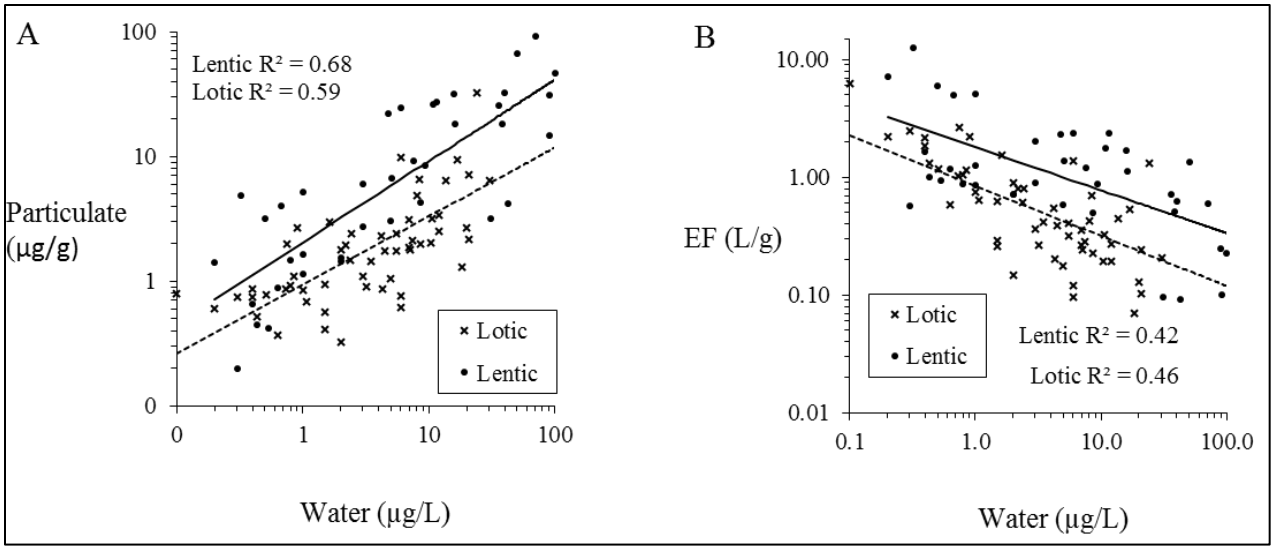


Figure 3.7. The Relationship between C_{water} and $C_{particulate}$, and C_{water} and *EF* for the 39 Lentic and 57 Lotic Aquatic Systems.

A: Relationship between C_{water} and $C_{particulate}$ by site category.
B: Relationship between C_{water} and *EF* by site category.
Solid line, ordinary least squares linear regression of logged data from lentic aquatic systems.
Dashed line, ordinary least squares linear regression of logged data from lotic aquatic systems.

Figure 3.8 shows the distribution of *EF* values grouped by lotic and lentic aquatic system categories. Although EPA derived the lentic and lotic *EF* values from aquatic sites with a similar range of water concentrations, the relative proportion of *EF* values collected at sites with higher water concentrations is larger for lentic sites than lotic sites. In particular, 6 of the 39 lentic *EF* values were from ponds in the Kesterson National Wildlife Refuge where C_{water} ranged from 38.6-196 µg/L (Saiki and Lowe 1987; Schuler et al. 1990). Despite the influence of selenium water concentration on *EF*, the median of *EF* values from lentic and lotic aquatic systems are significantly different from each other (Mann-Whitney U, P = 0.002). EPA concludes from these analyses that lentic and lotic aquatic system categories are appropriate categories for differentiating Se bioaccumulation. A listing of all aquatic-sites from which *EF*s were calculated is provided in Appendix H.

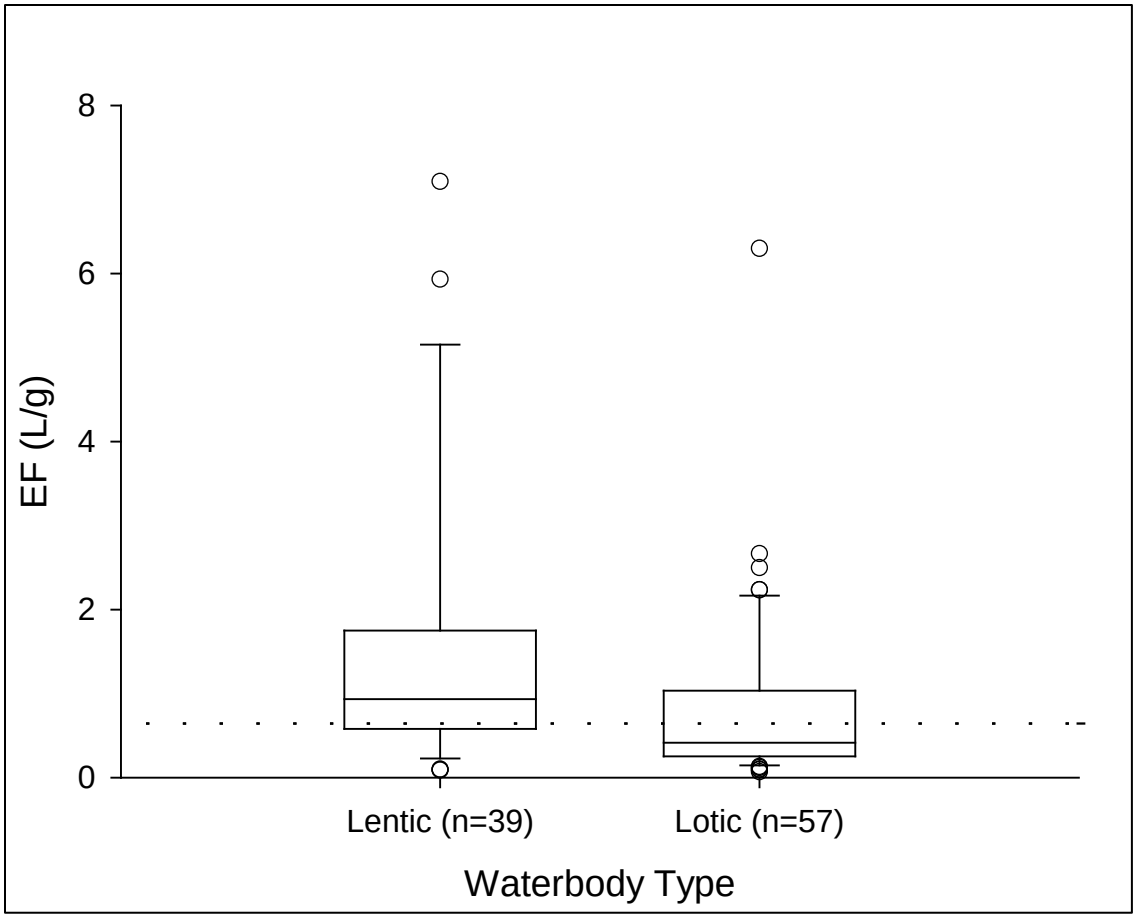


Figure 3.8. Distribution of *EF* Values for the Same 96 Aquatic Systems.
(As shown in **Figure 3.6** and **Figure 3.7** grouped by lentic and lotic aquatic system categories). Boxes show the 25th centile, median, and 75th centile *EF* values; whiskers show the 10th and 90th centiles. Circles represent *EF* values greater than 1.5 times the interquartile (25th-50th – lower circles; 50th-75th –upper circles) range. Dashed line represents the median *EF* of all 96 sites (0.63 L/g). The *EF* value of 12.48 L/g from Badin Lake (Lemly 1985) is off scale.

3.2.5 Deriving Protective Water Column Concentrations for Lentic and Lotic System Categories

To derive the water column element of the selenium criterion, EPA translated the egg-ovary criterion element to a distribution of water column concentration values for lentic and lotic aquatic systems. EPA uses the *EF* values calculated for 96 aquatic sites, available information about the fish species present at those sites, and food web models of those fish species. Because translation of the egg-ovary criterion element is site- and species-specific, several studies identifying more than one species of fish could potentially provide more than one translated water column concentration (one translated water value for each species). EPA considered using

all water column values for all species present to generate distributions of translated water column values from lentic and lotic aquatic sites. However, the number of reported fish species at aquatic sites with an *EF* value varied from one to six fish species. Furthermore, the studies providing data for 31 of the 96 sites with *EF* values do not provide information on the species of fish that may have been present at the aquatic site. Because the number of fish species at an aquatic site was not consistently reported, and because the number of reported fish species does not necessarily indicate the number of species present at a site, EPA calculated one translated egg-ovary criterion element to water column value for each aquatic site with both an *EF* value and at least one reported fish species. When more than one species was reported at a site, the EPA used the lowest translated water value for that site. Using this methodology, EPA translated the egg-ovary FCV into water column concentrations at 26 lentic and 39 lotic aquatic sites. EPA used these distributions of water concentration values translated from the egg-ovary criterion element to derive chronic water column criterion element values for lentic and lotic aquatic systems. **Table 3.13** shows the model parameter values used to translate the egg-ovary criterion element to site-specific water concentrations, and **Figure 3.9** shows the distribution of the translated values.

Table 3.13. Data for the 65 Site Minimum Translations of the Egg-Ovary Criterion Concentration Element to a Water Column Concentration.

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Birkner 1978	East Allen Reservoir, Medicine Bow WY	Iowa darter	Lentic	2.31	1.45	2.87	1.57
Birkner 1978	Galett Lake, Laramie WY	Iowa darter	Lentic	0.88	1.45	2.87	4.15
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	northern plains killifish	Lentic	1.70	1.20	2.44	3.04
Birkner 1978	Meeboer Lake, Laramie WY	northern plains killifish	Lentic	0.58	1.20	2.44	8.96
Birkner 1978	Miller's Lake, Wellington CO	Iowa darter	Lentic	2.37	1.45	2.87	1.53
Birkner 1978	Sweitzer Lake, Delta CO	fathead minnow	Lentic	0.87	1.40	2.78	4.45
Birkner 1978	Twin Buttes Reservoir, Laramie WY	Iowa darter	Lentic	1.21	1.45	2.87	3.01
Bowie et al. 1996	Hyco Reservoir	bluegill	Lentic	2.35	2.13	2.00	1.51
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	brown trout	Lentic	1.26	1.45	2.78	2.98
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	fathead minnow	Lentic	2.00	1.40	2.78	1.94
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	smallmouth bass	Lentic	5.15	1.42	1.93	1.07
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	Lentic	0.90	1.40	2.78	4.29
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	Lentic	0.86	1.40	2.78	4.49
Lemly 1985	Badin Lake	red shiner	Lentic	12.48	1.95	2.27	0.27
Lemly 1985	Belews Lake	red shiner	Lentic	1.75	1.95	2.27	1.94
Lemly 1985	High Rock Lake	red shiner	Lentic	4.99	1.95	2.27	0.68
Muscatello and Janz 2009	Vulture Lake	northern pike	Lentic	1.01	2.39	4.02	1.56
Orr et al. 2012	Clode Pond 11	cutthroat trout	Lentic	0.71	1.96	2.29	4.70
Orr et al. 2012	Elk Lakes 14	cutthroat trout	Lentic	1.64	1.96	2.29	2.05
Orr et al. 2012	Fording River Oxbow 10	cutthroat trout	Lentic	1.34	1.96	2.29	2.50
Orr et al. 2012	Henretta Lake 27	cutthroat trout	Lentic	0.50	1.96	2.29	6.72
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	Lentic	0.51	1.20	2.37	10.52
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	Lentic	0.32	1.20	2.37	16.83
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	Lentic	0.60	1.20	2.37	8.84
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	Lentic	0.93	1.20	2.37	5.69

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Stephens et al. 1988	Marsh 4720	common carp	Lentic	0.10	1.92	1.58	52.02
Butler et al. 1991	Uncompahgre River at Colona	rainbow trout	Lotic	0.63	2.44	2.33	4.21
Butler et al. 1993	Spring Cr. at La Boca	brown trout	Lotic	0.18	1.45	2.78	20.97
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	Lotic	0.15	1.40	2.78	26.04
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	fathead minnow	Lotic	0.90	1.40	2.78	4.32
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	fathead minnow	Lotic	0.37	1.40	2.78	10.57
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	red shiner	Lotic	0.12	1.95	2.27	28.34
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	Lotic	0.10	1.95	2.27	35.60
Butler et al. 1995	Navajo Wash near Towaoc	speckled dace	Lotic	0.20	1.95	1.36	29.07
Butler et al. 1995	San Juan River at Four Comers	red shiner	Lotic	0.26	1.95	2.27	12.97
Butler et al. 1995	San Juan River at Mexican Hat Utah	common carp	Lotic	0.29	1.92	1.58	17.24
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	Lotic	0.40	1.40	2.78	9.60
Butler et al. 1997	Cahone Canyon at Highway 666	green sunfish	Lotic	0.20	1.45	2.29	23.22
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	Lotic	0.07	1.40	2.78	55.27
Casey 2005	Deerlick Creek	rainbow trout	Lotic	2.24	2.44	2.33	1.18
Casey 2005	Luscar Creek	rainbow trout	Lotic	0.33	2.44	2.33	8.14
Formation 2012	Crow Creek - 1A	brown trout	Lotic	0.80	1.45	2.96	4.42
Formation 2012	Crow Creek - 3A	brown trout	Lotic	0.81	1.45	2.97	4.37
Formation 2012	Crow Creek - CC150	brown trout	Lotic	1.04	1.45	2.91	3.44
Formation 2012	Crow Creek - CC350	brown trout	Lotic	1.16	1.45	2.97	3.02
Formation 2012	Crow Creek - CC75	brown trout	Lotic	1.19	1.45	2.87	3.07
Formation 2012	Deer Creek	brown trout	Lotic	1.55	1.45	3.00	2.25
Formation 2012	Hoopes Spring - HS	brown trout	Lotic	0.24	1.45	3.86	11.06
Formation 2012	Hoopes Spring - HS3	brown trout	Lotic	0.54	1.45	2.63	7.40
Formation 2012	Sage Creek - LSV2C	brown trout	Lotic	0.45	1.45	3.01	7.76
Formation 2012	Sage Creek - LSV4	brown trout	Lotic	0.69	1.45	2.88	5.22
Formation 2012	South Fork Tincup Cr.	brown trout	Lotic	1.32	1.45	3.05	2.58
Hamilton and Buhl 2004	Lower East Mill Creek	cutthroat trout	Lotic	1.32	1.96	2.29	2.55
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	mountain whitefish	Lotic	6.30	7.39	2.97	0.11
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	cutthroat trout	Lotic	0.23	1.96	2.29	14.91
Orr et al. 2012	Elk River 1	cutthroat trout	Lotic	0.55	1.96	2.29	6.14

Identification				Model Parameters			Translation
Reference	**Site**	**Species**	**Type**	***EF*[a]**	***CF*[b]**	***TTF*[composite-c]**	**C_{water}[d]**
Orr et al. 2012	Elk River 12	cutthroat trout	Lotic	2.67	1.96	2.29	1.26
Orr et al. 2012	Fording River 23	cutthroat trout	Lotic	0.21	1.96	2.29	16.20
Orr et al. 2012	Michel Creek 2	cutthroat trout	Lotic	0.28	1.96	2.29	11.85
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	Lotic	0.36	1.20	2.37	14.81
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	Lotic	1.03	1.20	2.37	5.17
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	Lotic	1.37	2.13	1.47	3.53
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	Lotic	0.43	2.13	1.47	11.29
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	Lotic	0.36	2.13	1.47	13.50
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	Lotic	0.75	2.13	1.47	6.46

a - Geometric mean of the median enrichments functions (*EF*) for all available food types (algae, detritus, and sediment). *EF* (L/g) = C_{food}/C_{water}.
b - Taxa-specific conversion whole-body to egg ovary conversion factor (*CF*; dimensionless ratio).
c - Composite trophic transfer factor ($TTF^{composite}$). Product of *TTF* values for all trophic levels.
d - Translated water concentration corresponding to an egg-ovary criterion element of 15.1 mg Se/kg dw, calculated by Equation 18.

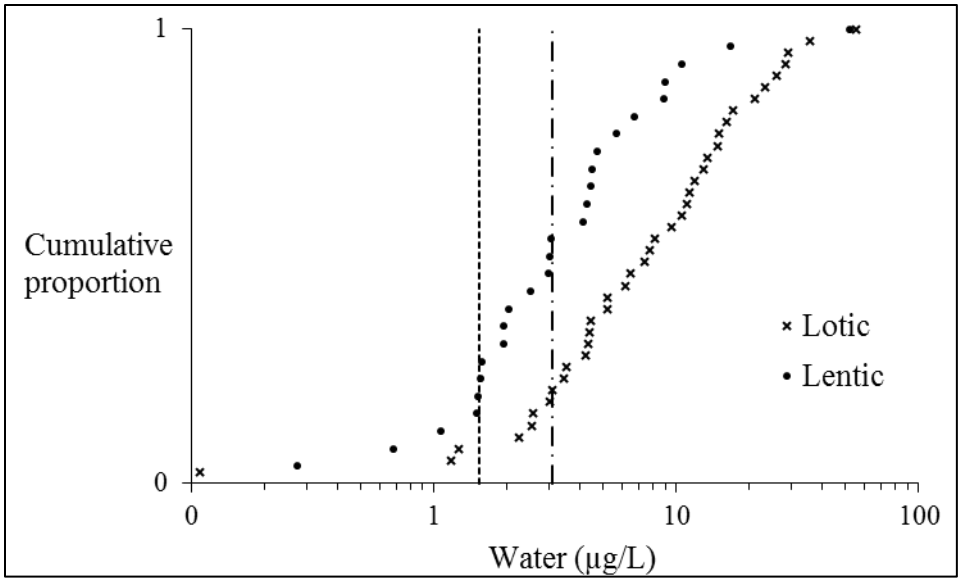


Figure 3.9. Probability Distribution of the Water Column Concentrations Translated from the Egg-Ovary Criterion Element at 26 Lentic and 39 Lotic Aquatic Sites.
Dashed and dash-dot lines show the 20th percentiles of the lentic and lotic distributions, respectively.

EPA selected the 20th percentile from the distribution of translated water column values of each category as the final national water column criterion element concentrations (3.1 µg/L for lotic waters and 1.5 µg/L for lentic waters) because the 20th percentile is consistent with past practice as it provides a high probability of protection for most aquatic systems in both lentic and lotic categories. **Table 3.14** provides the 20th percentile of the water concentration values translated from the egg-ovary criterion element value.

Table 3.14. Water Column Criterion Element Concentration Values Translated from the Egg-Ovary Criterion Element.

	Lentic	Lotic
20th percentile (final EPA-recommended water column criterion element)	**1.5 µg/L**	**3.1 µg/L**

As discussed in **Section 2.2.2**, selenium bioaccumulation potential depends on several biogeochemical factors that characterize a particular aquatic system. Uncertainty in the translation of the egg-ovary criterion element to the water column element can be reduced by deriving a site-specific criterion that uses site-specific selenium data and information on food-web dynamics from a biological assessment of the aquatic system. The general considerations are provided in Appendix K. The derivation of water column criterion element values described

above is constrained by the need to apply a national criterion value to a large number of aquatic systems for lentic and lotic systems.

3.2.6 Derivation of Averaging Period for Chronic Water Criterion Element

In the context of selenium bioaccumulation in a single trophic level, k would be the first-order depuration coefficient, and 1/k would equal the time needed to depurate to a concentration of 1/e times the initial concentration (where e=2.718). For depuration of two trophic levels sequentially, invertebrates and fish, the characteristic time is likewise the time needed for c/c_o to reach a value of 1/e. This differs from typical criteria averaging periods based on U.S. EPA (1995), where the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action due to direct waterborne toxicity of metals (i.e., where characteristic time = 1/k, where k is the first-order kinetic coefficient in a toxicokinetic model fitted to the relationship between LC_{50} and exposure duration). For the first trophic level, the kinetics for algal bioaccumulation and depuration were assumed to be rapid compared to the kinetics for larger organisms at higher trophic levels; that is, the characteristic time for algae was assumed to be negligible.

For the second trophic level, invertebrates, values for k_{TL2} are tabulated elsewhere in the document. A value of 0.1/day appears to be environmentally conservative, considerably higher than those for *Lumbriculus*, Asian clam, and zebra mussel, but slightly lower than copepods, which are very small in size. This corresponds to a characteristic time of 10 days.

For fish, the median depuration coefficient measured by Bertram and Brooks (1986) for 6-9 month-old (early adult) fathead minnows is applied, providing a k_{TL3} value of 0.02/day. This corresponds to a characteristic time of 50 days. Because of the small size of adults of this species, this represents faster kinetics than would likely be applicable to the salmonids and centrarchids of greatest concern for selenium toxicity, consonant with the Newman and Mitz (1988) inverse relationship between depuration rate and organism size. The striped bass k value of Baines et al. (2002) is inapplicable here because it was measured in the early juvenile life stage, a size that is too small to be relevant to reproductive impairment stemming from exposure of adult females.

As shown in Appendix J, the characteristic time for the combined second and third trophic levels (invertebrates and fish) is the approximate sum of the above two characteristic times, or 60 days. The analysis of the protectiveness of a 30-day averaging period, shorter than

the characteristic time, was performed and is shown in Appendix J. That analysis demonstrated that a 30-day averaging period for the chronic water criterion element affords protection under all conditions, and is therefore the duration recommended for the chronic water column criterion element.

3.3 Intermittent-Exposure Water Criterion Element: Derivation from the Chronic Water Criterion Element

Chapman et al. (2009) noted that selenium acute toxicity has been reported rarely in the aquatic environment and that traditional methods for predicting effects based on direct exposure to dissolved concentrations do not work well for selenium. As demonstrated in Appendix J, the kinetics of selenium accumulation and depuration are sufficiently slow that attainment of the water criterion element concentration by ambient 30-day averages will protect sensitive aquatic life species even where concentrations exhibit a high degree of variability.

To address situations where pulsed exposures of selenium could result in bioaccumulation in the ecosystem and potential chronic effects in fish, EPA is providing an intermittent exposure water criterion element concentration intended to limit cumulative exposure to selenium, derived from the chronic 30-day water criterion element magnitude and from its duration, which was obtained from the kinetic analysis of Appendix J. That is, the intermittent criterion element is based on the same kinetic analysis used to derive the water chronic averaging period (30 days).

To illustrate the concept of the intermittent criterion element and its dependence on the 30-day criterion element magnitude and duration, **Figure 3.10** shows a possible sequence of exposures over a 30-day period.

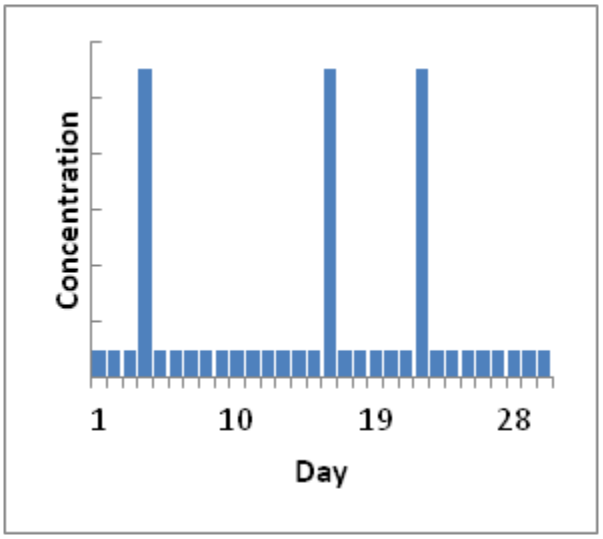


Figure 3.10. Illustration of Intermittent Spike Exposure Occurring for a Certain Percentage of Time (e.g., 10%) Over a 30-Day Period, and Background Exposure Occurring for the Remaining Percentage of Time (e.g., 90%).

The 30-day average concentration, $C_{30\,day}$, is given by Equation 20:

$$C_{30-day} = C_{int} f_{int} + C_{bkgrnd}(1 - f_{int}) \qquad \text{(Equation 20)}$$

Where:

C_{int} = the intermittent spike concentration (µg/L)

f_{int} = the fraction of any 30-day period during which elevated selenium concentrations occur

C_{bkgrnd} = the average daily background concentration occurring during the remaining time, integrated over 30 days.

$C_{30\text{-}day}$ is not to exceed the chronic criterion element, $WQC_{30\text{-}day}$. If the intent is to apply a criterion element, WQC_{int}, to the intermittent spike concentrations, then replacing C_{int} with WQC_{int} and $C_{30\text{-}day}$ with $WQC_{30\text{-}day}$ in the above equation, and then solving for WQC_{int} yields Equation 21:

$$WQC_{int} = \frac{WQC_{30\,day} - C_{bkgrnd}(1-f_{int})}{f_{int}} \qquad \text{(Equation 21)}$$

The equation expresses the intermittent exposure water criterion element in terms of the 30-day average chronic water criterion element, for a lentic or lotic system, as appropriate, while accounting for the fraction in days of any 30-day period the intermittent spikes occur and for the concentration background occurring during the remaining time. The reasonable worst-case assumption inherent in this approach is that selenium bioaccumulation is linear over a very wide range of concentrations, that is, *EF* and *TTF* values do not decrease significantly as concentrations increase.

If the heights of three spikes in **Figure 3.10** were to differ somewhat among each other, the intermittent element would apply to the arithmetic mean of the three. If the background concentrations were to vary somewhat, then the arithmetic mean background would be entered into the equation. Where concentrations vary smoothly over time, it does not matter where the line is drawn defining elevated versus background concentrations. The intermittent element will yield the same level of protection as the 30-day average element, provided that the equation uses (a) the average of the concentrations occurring for the fraction of time defined as being intermittently elevated, and (b) the average of the concentrations occurring for the remaining time, defined as being background. The intermittent element will only be exceeded under conditions that would have caused the 30-day element to be exceeded, had it been applied.

Table 3.15 illustrates example values for the intermittent element. The bottom row of the lotic and lentic values and the right column are to emphasize that WQC_{int} is not an independent element but a re-expression of the 30-day average water criterion element concentration. WQC_{int} converges to $WQC_{30\text{-}day}$ when the background concentration is already at $WQC_{30\text{-}day}$ or when the intermittent exposure is said to occur throughout the 30-day period.

Table 3.15. Representative Values of the Intermittent Water Criterion Element Concentration.

Bkgrnd Conc, C_{bkgrnd} (µg/L)		Fraction of Time, f_{int} in a 30-day period				
	0.03333 (1 day)	0.05 (1.5 days)	0.1 (3 days)	0.2 (6 days)	0.5 (15 days)	1 (30 days)
	Lotic Intermittent Criterion Element, WQC_{int} (µg/L)					
0	93	62	31	15.5	6.2	3.1
1	64	43	22	11.5	5.2	3.1
2	35	24	13	7.5	4.2	3.1
2.5	20.5	14.5	8.5	5.5	3.7	3.1
3.1	3.1	3.1	3.1	3.1	3.1	3.1
	Lentic Intermittent Criterion Element, WQC_{int} (µg/L)					
0	45	30	15	7.5	3	1.5
0.5	30.5	20.5	10.5	5.5	2.5	1.5
1	16	11	6	3.5	2	1.5
1.25	8.8	6.3	3.8	2.5	1.8	1.5
1.5	1.5	1.5	1.5	1.5	1.5	1.5

If the value of f_{int}, the intermittent exposure fraction of the month, is assigned a value less than one day, the intermittent criterion element value could exceed water concentrations that have been shown to be acutely toxic to sensitive species in 2- or 4-day toxicity tests (compiled in U.S. EPA 2004). Because the concentrations that would be acutely toxic in exposures of less than one day might not be much greater than those observed to be toxic in 2-4 day exposures, the intermittent fraction of the month must *not* be assigned a value less than 0.033, corresponding to one day.

4 NATIONAL CRITERION FOR SELENIUM IN FRESH WATERS

The available data indicate that freshwater aquatic life would be protected from the toxic effects of selenium by applying the following four-part criterion, recognizing that fish tissue elements supersede the water elements (except in special situations, see footnotes 3 and 4, Table 4.1) and that the egg-ovary tissue element supersedes all other tissue elements:

1. The concentration of selenium in the eggs or ovaries of fish does not exceed 15.1 mg/kg, dry weight; [1]
2. The concentration of selenium (a) in whole-body of fish does not exceed 8.5 mg/kg dry weight, or (b) in muscle tissue of fish (skinless, boneless fillet) does not exceed 11.3 mg/kg dry weight; [2]
3. The 30-day average concentration of selenium in water does not exceed 3.1 µg/L in lotic (flowing) waters and 1.5 µg/L in lentic (standing) waters more than once in three years on average;
4. The intermittent concentration of selenium in either a lentic or lotic water, as appropriate, does not exceed $WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1-f_{int})}{f_{int}}$ more than once in three years on average.[3]

Table 4.1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	**Fish Tissue**[1]		**Water Column**[4]	
Criterion Element	**Egg/Ovary** [2]	**Fish Whole Body or Muscle** [3]	**Monthly Average Exposure**	**Intermittent Exposure**[5]
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with fint assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

EPA recommends that states and tribes adopt into their water quality standards a selenium criterion that includes all four elements, and express the four elements as a single criterion composed of multiple parts, in a manner that explicitly affirms that the whole-body or muscle elements supersede the water column element, and the egg-ovary element supersedes any other element. The magnitude of the fish egg-ovary element is derived from analysis of the

available toxicity data. The magnitudes of the fish whole-body element and fish muscle elements are derived from the egg-ovary element coupled with data on concentration ratios among tissues. The magnitudes of the water column elements are derived from the egg-ovary element coupled with bioaccumulation considerations. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements into the selenium criterion ensures protection when neither fish egg-ovary nor fish whole-body nor muscle tissue measurements are available. To ensure that the contribution of short-term exposures to the bioaccumulation risks is accounted for in all situations, EPA is also recommending that the intermittent exposure element be included in the selenium criterion, as noted above. EPA is not recommending a separate acute criterion derived from the results of toxicity tests having water-only exposure because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. Application of the intermittent exposure criterion element values to single day, high exposure events will provide protection from the most important selenium toxicity effect, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high exposure events. It is unnecessary to have an additional acute water column criterion element because the intermittent exposure criterion element will be more stringent than an acute criterion element. Further, as noted in this document, there have been few if any acute exposure, water column-only selenium aquatic toxicity events documented in the literature.

In implementing the water quality criterion for selenium under the NPDES permits program, states may need to establish additional procedures due to the unique components of the selenium criterion. Where states use the selenium water column concentration criterion element values only (as opposed to using both the water column and fish tissue elements) for conducting reasonable potential (RP) determinations and establishing water quality-based effluent limitations (WQBELS) per 40 CFR 122.44(d), existing implementation procedures used for other acute and chronic aquatic life protection criteria may be appropriate. However, if states also decide to use the selenium fish tissue criterion element values for NPDES permitting purposes, additional state WQS implementation procedures (IPs) will be needed to determine the need for and development of WQBELs necessary to ensure that the fish tissue criterion element(s) are met.

EPA recommends that states use the default monthly average exposure water column elements of the criterion, adopted as part of the state's water quality criterion. Alternatively, states may want to develop and adopt, and submit for EPA approval, either a site-specific water column criterion (see Appendix K for details), or a procedure to facilitate the translation of a fish tissue criterion element concentration into site-specific water concentration values. A site-specific water column criterion element or set of lentic/lotic criterion element values may be developed using a mechanistic modeling approach (Presser and Luoma 2010) or using the empirical bioaccumulation factor approach, both described in Appendix K, for the specific waterbody or waterbodies, or a on a state-wide basis. A translation procedure must be scientifically defensible and able to produce repeatable and predictable outcomes, and must be consistent with either the mechanistic modeling approach or the empirical bioaccumulation factor approach described in Appendix K. The chronic selenium criterion is derived to be protective of the entire aquatic community, including fish, amphibians, and invertebrates. Fish are the most sensitive to selenium effects. Selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) sample data override the criterion elements based on water column selenium data due to the fact, noted above, that fish tissue concentrations provide the most robust and direct information on potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) in “fishless” waters, and 2) in areas with new selenium inputs.

Fishless waters are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (i.e., extirpation) due to temporary or permanent changes in water quality (e.g., due to selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas.

New inputs are defined as new activities resulting in selenium being released into a lentic or lotic waterbody. New inputs will likely result in increased selenium in the food web, likely resulting in increased bioaccumulation of selenium in fish over a period of time until the new or

increased selenium release achieves a quasi-"steady state" balance within the food web. EPA estimates that concentrations of selenium fish tissue will not represent a "steady state" for several months in lotic systems, and longer time periods (e.g., two to three years) in lentic systems, depending upon the hydrodynamics of a given system such as the location of the selenium input related to the shape and internal circulation of the waterbody, particularly in reservoirs with multiple riverine inputs, hydraulic residence time, and the particular food web. Estimates of steady state under new or increased selenium input situations are expected to be site dependent, so local information should be used to better refine these estimates for a particular waterbody. Thus, EPA recommends that fish tissue concentration not override water column concentration in these situations until these periods of time have passed in lotic and lentic systems, respectively, or steady state conditions can be estimated.

4.1 Protection of Downstream Waters

EPA regulations at 40 CFR 131.10(b) provide that "[i]n designating uses of a waterbody and the appropriate criteria for those uses, the state shall take into consideration the water quality standards of downstream waters and ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." Especially in cases where downstream waters are lentic waterbody types (e.g., lakes, impoundments), or harbor more sensitive species, a selenium criterion more stringent than that required to protect in-stream uses may be necessary to ensure that water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

5 SITE-SPECIFIC CRITERIA

All four elements of the freshwater selenium criterion may be modified to reflect site-specific conditions where the scientific evidence indicates that different values will be protective of aquatic life and provide for the attainment of designated uses.

Since the fish egg-ovary criterion element is based on toxicity data, a state may modify that element by applying the Recalculation Procedure (U.S. EPA 2013b) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while including tested surrogates for untested resident species.

It is important to note that species in the national data set that are not present at a site should not be deleted from the data set because those species serve as surrogate(s) for other species known or expected to be present at a site. Confidence in the applied tissue criterion element can be improved by further testing of fish species resident at the site. The most relevant testing would measure the survival and occurrence of deformities in offspring of wild-caught female fish to determine an EC_{10} for selenium in the eggs or ovaries (e.g., following Janz and Muscatello 2008).

Using either the EPA national recommended egg-ovary, whole-body, or muscle criterion concentration element or a site-specific egg-ovary, whole-body, or muscle criterion element, translation of the fish tissue criterion to a protective water concentration can be performed in a manner that accounts for site-specific conditions. Appendix K provides a step-wise process for deriving each parameter used in Equation 18 to perform a site-specific translation. These steps include:

1. selecting a target fish species for the waterbody,
2. determining the primary food source for the target species
3. determining the appropriate *TTF* values,
4. determining the appropriate *EF* value, and
5. determining the appropriate *CF* value.

Appendix K also provides information on the data necessary to derive a site-specific criterion, as well as scientifically defensible methods, including the use of traditional Bioaccumulation Factors (BAFs), in addition to the more comprehensive mechanistic modeling.

6 EFFECTS CHARACTERIZATION

6.1 FISH AND AMPHIBIANS

6.1.1 Principles for Using Studies for which EC_{10}s Cannot Be Calculated

When the data from an acceptable chronic test met the conditions for logistic regression analysis, the EC_{10} was used. When data did not allow calculation of ECs but did allow calculation of closely spaced NOECs and LOECs, the NOEC was used to approximate the EC_{10}. No NOEC values were used in calculating the tissue criterion element values.

When significant effects were observed at all treatment concentrations, such that no treatment concentration was classified as a NOEC, then the chronic value was assigned as "less than" (<) the lowest tested concentration. When no significant effects were observed at any concentration, such that no treatment concentration was defined as an LOEC, then the chronic value was assigned as "greater than" (>) the highest tested concentration.

A number of the chronic values in **Sections 3.1.1** and **6.1.2** (reproductive effects) and in **Section 6.1.9** (nonreproductive effects) include a "greater than" (>) or "less than" (<) sign because of an inability to resolve an exact value when all exposure concentrations of a study yielded either too little or too much effect to provide a point estimate of a chronic value. The decision to use chronic values with a "greater than" or "less than" sign in calculating an SMCV followed a rule based on whether these values add relevant information to the mean, as described below. None of these values were used in this assessment to derive the tissue criterion element values.

6.1.1.1 Evaluation Approach

- Neither a low "greater than" value nor a high "less than" value were used to calculate the SMCV;
- Both a low "less than" value and a high "greater than" value were included in the SMCV calculation. However, none of these values were used in this assessment to calculate the numeric criterion values for fish tissue.

For example, a chronic value reported here as ">15 mg Se/kg" is ignored if the tentative SMCV is 20 mg Se/kg. The ">15 mg Se/kg" value indicates that no significant effects were

observed at the study's highest tested concentration of 15 mg Se/kg. As this is consistent with what would be expected if the SMCV were 20 mg Se/kg, it provides no information to support modifying the SMCV. However, a different study showing no effects at its highest tested concentration and yielding the value ">25 mg Se/kg" is not consistent with an SMCV of 20 mg Se/kg, and indicates that the ">25 mg Se/kg" value provides information for modifying the mean upwards. Conversely, a chronic value reported here as "<15 mg Se/kg" indicates that significant effects were observed even at the study's lowest tested concentration of 15 mg Se/kg. As this is not consistent with a 20 mg Se/kg SMCV, it indicates the utility of the "<15 mg Se/kg" information for modifying the SMCV downwards. On the other hand, a value reported here as "<25 mg Se/kg" would not be used to recalculate a 20 mg Se/kg SMCV. The intent of the approach is to use all quality information that is relevant and appropriate for calculating the SMCVs.

6.1.2 Acceptable Studies of Fish Reproductive Effects of Genera that were not among the Four Most Sensitive Genera

The following is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the genera that were not the four most sensitive but were included in the number of GMCVs in the dataset (see **Section 3.1.3**). The studies in this section involve effects on the offspring of exposed female fish. Data are summarized in **Table 3.1**. Details of these studies are contained in Appendix C.

6.1.2.1 Cyprinidae

Pimephales promelas (fathead minnow)

Schultz and Hermanutz (1990) examined the effects of selenium transferred from parental fish (females) on fathead minnow larvae. The parental fathead minnows were first exposed to selenite (10 µg/L) that was added directly to the water in artificial streams in a mesocosm study. The selenite entered the food web and contributed to exposure via diet. Spawning platforms were submerged into treated and control streams. Embryos were collected from the spawning platforms and transferred to a proportional diluter where they were reared in incubation cups for observation. Treated embryos in the egg cups were exposed to 10 µg/L selenium. Edema and lordosis were observed in approximately 25 percent of the larvae spawned and reared in natural water spiked with 10 µg Se/L and in ≤ 6 percent of control larvae. Although

a case can be made that the selenium treatment had a higher rate of edema and lordosis, there are some issues that add uncertainty to the estimation of an effect concentration (R. Erickson, personal communication). Heavy mortality/loss of embryo/larvae during monitoring and the erratic occurrence of the abnormalities (e.g., significant incidence of edema in only 3 of 10 replicates for the Se treatment) led to the conclusion that results should not be used for criterion derivation. The data from this study support the range of reproductive effect levels determined in other fish studies. The Se concentration in embryos from the 10 µg/L treatment stream of 3.91 mg/kg ww converts to 25.6 mg/kg dw using 15.3% dw (N=3 range 14.7 – 15.6%) for fathead minnow eggs (R. Erickson, personal communication).

Two other studies suggest fathead minnows are less sensitive to selenium than other fish. Young et al. (2010) observed that fathead minnow populations remained after selenium contamination of Belews Lake had eliminated most other fish species, including bluegill and largemouth bass. In a maternal transfer laboratory study with fathead minnows, GEI (2008) estimated EC_{10}s for larval survival and deformities that ranged from 35 – 65 mg Se/kg dw expressed as maternal whole body, as noted in Appendix E, Figures E-2 and E-3.

6.1.2.2 Esocidae

Esox lucius (northern pike)

Muscatello et al. (2006) collected spawning northern pike from four sites near a uranium milling operation in north-central Saskatoon, Canada, with egg concentrations ranging from 2.7 to 48 mg Se/kg dw. The four sites included a reference site and three sites 2, 10 and 15 km downstream of the effluent discharge, representing a gradient of selenium exposure. Milt and ova were stripped from gravid fish. Eggs were then fertilized in the field and incubated in the laboratory for observations and measurements. The test was terminated when the majority of the fry exhibited swim-up and had absorbed the yolk.

Mean egg diameter, fertilization success and cumulative embryo mortality were not significantly different among the sites. Significant increases in percent total deformities including edema, skeletal deformities, craniofacial deformities and fin deformities were observed in fry originating from pike collected at the medium exposure site. The concentrations of selenium in the northern pike eggs collected at the reference and low exposure site were very similar, as were the percent total deformities in embryos/fry. The geometric mean of selenium in

the eggs of the adult females at the reference and low exposure sites was 3.462 mg Se/kg dw and the corresponding arithmetic mean of the percent total deformities was 13.20%. There were only 4 adult females from exposed sites, and all had relatively similar concentrations in their eggs, all close to the geometric mean concentration of 34.00 mg Se/kg dw. Likewise, all four exposed females had relatively similar percent total deformities, not far from their arithmetic mean of 33.40%. This is not a sufficient level of effect for applying TRAP to determine an EC_{10}. Furthermore, the relatively large spread between the two clusters of exposure concentrations (3.462 and 34.00 mg Se/kg dw) would render a NOEC and LOEC unreliable and unsuitable for defining a threshold. That is, the NOEC and LOEC would be "greater than" and "less than" values, >3.462 and <34.00 mg Se/kg dw respectively, providing little information on the sensitivity of northern pike compared to other species.

Instead, making use of the clustering of data at low exposure and effects and at elevated exposure and effects, the effect level for the elevated exposure eggs was normalized to the low exposure condition and rescaled to a 0-100% range. The rescaled (i.e., Abbott-adjusted) percent of total deformities for the elevated exposure eggs was 24% (relative to the low exposure eggs). Thus the concentration of selenium in the elevated exposure eggs (34 mg Se/kg dw) was equivalent to an EC_{24}, and is *the only effects concentration that can be calculated for this test*, given the limitations in the range of concentrations tested and effects observed. Although the EC_{24} is not directly translatable to an EC_{10} for use in determining the criterion, it is useful for comparison with the EC_{24} in other species in order to determine species sensitivity rank. The EC_{24} for skeletal deformities from the Holm et al. (2005) study of rainbow trout, calculated to be 30.9 mg Se/kg dw in eggs, is slightly lower than the northern pike value, indicating these two species may be similar in tolerance, with the northern pike being slightly more tolerant (see Appendix C for more details.)

6.1.2.3 Salmonidae

Seven publications provide quantitative data on the effects of selenium on salmonid embryo/larval survival and deformity that were used in calculating criterion values. All involve wild-caught adults taken from selenium contaminated streams and spawned for effects determination. Exposure for all studies was therefore through the parents. Data are available for rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*), Dolly Varden

(*Salvelinus malma*) and brown trout (*Salmo trutta*). The studies with *Salvelinus* are discussed below; *Oncorhynchus* and *Salmo* were previously discussed in **Section 3.1.2**.

Salvelinus fontinalis (brook trout)

These data were not used directly in the criterion calculations. See **Section 6.1.5** for discussion of the available data.

Salvelinus malma (Dolly Varden)

Golder (2009) collected adult Dolly Varden from a reference site and two sites downstream from the Kemess Mine in northern British Columbia, one with a high and one with a moderate selenium exposure in the fall of 2008. Fertilized eggs were taken to the laboratory where they were monitored for survival and deformities until 90% of the larvae reached swim-up, approximately five months after fertilization. Alevin mortality was <1% in the treatments collected from the exposed sites and not considered an effect. The prevalence of deformities increased sharply after the selenium egg concentration exceeded 50 mg/kg dw (Appendix C). The proportion of Dolly Varden larvae without any type of deformity (skeletal, craniofacial, and finfold as well as edema), as a function of the log of the selenium concentration in the eggs using TRAP, produced an EC_{10} value of 56.22 mg Se/kg dw and an EC_{20} value of 60.12 mg Se/kg dw.

6.1.2.4 Salmonidae SMCV and GMCV Summary

As given in **Section 3.1.2**, the SMCV for cutthroat trout, *Oncorhynchus clarkii*, is 26.2 mg Se/kg dw in eggs derived from Rudolph et al. (2008), and Nautilus Environmental (2011), (24.7, and 27.7 mg Se/kg dw respectively). The GMCV for the genus *Oncorhynchus* is 25.3 mg Se/kg dw in eggs, derived from the 24.5 mg Se/kg dw EC_{10} from the combined Holm (2002) and Holm et al. (2005) rainbow trout data, the above mean of the Rudolph et al. (2008) and Nautilus Environmental (2011) Westslope cutthroat trout studies. The GMCV for the genus *Salvelinus* is the EC_{10} value of 56.22 mg Se/kg dw for Dolly Varden (*S. malma*) from the Golder (2009) study.

6.1.2.5 Poeciliidae

Data are available for two species in this family. These studies are not represented in **Table 3.1** because they are live-bearing rather than egg-laying, but the relative tolerance of these species is accounted for in derivation of the criterion.

Gambusia holbrooki (eastern mosquitofish)

Staub et al. (2004) collected male and gravid female eastern mosquitofish from a contaminated ash basin and a reference pond in July 1999. Male fish were used for measuring standard metabolic rate and the reproductive endpoints. Brood size and percent viability of live offspring at parturition were measured using the live-bearing females. Standard metabolic rates of males, brood size of females, and offspring viability were not significantly different between sites. Average concentrations of selenium in females were 11.85 and 0.61 mg/kg dw in the contaminated ash basin and reference sites, respectively. The chronic value in whole body tissue is >11.85 mg Se/kg dw whole-body (Appendix C). In a community of equally exposed fish taxa (fish taxa having whole body tissue concentrations >11.85 mg Se/kg dw), the median egg-ovary concentration among egg-laying fish would be expected to be 1.71 higher, or >20.26 mg Se/kg dw.

Gambusia affinis (western mosquitofish)

Western mosquitofish were collected in June and July 2001 from sites in the grassland water district in Merced County, California. Mosquitofish were collected from two sites that were contaminated with selenium and from two reference sites in the same area with relatively low selenium water concentrations (Saiki et al. 2004). Seventeen to 20 gravid females (mosquitofish are live-bearers) from each site were held in the laboratory for two weeks to quantify live and dead births and to make other measurements. Live and dead fry were visually examined under low magnification with a binocular microscope for evidence of external abnormalities (teratogenic symptoms such as spinal curvature, missing or deformed fins, eyes and mouths and edema). The percentage of live births was high at both selenium-contaminated sites (96.6 to 99.9%) and reference sites (98.8 to 99.2%). There were no obvious anomalies (e.g., deformities, edema) observed during the study. The concentration of selenium in four postpartum females from the site with the highest selenium concentration ranged from 13.0 to 17.5 mg Se/kg dw (geometric mean of the high and low is 15.1 mg Se/kg dw). The chronic value in whole body tissue is >15.1 mg Se/kg dw (Appendix C). Similar to Staub et al. (2004), this value can be converted to egg-ovary concentrations that would be expected in accompanying egg-laying fish, by multiplying by the median fish egg-ovary to whole-body concentration ratio, 1.71. This yields a >25.82 mg Se/kg dw equivalent egg-ovary concentration.

Gambusia, which have been reported to be tolerant to selenium contamination, are often one of the few remaining species at sites with high levels of selenium contamination (Cherry et al. 1976; Lemly 1985a; Saiki et al. 2004; Young et al. 2010; Janz et al. 2010). The two studies discussed above support this observation with a GMCV of >13.4 mg Se/kg dw in whole body tissue, combining these "greater than" values as described in **Section 6.1.1**. It may be concluded that this genus is not among the most sensitive to selenium.

6.1.2.6 Cyprinodontidae

Cyprinodon macularius (desert pupfish)

Besser et al. (2012), using a diet of oligochaete *Lumbriculus* that had fed on selenized yeast, exposed desert pupfish to six levels of dietary and waterborne selenium. Five-week old juveniles (F_0) were exposed for 85 days, during which time survival and growth were measured. Upon reaching maturity at the end of this exposure period, the 60-day reproductive study was begun, during which F_1 eggs were collected, counted, and further tested for percent hatch, survival, growth, and deformities. The authors observed no significant differences in pupfish survival, growth, total egg production, hatch, or deformities among treatments. Although the authors noted a potential interaction between the timing of egg production and treatment, a comprehensive re-analysis of this data, described in Appendix C, indicated that the phenomenon was neither statistically nor biologically significant. It is concluded that the egg concentration, 27 mg Se/kg (dw), for the test's highest treatment was not sufficiently high to define a concentration-response curve. Although desert pupfish is thus not among the most sensitive species, the slightly reduced survival observed at 27 mg Se/kg egg dw egg suggests that the EC_{10} may be close to that concentration, as also noted by the authors.

6.1.2.7 Centrarchidae

Micropterus salmoides (largemouth bass)

A laboratory study was conducted by Carolina Power & Light (1997) in which adult largemouth bass obtained from a commercial supplier were fed an artificial diet spiked with a gradient of selenomethionine concentrations for several months. Approximately 100 eggs from each spawn were monitored for mortality and deformities up to the larval swim-up stage. The authors combined survival and deformities into a single metric (i.e., survival as normal

offspring). The average concentration of selenium in the ovaries ranged from 3.1 mg/kg dw in the control to 77.6 mg/kg dw in the high dietary treatment (53.1 mg/kg dw). A plot of the percent survival of larval largemouth bass as a function of the selenium concentration in the parental female ovary shows two groups of data; one at background survival with considerable variability (mean 90.3%, standard deviation 10.9%) and one with <10% survival, with most of the data being at 0% survival (see *Micropterus* summary in Appendix C, Figure 1). Due to inadequate partial effects, a TRAP interpolation was used to estimate an EC_{10} value. Based on a risk management decision that the LOEC cannot be any higher than the lowest concentration with 0% survival (32.9 mg/kg) and that any ECx should be below this, this establishes the higher concentration point for the interpolation (an EC_{100} of 32.9 mg/kg) and requires that the highest 4 NOECs not be considered in setting the EC_0. The lower concentration point for the interpolation is therefore set here to 24.6, the next highest NOEC with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg (and a steep slope of 16). Please see Appendix C for more detailed information.

6.1.3 Reproductive Effects in Catfish (Ictaluridae)

Some important families of fish are not represented in the effects assessment, such as the catfish family (Ictaluridae). In their compilation of egg-ovary versus whole-body ratios, Osmundson et al. (2007) found comparatively high concentrations of selenium in egg-ovary compared to whole body in black bullhead, *Ameiurus melas*, which are related to the Ictaluridae. This raises a question about the potential risks of reproductive effects in this species and possibly in related Ictaluridae. In addition to this concern about how much selenium such species may accumulate in their eggs, U.S. Fish and Wildlife Service (2005) has suggested that offspring of channel catfish (*Ictalurus punctatus*) and related species might be affected at unusually low egg concentrations. This is based on results of a study in which adult female catfish were injected with seleno-L-methionine (Doroshov et al. 1992b). Effects were found in the offspring at egg concentrations between 3.2 mg/kg (NOEC) and 6.3 mg/kg (LOEC), below levels observed in the studies summarized in **Section 3.1.2** and documented in Appendix C. These data were not included in derivation of the criterion because the injection route of exposure is not an acceptable experimental protocol for studies used in criterion derivation due to its difference from exposure routes in the environment (water column and diet).

In the absence of valid tests yielding an Ictaluridae EC_{10} or chronic value, EPA evaluated the potential vulnerability of the taxonomic group that includes catfish by examining comparative fisheries observations of Ictaluridae and Centrarchidae sharing the same selenium-contaminated water body. Crutchfield (2000) reports results of annual cove rotenone sampling performed from 1982 to 1997 in Hyco Reservoir, North Carolina. The sampling was begun after centrarchid populations in this reservoir had collapsed due to the release of ash pond selenium from a coal-fired power plant. The plant began operating a dry fly ash handling system in January 1990, thereby eliminating the aquatic discharge of selenium; the sampling continued through the recovery period.

Crutchfield (2000) reports abundance data (kg/ha) for 20 fish taxa, including four Ictaluridae and three Centrarchidae. These data were examined to determine the relationship between the Ictaluridae and the selenium-affected Centrarchidae populations. The correlation matrix between annual measured abundance of the seven taxa is shown below in **Table 6.1**. Correlation with the reciprocal of measured average concentrations of selenium in invertebrates is also shown. Because the reciprocal of the selenium concentration is used, a positive correlation means that abundance decreases as selenium concentration increases. Conversely, a negative correlation means abundance decreases as selenium concentration decreases.

Table 6.1. Correlation Matrix (Values of r) for Ictaluridae and Centrarchidae Abundance and for Selenium Food Chain Contamination for the Hyco Reservoir.
(Data Reported by Crutchfield 2000).

	Ictaluridae				**Centrarchidae**			
	Channel catfish	White catfish	Flat bullhead	*Ameiurus* spp.	Bluegill	Large-mouth bass	*Pomoxis* spp. (crappie)	1 ÷ Inverteb. Se Conc
Channel catfish	1.00	-0.36	0.18	0.68	0.08	-0.33	-0.08	-0.44
White catfish	-0.36	1.00	0.02	-0.32	-0.31	-0.24	-0.15	-0.06
Black bullhead	0.18	0.02	1.00	0.40	0.32	-0.08	0.08	-0.03
Ameiurus spp.	0.68	-0.32	0.40	1.00	0.22	-0.24	-0.05	-0.31
Bluegill	0.08	-0.31	0.32	0.22	1.00	0.78	0.76	0.80
Largemouth bass	-0.33	-0.24	-0.08	-0.24	0.78	1.00	0.78	0.92
Pomoxis spp. (crappie)	-0.08	-0.15	0.08	-0.05	0.76	0.78	1.00	0.69
1 ÷ Inverteb. Se Conc.	-0.44	-0.06	-0.03	-0.31	0.80	0.92	0.69	1.00

The centrarchid abundances are well correlated with each other and are closely related to selenium concentrations in the food chain, with fish abundance decreasing as selenium concentrations increase. Ictaluridae abundances, however, are unrelated either to the selenium-sensitive centrarchid abundances or to the selenium concentrations in the food chain.

Figure 6.1 shows abundance as both mass and numbers of individuals of channel catfish (CCF) and largemouth bass (LMB) observed by Crutchfield (2000) during the period 1982-1997. Both species are long lived. Crutchfield (2000) notes that the decline of reproductive success and abundance of Hyco's largemouth bass (and bluegill) was first documented in the mid-1970s. Because this study was initiated after the largemouth bass recreational fishery had collapsed, **Figure 6.1** does not show the largemouth bass decline, only the period of its depression and subsequent recovery.

Numbers of largemouth bass were very low at the beginning of the study period; their numbers and mass do not begin to recover until invertebrate selenium drops below 30 mg Se/kg dw. In the later portion of the study period, 1989-1997, largemouth bass numbers and mass increase 100-fold. These observations are fully consistent with the premise that the earlier observations of elevated selenium concentrations had been impairing reproduction of largemouth bass.

In contrast, the ups and downs of channel catfish numbers, mass, and size seem to vary randomly throughout the period of study. In 1984 catfish numbers reached their third highest value while their average size was at its minimum: that is, there were many young individuals. Simultaneously, largemouth bass was near its minimum for both numbers and mass. The next year (1985) catfish numbers jumped to their maximum for the study period, and mass reached near maximum. Such observations are easily explained if reproduction is taking place. But they seem inexplicable under a premise that channel catfish reproduction was even more impaired than largemouth bass reproduction, and its population merely a senescent non-reproducing remnant of the pre-contamination population. Rather the observations indicate that *if* selenium was having *any* effect on catfish reproduction, it was far less than on largemouth bass reproduction and was no hindrance to rapid population increases.

Observations of selenium-contaminated Belews Lake accord with the above observations of Hyco Reservoir. Young et al. (2010) indicate that as many as 29 resident fish species were documented prior to contamination, but only common carp, catfish, and fathead minnows

remained after contamination. The Doroshov et al. (1992b) injection study results suggesting that channel catfish is sensitive at egg concentrations of 5 mg Se/kg dw, four-fold below the largemouth bass Chronic Value, thus conflict with field observations. As demonstrated in the Appendix C discussion of the Cleveland et al. (1993) toxicity tests with juvenile bluegill, the exposure route by which selenium was accumulated can have a dramatic influence on the potency of a given tissue concentration. That is, to accord with the Cleveland et al. (1993) data, the whole-body EC_{50} would be expected to be at least four-fold higher when accumulated via diet than when accumulated via water. For this reason, the criterion is derived only from tests using the environmentally relevant exposure route of diet.

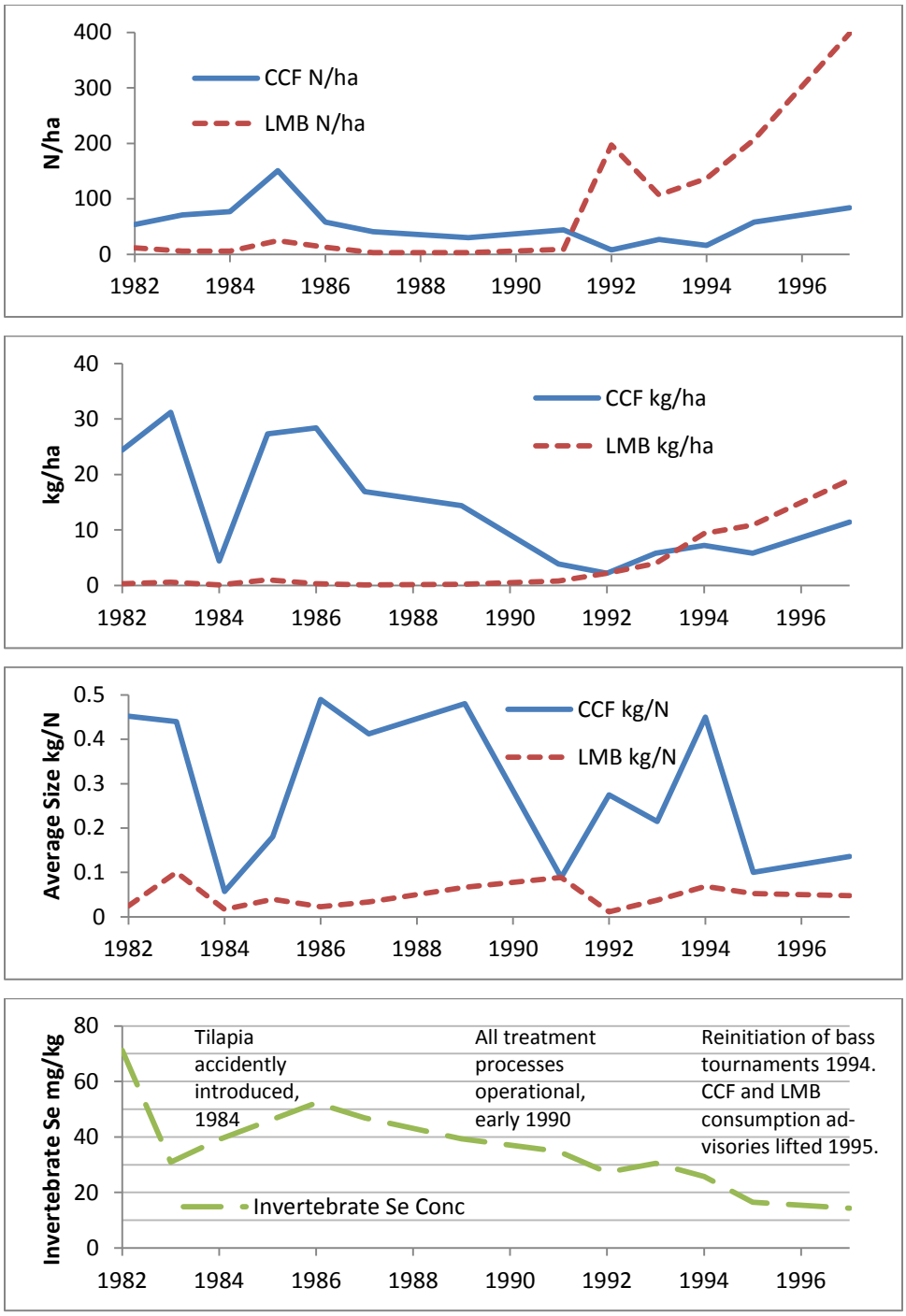


Figure 6.1. Crutchfield (2000) Observations of Channel Catfish (CCF) and Largemouth Bass (LMB) in Hyco Reservoir Beginning a Few Years after Populations of Largemouth Bass had been Reduced by Se Contamination.
(A) number of individuals/ha, (B) mass/ha, (C) mass/ha divided by number/ha, yielding average weight per individual, and (D) invertebrate Se concentration (mg Se/kg dw), and noting other events relevant to management of the fishery.

6.1.4 Reproductive Effects in Amphibians (*Xenopus laevis*)

Massé et al. (2015) has conducted the only maternal transfer study conducted with an amphibian under controlled laboratory conditions. The African clawed frog (*Xenopus laevis*) was fed a control diet (0.73 mg/kg Se dw) and three spiked diets containing selenium concentrations of 10.92, 30.4 and 94.2 mg/kg dw. Trophic transfer to the frog's eggs was approximately 1:1 with measured selenium concentrations in the control and three spiked diets of 1.6, 10.82, 28.13, and 81.66 mg/kg egg dw, respectively. Deformities were assessed in 200 tadpoles per female (1800 – 2000 tadpoles per treatment group). EC_{10} values determined by the authors for abnormal spinal curvature, abnormal craniofacial structure and abnormal lens structure were 57.3, 38.4, and 34.5 mg/kg Se egg dw, respectively. The EC_{10} value for total deformities of 44.9 mg/kg Se egg dw is in the upper-range of EC_{10} values for fish (see **Table 3.2**). Although *X. laevis* is a non-native amphibian with a different reproductive strategy, their upper-range sensitivity suggests amphibians are protected by the fish chronic criterion elements.

6.1.5 Reproductive Studies Not Used in the Numeric Criterion Derivation

Danio rerio (zebrafish)

Two studies (Penglase et al. 2014; and Thomas and Janz 2014) have shown the zebrafish, *Danio rerio* (family Cyprinidae), to be sensitive to selenium. Penglase et al. (2014) assessed the interaction of selenium with mercury through a maternal transfer study but did have two treatments with selenium exposures resulting in 1.17 mg/kg egg dw (control) and 6.24 mg/kg egg dw. The higher Se egg concentration had significantly reduced embryo survival and fecundity relative to the control, however embryo survival in the controls was low at 54%. With only one selenium treatment exposure, the data were not amenable to TRAP analysis. Thomas and Janz (2014) conducted a maternal transfer study using adult zebrafish that were fed a control diet and three levels of selenomethionine, 3.7, 9.6, and 26.6 mg/kg Se dw for 90 days before breeding the exposed fish and collecting the fertilized embryos for assessment. TRAP analysis of larval survival and larval deformities of 2-6 days post fertilization fish produced very low EC_{10} values. The lowest EC_{10} was for deformities at 7.0 mg/kg egg dw. This value is markedly lower than any of the EC_{10}'s in the current data set. The slope of the concentration-response curve for both deformities and larval survival was very shallow, which was different than the selenium responses for all other fish species for which data were available (see Figure E-6 in Appendix E). Further, the control mortality in the experiment continuing over 160 days was high, over 40%.

This zebrafish EC_{10} for deformities contrasts with the absence of deformities in the related species, fathead minnow, observed by GEI (2008) at concentrations up 40 mg/kg in adult whole body (dw) as presented in Figure E-3 in Appendix E. The GEI (2008) fathead minnow study was not directly used for criterion derivation because the offspring survival data for Sand Creek appeared to be confounded by multiple stressors in this industrial waterway. However, its deformity data appear unequivocal, indicating that the fathead minnow deformity endpoint is relatively insensitive to selenium.

Since the zebrafish is a non-native cyprinid species, EPA assessed the information available on zebrafish sensitivity to selenium compared to the sensitivity of native cyprinid (minnow) species across the U.S. (Appendix E in the 2016 criterion document), including several studies where native cyprinids were investigated in selenium-impacted waters (NAMC 2008). Data from these studies suggest that native cyprinids are likely less sensitive to selenium than the non-native zebrafish.

The anomalous nature of the concentration-response curve, with the very low value coupled with field and other laboratory data suggesting that cyprinids are not particularly sensitive to selenium was the basis for not including the zebrafish EC_{10} in the data for deriving the criterion. A detailed write up of this study and a summary of field and laboratory studies indicating native cyprinids are not one of the more sensitive families are provided in Appendix E.

Oncorhynchus clarkii (cutthroat trout)

Kennedy et al. (2000) reported no significant differences in mortality and deformity in eggs, larvae, and fry from wild-caught cutthroat trout between a reference and an exposed site (Fording River, British Columbia, Canada). The observations were made on eggs reared in well water from spawning age females collected from the two locations (N = 17 and 20, respectively) and fertilized by one male collected at each site. The mean selenium content in eggs from fish collected from the reference site was 4.6 mg/kg dw and from fish collected from the Fording River was 21.2 mg/kg dw. The chronic value for eggs is >21.2 mg Se/kg dw. These values were not used in the criterion derivation because they represent high “greater than” values, as discussed above, and provide no additional important quantitative data for the analyses.

Hardy (2005) fed cutthroat trout experimental diets containing a range of selenomethionine (0-10 mg/kg dw) for 124 weeks. No significant growth or survival effects were

observed in the adult fish over the 124 weeks. The whole body concentration reached 12.5 mg/kg dw selenium after 44 weeks. Embryo-larval observations (percent hatch and percent deformed) were not related to whole body selenium concentrations in the spawning females (9.37 mg/kg dw) fed the selenium-laden diet for 124 weeks. The concentration of selenium in eggs from these females was 16.04 mg/kg dw. For this study the chronic value, an unbounded NOEC, is thus >16.04 mg Se/kg dw in eggs. This value was not used in the criterion derivation.

Salvelinus fontinalis (brook trout)

Holm et al. (2005) collected spawning brook trout from streams with elevated selenium contaminated by coal mining activity and from reference streams in 2000, 2001 and 2002. Similar to procedures described by these authors for rainbow trout, above, fertilized eggs were monitored in the laboratory for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. Embryos from the contaminated stream had on average a higher frequency of craniofacial deformities than fry from the reference stream (7.9% for the contaminated stream compared to 2.1% in the reference stream). Although this increased rate of craniofacial deformities was calculated to be statistically significant when compared across sites, the Abbott-adjusted effect is only 6% and is thus below the 10% effect represented by an EC_{10}. But more important, when comparing across adult females (the more reliable analysis for selenium reproductive toxicity studies of this type, and the one used to obtain the related rainbow trout EC_{10} for these authors' studies), there is no apparent relationship between brook trout craniofacial deformities and exposure across a broad range of concentrations, as illustrated in Appendix C. An environmentally conservative estimate of the NOEC might be considered to be the average concentration of selenium in eggs from the high exposure site (Luscar Creek), >7.78 mg Se/kg ww or >20.5 mg Se/kg dw using the 61.2% moisture content for rainbow trout eggs cited above. However, the effect threshold appears to be substantially higher based on the absence of any consistent concentration-response relationship up to the maximum observed egg concentration of 18.9 mg Se/kg ww or 48.7 mg Se/kg dw, as shown in the Appendix C graphs. Given the point estimate EC_{10} available for the related species, *Salvelinus malma* (Dolly Varden, **Section 6.1.2.3**), the "greater than" chronic value for brook trout is not used to obtain the GMCV, in accordance with the principles listed in **Section 6.1.1**.

Lepomis macrochirus (bluegill)

Applicable chronic reproductive data for bluegill can be grouped by exposure type: field and laboratory. In some field studies, chronic value estimates were "less than" fairly high selenium concentrations (Bryson et al. 1984, 1985a; Gillespie and Baumann 1986). This low resolution is due to the observed effect occurring at a single observed high exposure concentration relative to a reference condition. In the Bryson et al. (1984, 1985a) and Gillespie and Baumann (1986) studies, the artificially crossed progeny of females collected from a selenium contaminated reservoir (Hyco Reservoir, Person County, NC) did not survive to swim-up stage, irrespective of the origin of milt used for fertilization. Measured waterborne selenium concentrations prior to the experiments ranged from 35 to 80 µg/L. The ovary tissue selenium concentration associated with this high occurrence of mortality of hatched larvae was <30 mg/kg dw tissue, as reported by Bryson et al. (1985a), and <46.30 mg/kg dw tissue, as reported by Gillespie and Baumann (1986). In the case of the latter, nearly all swim-up larvae from the Hyco Reservoir females were edematous, none of which survived to swim-up.

Bryson et al. (1985b) examined percent hatch and percent swim-up larvae from spawns using bluegills collected from Hyco Reservoir and a control site. There were no differences in the Hyco measurements relative to the control. The concentration of selenium in the liver of the parental Hyco bluegill was 18.6 mg/kg dw. The chronic values for this embryo-larval development test was >18.6 mg Se/kg dw liver. The high "less than" and low "greater than" chronic values obtained from Bryson et al. (1984, 1985a, b) and Gillespie and Baumann (1986) were not used in the SMCV calculation because these values are consistent with and yet provide no numeric basis for modifying the SMCV obtained from the EC_{10}s.

6.1.6 *Salmo* GMCV: EPA Re-analysis of a Key Study Used in Criterion Derivation

Previously, in the draft selenium criterion document submitted for external peer review in May 2014, the lowest GMCV in the reproductive effects dataset was for *Salmo* (15.91 mg/kg dw) based on larval deformities. Subsequently, in 2015, EPA conducted a careful re-analysis in response to stakeholder comments to confirm the validity of the approach used in 2014, resulting in the calculation of an EC_{10} at 18.09 mg/kg dw based on larval mortality from hatch through swim up, prior to a lab overflow accident during the post swim up feeding portion of the test. The dataset was constrained to hatch through swim up information due to uncertainty introduced by the loss of larvae from an overflow event caused by clogged drains during the post swim-up

portion of the test (Formation 2011). The hatch through swim up deformity endpoint was not considered because of the preferential selection of visibly non-deformed fish for the post swim up portion of the test (Response letter to EPA, J.R. Simplot Company 2014). This is important because the primary endpoint of interest during the post-swim up phase is deformity rate. Random selection of living fish would have been more appropriate since visibly healthy fish may be less likely to express deformities in this later stage of the test.

Following the release of the 2015 draft selenium criterion document, the larval survival from hatch through swim up dataset was reanalyzed, and it was determined that the TRAP model resulting in an EC_{10} of 18.09 mg/kg was not appropriate, because the EC_{10} was lower than one of the treatment levels within the background no-effect range. In order to calculate an EC_{10} that would not fall below the highest background concentration, a weighted least squares nonlinear regression was calculated in TRAP, resulting in an EC_{10} of 21.0 mg/kg. Additional details describing this weighted nonlinear regression approach are described in Appendix C.

6.1.7 Impact of Number of Tested Species on Criterion Derivation

Many of the species used for testing the toxicity of selenium are those observed to be affected at contaminated sites or otherwise suspected to be particularly sensitive. Six of the eight minimum data requirements were met, and the other two (for planktonic and benthic crustaceans) were waived (see **Section 2.6**). Of the N=15 genera used for the calculation of the criterion, ten are fish, which are more sensitive than invertebrates, based on the available data. Of the ten fish genera, five are either salmonids or centrarchids. Had a broader array of expected insensitive taxa been included, the four most sensitive genera would not likely change, but N would increase. The criterion calculation for selenium is relatively insensitive to the effect of increasing the value of N by adding more tests with different genera than those already represented. Setting N=20 (leaving the four most sensitive the same) would only raise the egg-ovary criterion element from 15.1 mg Se/kg to 16.0 mg Se/kg. This insensitivity occurs because the four lowest GMCVs are closely spaced, such that the calculated egg-ovary criterion element is never distant from the lowest GMCV.

6.1.8 Comparisons between Concentrations in Different Tissues

Researchers often report concentrations of selenium in fish eggs or ovaries (e.g., Formation Environmental 2011, 2012; Holm et al. 2005; Osmundson et al. 2007). Osmundson et

al. (2007) found reduced levels of selenium in ovaries after spawning, presumably due to the loss of selenium through spawning and release of eggs with relatively high concentrations of selenium. Of the 14 chronic values determined from the maternal transfer reproductive studies, 12 values represent selenium measured in eggs. Two values represent selenium measured in the ovaries: Hermanutz et al. (1992, 1996) and Carolina Power & Light (1997). Hermanutz et al. (1992, 1996) sampled adult female bluegill just prior to spawning and at the end of the test (post spawning) and found no decreases in the concentration of selenium in the post-spawned fish. In the Carolina Power & Light (1997) study, selenium in ovaries of largemouth bass was measured from fish sampled just after spawning. No comparison to prespawning fish or selenium in eggs can be made for the largemouth bass study, however, the EC_{10} of 26.3 mg Se/kg ovary dw was mid-range in the SSD indicating this test was not overly conservative due to lower selenium measurements in post spawning ovaries. Based on the observations stated above, egg selenium and ovary selenium were considered equal for the toxicity data set. Any potential error resulting from this assumption would be conservative since the effect of spawning only lowers the selenium concentration in the ovary. EPA recognizes selenium ovary concentrations may vary in field collected samples due to fish reproductive cycles and will address such concerns in the implementation information.

6.1.9 Studies of Non-Reproductive Effects

Non-reproductive effect studies do *not* involve effects on the offspring of exposed female adults, and their results are *not* expressed as selenium concentrations in egg or ovary tissue. Because selenium concentrations in whole body and muscle are generally lower than in egg and ovary, with observed egg-ovary to whole-body ratios ranging from 1.3 to 7.4, and egg-ovary to muscle ratios ranging from 1.0 to 5.8, *whole-body, muscle, and egg-ovary effect concentrations can only be compared after accounting for the inherent differences in the selenium concentrations in these different media*. Non-reproductive effects were determined to provide a less reliable basis for a criterion, in part because comparatively few of such studies provided sigmoidal concentration-response curves. Non-reproductive SMCVs and GMCVs are shown in **Table 6.2** below and summaries of the acceptable non-reproductive studies are included in Appendix D.

Table 6.2. Freshwater Chronic Values from Acceptable Tests - Non-Reproductive Endpoints.
(Parental Females Not Exposed).

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Acipenser transmontanus white sturgeon	Tashjian et al. 2006	dietary (lab) 8 weeks	seleno-L-methionine in artificial diet	EC_{10} juvenile growth	15.08 WB 27.76 M	EC_{10} 15.1 WB 27.8 M	15.1 WB 27.8 M
			seleno-L-methionine in artificial diet	EC_{20} juvenile growth	17.82 WB 32.53 M	EC_{20} 17.8 WB 32.5 M	
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	dietary (lab) 9 months	selenized-yeast	NOEC	10.1 M	10.1 M 15.1 M 12.3 M	10.1 M 15.1 M 12.3 M
				LOEC	15.1 M		
				MATC juvenile deformities (juvenile exposure only)	12.34 M		
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab) 9 to 19 days	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40 WB	51.40 WB 69.83 M	51.40 WB 69.83 M
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab) 8 days	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOEC for larval fish dry weight after 8 d	<73 WBb		
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>12.9 WBb	see text	see text
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001b	dietary and waterborne (lab) 28 days	water: site waters; diet: algae exposed to site water then fed to rotifers which were fed to fish	NOEC for survival and growth	>42 WBb		
Catostomus latipinnis flannelmouth sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>10.2 WB	>10.2 WB	>10.2 WB
Oncorhynchus tshawytscha	Hamilton et al. 1990	dietary (lab) 60 days	mosquitofish spiked with seleno-DL-methionine	EC_{10} for juvenile growth	7.355 WB	EC_{10} 9.052 WB	EC_{10} 9.052 WB

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
chinook salmon				EC_{20} for juvenile growth	10.47 WB	EC_{20} 12.83 WB	
			mosquitofish spiked with SLD diet	EC_{10} for juvenile growth	11.14 WB		
				EC_{20} for juvenile growth	15.73 WB		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab) 16 weeks	sodium selenite in food preparation	juvenile growth NOEC	21 Liver	NOAEC 28.98 L	
				LOEC	71.7 Liver		
				MATC	38.80 Liver	LOAEC 84.68 L	
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juvenile survival and growth NOEC	40 Liver		
				LOEC	100 Liver	MATC 49.52 L	
				MATC	63.25 Liver		
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab) 80 days	Se-laden shiners from Belews Lake, NC	LOEC for survival of yearling bass	<16.2 M[c]	<16.2 M	<16.2 M
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab) 180 days 20 to 4°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOEC for juvenile mortality at 4°C	<7.91 WB	4°C EC_{10}-NOAEC 8.15 WB	4°C & 9°C 9.33 WB
				Threshold prior to "winter stress"	5.85 WB		
		dietary and waterborne (lab) 180 days 20°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC for juvenile mortality at 20°C	>6.0 WB		
Lepomis macrochirus bluegill	McIntyre et al. 2008	dietary and waterborne (lab) 182 days 20 to 4°C (ES1)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES1	9.27 WB	4°C EC_{20}-LOAEC 8.80 WB	
				EC_{20} juv. survival ES1	9.78 WB		
		dietary and waterborne (lab) 182 days 20 to 9°C (ES3)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES3	14.00 WB	9°C EC_{10} 14.0 WB	
				EC_{20} juv. survival ES3	14.64 WB	9°C EC_{20} 14.6 WB	
		dietary and waterborne (lab) 182 days 20 to 4°C (ES2)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC juv. surv. ES2	>9.992 WB		

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic Value mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary (lab) 60 days	seleno-DL-cysteine	NOEC for juvenile growth	>3.74 WB[b]		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary (lab) 90 days	seleno-L-methionine	NOEC for juvenile survival	>13.4 WB[b]		

[a] All chronic values reported in this table are based on the measured concentration of selenium in whole body (WB), muscle (M) or liver (L) tissues.

[b] Chronic value not used in SMCV calculation (see text).

[c] Tissue value converted from ww to dw. See Appendix C for conversion.

6.1.9.1 Comparison of Fish Chronic Reproductive Effects and Chronic Non-Reproductive Effects

A chronic criterion element concentration of 15.1 mg/kg dw in the egg/ovary addresses the toxic effect identified by the Chapman et al. (2009, 2010) expert workshop to be of greatest concern, reproductive effects, and is expected to be protective of non-reproductive endpoints such as juvenile survival and growth.

If the information in the reproductive-effect GMCV **Table 3.2** (expressed as whole-body) were combined with the information in the nonreproductive-effect **Table 6.2**, and the lower of the reproductive or nonreproductive GMCVs for each taxon were used to construct a combined distribution of whole-body chronic values, the resulting criterion element (corresponding to N=18, accounting for three additional fish genera only having nonreproductive-effect GMCVs), the FCV would be calculated to be 9.1 mg Se/kg WB dw, similar to the 8.5 mg Se/kg WB dw FCV for reproductive effects expressed as whole-body **Figure 6.2**.

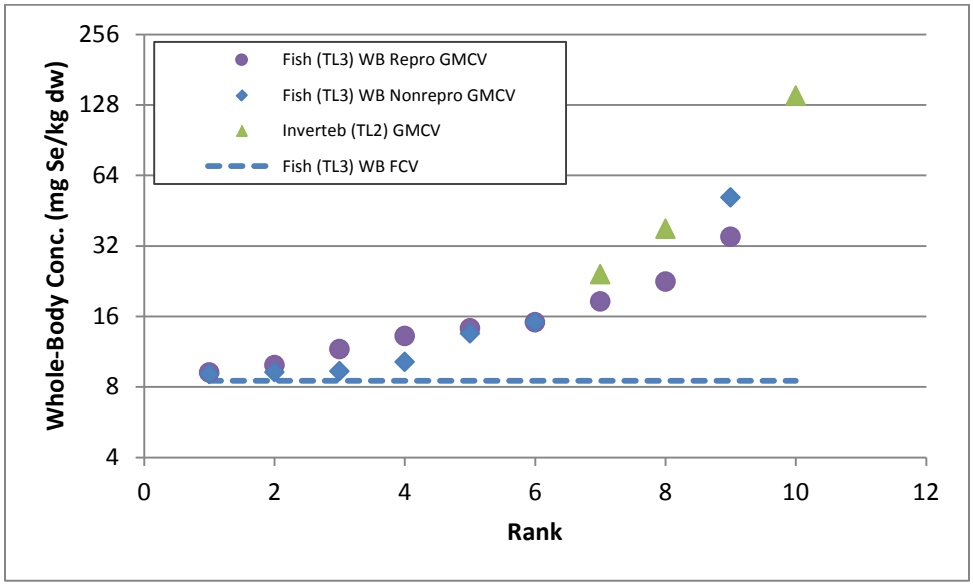


Figure 6.2. Distribution of Fish Reproductive Effect GMCVs from Figure 3.2 and Distribution of Fish Nonreproductive Effect GMCVs and Invertebrate GMCVs.

For establishing a reliable criterion, the sufficiency of and consistency among the data underlying the reproductive-endpoint GMCVs favor their use over any non-reproductive endpoint data (see **Section 3.1.1** and Appendix C). Most of the reproductive studies involved

examining the offspring of wild-caught females, exposed under real-world conditions. Most had concentration-response curves that supported EC_{10} estimates.

In contrast, the non-reproductive endpoint studies provide fewer data for supporting a criterion, and fewer of these studies yielded the type of concentration-response data that could support EC_{10} estimates. Furthermore, the non-reproductive data are not as consistent, as noted by Janz et al. (2010). The reproductive effect data also show more clear-cut concentration-response relationships than the non-reproductive effect data (11 of the reproductive chronic values are specific ECs, compared to only five of the non-reproductive chronic values), are more readily reproducible, and are better corroborated by field observations. Reproductive effects represent the endpoint of greatest concern (Chapman et al. 2009, 2010); all non-reproductive GMCVs are protected by a criterion derived from the reproductive GMCVs. The reproductive endpoint data, expressed relative to selenium concentrations in fish eggs and ovaries, thus provide a more reliable and protective basis for the criterion. Because the data set used to derive the criterion is comprised primarily of the aquatic species considered most sensitive to selenium (salmonids and centrarchids) and because the criterion is designed to protect 95% of the genera, the egg-ovary criterion element concentration of 15.1 mg/kg dw ovary/egg should be protective of aquatic populations of fish and invertebrates.

6.1.10 Special conditions for consideration of primacy of water column criterion elements over fish tissue criterion elements

The chronic selenium criterion is derived to be protective of the entire aquatic community, including fish, amphibians, and invertebrates. Fish are the most sensitive taxa to selenium effects. Selenium water quality criterion elements based on fish tissue (egg-ovary, whole body, and/or muscle) sample data supersede the criterion elements based on water column selenium data, when measured in the same approximate time frame (approximately one year) and site. This is due to the fact, noted above, that fish tissue concentrations provide the most robust and direct information on potential selenium effects in fish. However, because selenium concentrations in fish tissue are a result of selenium bioaccumulation via dietary exposure, there are two specific circumstances where the fish tissue concentrations do not fully represent potential effects on fish and the aquatic ecosystem: 1) In “fishless” waters, and 2) new selenium inputs. *Fishless waters* are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported

populations of one or more fish species but no longer support fish (i.e., extirpation) due to temporary or permanent changes in water quality (e.g., due to selenium pollution), flow or instream habitat. Because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish within such waters, water column concentrations will best represent selenium levels required to protect aquatic communities and downstream waters in such areas. *New inputs* are defined as new activities resulting in selenium being released into a lentic or lotic waterbody. New inputs will likely result in increased selenium in the food web, resulting in increased bioaccumulation of selenium in fish over a period of time until the new selenium release achieves a quasi-"steady state" balance within the food web. EPA estimates that concentrations of selenium fish tissue will not represent a "steady state" for several months in lotic systems, and longer time periods (e.g., 2 to 3 years) in lentic systems, dependent upon the hydrodynamics of a given system; the location of the selenium input related to the shape and internal circulation of the waterbody, particularly in reservoirs with multiple riverine inputs; and the particular food web. Estimates of time to achieve steady state under new selenium input situations are expected to be site dependent, so local information should be used to better refine these estimates for a particular waterbody. Thus, EPA recommends that fish tissue concentration not supersede water column concentration until these periods of time have passed in lotic and lentic systems, respectively, or until steady state concentrations can be determined.

6.2 WATER

6.2.1 Validation of Translation Equation for Developing Water Column Concentrations

EPA evaluated the efficacy of the equation used to translate the egg-ovary criterion element to a water column concentration. EPA's translation equation is given as:

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \text{(Equation 18)}$$

Because selenium levels in fish are relatively stable over a long time period if the ecosystem is at steady state with respect to selenium concentration, single measurements of selenium in fish tissue are likely to be less variable and a better representation of selenium loads to the aquatic system than single measurements of selenium in the water column. Thus, EPA used a validation approach based on fish tissue measurements rather than single water measurements.

Rearranging Equation 18 to solve for egg-ovary concentration yields:

$$C_{egg-ovary} = C_{water} \times TTF^{composite} \times EF \times CF \qquad \text{(Equation 22)}$$

EPA used Equation 22 to calculate the predicted concentration of selenium in the eggs and ovaries of fish from all spatially and temporally relevant measurements in the water column. EPA then compared those predicted values to the measured concentration in the fish.

EPA searched its collection of selenium measurements in fish tissue taken from aquatic sites with a previously calculated *EF* value. Identified tissue measurements from other than eggs or ovaries were converted to equivalent egg-ovary concentrations using species-specific conversion factors as described previously. For each tissue measurement, EPA searched its collection of selenium measurements again for water column measurements that were taken from the same aquatic site and within one year of the tissue measurement. If more than one water column measurement was matched to a tissue measurement, the median water column measurement was used. For each matched pair of tissue and water measurements, appropriate species-specific *TTF* and *CF* values were identified as described previously, and the *EF* value from the site samples were taken. EPA then used Equation 22 to calculate the predicted egg-ovary concentration from the observed water column concentration. Finally, EPA compared the predicted egg-ovary concentrations with the observed egg-ovary concentrations.

EPA identified 317 tissue measurements associated with one or more water column measurements. A predicted egg-ovary concentration was calculated for each water column concentration as described above. **Figure 6.3** shows all 317 predicted egg-ovary concentrations plotted against the measured egg-ovary concentrations. Because both the predicted and observed selenium concentrations exhibited substantial heteroscedasticity (the variability of one variable is unequal across the range of values of a second variable that predicts it), they are plotted and analyzed on a log scale. The predicted and measured concentrations are highly correlated ($r=0.82$, $t_{(315)}=25.30$, $P<0.001$). Data used to generate **Figure 6.3** can be found in Appendix I.

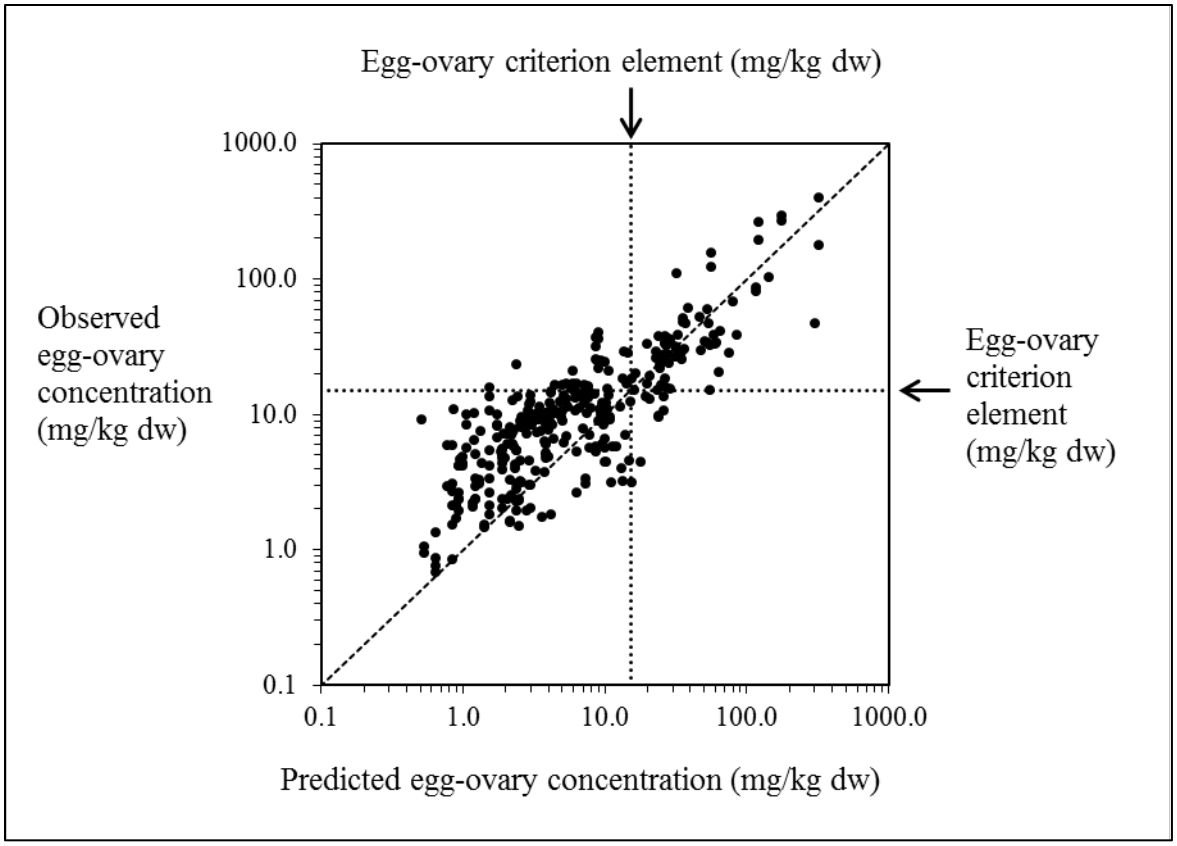


Figure 6.3. Scatter Plot of Predicted Versus Measured Concentrations of Selenium in Fish. Solid line shows unity y = x line; dashed lines show the egg-ovary criterion element value.

Although there is a strong correlation between predicted and observed egg-ovary concentration values, **Figure 6.3** shows more data points above the y = x line at low selenium concentrations. This result suggests the model underestimates bioaccumulation at low selenium concentrations. Such behavior is likely the result of the inherent model assumption of constant bioaccumulation rates regardless of selenium concentration, whereas selenium bioaccumulation has been shown to be inversely related to water concentration (see **Sections 3.2.2** and **3.2.4** for further discussion). Within the range of concentrations near the egg-ovary criterion element value, however, the relationship between predicted and observed selenium concentrations are evenly dispersed around the y = x line. Thus the model is unlikely to result in biased estimates near egg-ovary concentrations that may require regulatory action.

Dispersion around the unity line is likely attributable to several sources of uncertainty including small sample sizes, temporal or spatial variability in selenium exposure, and local variability in aquatic food webs. EPA limited this analysis to only those aquatic sites with at least

two particulate measurements available to calculate an *EF* value and with at least one of them from algae or detritus. The requirement of at least two particulate measurements was made because a single measurement was considered insufficient. The requirement that at least one of the measurements be for algae and/or detritus was made because selenium within these particulate types was more highly correlated to water (**Section 3.2.2.3**). Nevertheless, only one or two measurements of algae and/or detritus were available for 62 of the 96 aquatic sites evaluated. Although the minimum data requirements described above reduce uncertainty when applying Equation 22 to available data, EPA believes that two particulate measurements are only marginally sufficient. Another potential source of uncertainty is the frequent absence of site-specific information about the types and proportions of organisms ingested by fish. In most cases, EPA estimated the type and proportion of prey organisms using general knowledge of the fish species and aquatic system location. Notwithstanding the limitations in available data, the EPA concludes from this analysis that Equation 18 provides a reasonable translation of the egg-ovary criterion element to a site-specific water concentration.

6.2.2 Sulfate-Selenium Interactions

Several investigators (Brix et al. 2001; Ogle and Knight 1996; Williams et al. 1994) have previously evaluated the role of sulfate on the bioavailability and toxicity of selenium in freshwater organisms. A report from DeForest et al. (2014) notes that a sulfate-dependent selenium criteria would apply only to selenate-dominated, well-oxygenated streams, a subset of freshwater systems in the U.S. The DeForest publication discussed experiments to assess influence of sulfate on selenate uptake on one species of macrophyte (*Lemna minor*) and one algal species (*Pseudokirchnella subcapitata*), a limited data set of primary producers. The authors note that, “It does need to be emphasized here, however, the analysis currently does not include Se data for periphyton and benthic diatoms, as these data are not available.” The authors also note that, “due to methodological challenges and high costs, it is difficult to comprehensively evaluate the influence of sulphate on bioconcentration and transfer up the food chain.”

Including any type of sulfate relationship in the national criterion derivation would necessitate having sulfate measurements to accompany all observed selenium water concentrations included in the derivation database. That is, the absence of an accompanying sulfate observation would necessitate excluding the water observation. The resulting reduction in

the number of sites included in the database would reduce the confidence in its ability to represent the nation's waters. For the above reasons, EPA has not included a sulfate relationship in the 2016 selenium criterion.

6.3 UNCERTAINTY

This section examines several areas where EPA addressed uncertainty in the development of the selenium water quality criterion. This section represents a qualitative treatment of specific parts of the derivation process for the selenium freshwater chronic criterion where EPA has identified the potential for uncertainty, and also describes the approaches that the Agency used to reduce uncertainty.

EPA developed a tissue-based water quality criterion designed to be protective of aquatic life from the chronic effects of selenium. In general, EPA followed the procedure detailed in the document, Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the "Guidelines") (Stephan et al. 1985). The Guidelines sets forth a methodology for deriving ambient water quality criteria for the protection of aquatic life that includes a rigorous list of data quality requirements. Because selenium is a bioaccumulative chemical with maternal diet and transfer as the primary route of exposure for chronic toxicity, EPA included additional data quality requirements such as the requirement of a dietary exposure. See **Section 2.7.5** Analysis Plan for Derivation of the Chronic Fish Tissue-Based Criterion Elements for how chronic effect levels were determined for selenium. The Guidelines provide several recommended approaches that reduce uncertainty in the derivation of criterion. It provides a strict set of guidelines for the acceptance of data to be used in criteria derivation. It provides a minimum set of data requirements (MDRs) that define an assemblage of aquatic organisms that can be used in a genus sensitivity distribution to derive a criterion that is protective of 95% of aquatic species. The requirements in the Guidelines reduce the uncertainty in the ability of a criterion to be protective of aquatic life.

6.3.1 Tissue Criterion Element

The tissue criterion element is based on reproductive effects caused by selenium and is expressed in three different tissue types, egg/ovary, muscle and whole body. Non-reproductive effects were also determined but not used in the derivation of the criterion because of less certainty in the endpoints and effect levels (**Section 6.1.9** and **Table 6.2**). A comparison of fish

reproductive and non-reproductive effects and the conclusion that the reproductive criterion is protective of the non-reproductive effects is given in **Section 6.1.9.1**.

The dataset used to derive the tissue-based criterion consists primarily of fish species: 12 fish species representing 10 genera and 7 families. Although this might be viewed as a small number of the nearly 800 native freshwater fish species (36 families) of fish in the United States, it is a large number of species relative to chronic criteria derivations for other pollutants. Furthermore, the fish species that have been the focus of some of the research have been the species observed to be those first affected (most sensitive) at selenium-contaminated sites such as Belews Lake and Hyco Reservoir, i.e., bluegill sunfish and largemouth bass. The data set contains three acceptable chronic toxicity studies with bluegill sunfish, the second most sensitive genus in the dataset and one with largemouth bass, the fifth most sensitive genus. The three replicate chronic values for bluegill are 14.7, 22.6, and 26.3 mg Se/kg egg-ovary dw. Of these three values, 14.7 mg Se/kg ovary dw is likely the least certain because the study (Hermanutz et al. 1992, 1996) was not designed to minimize uncertainty in characterizing the tissue concentrations associated with its observed levels of effect.

Three genera representing five species from Salmonidae, a family considered to be generally sensitive to contaminants, are in the data set. Two salmonid genera, *Salmo* and *Oncorhynchus* are the third and fourth most sensitive taxa in the data set. *Salmo* is represented by a single study, but because the study included a large number of individuals across a broad spectrum of exposure, the uncertainty associated with its 21 mg Se/kg egg dw might not be viewed as particularly large. The chronic values for the three studies with *Oncorhynchus* are confined to the narrow range 24.5 – 27.7 mg Se/kg egg dw, and on that basis may be considered to have low uncertainty. Although the numbers of species and families of fish in the data set are a fraction of what are native to the United States, the fish species contained in the data set are known to be those most sensitive to selenium based on field observations or known to be sensitive in general to contaminants. With the lowest six chronic values falling in the relatively narrow range 15.6 – 27 mg Se/kg egg-ovary dw, the selenium tissue criterion element should probably be considered to have the smallest amount of uncertainty among the existing aquatic life criteria.

As stated in the previous paragraph, the data set primarily consists of fish species and contains only three invertebrate species. The cases in the field where adverse effects have been

observed to fish and water birds (e.g., Belews Lake, Hyco Reservoir, Kesterson Reservoir) have not documented any adverse effects on macroinvertebrates either on a species or community level (Janz et al. 2010). The effect levels determined for the three invertebrate species contained in the data set are consistent with the field observations that macroinvertebrates are in general less sensitive to selenium than fish species. EPA recognizes that there may be more sensitive oviparous taxa (fish and amphibians), as well as macroinvertebrate taxa than those in the current data set and supports the testing of different species.

6.3.1.1 Reproductive Endpoints

Reproductive endpoints were determined from studies in which adults were exposed to selenium either in the laboratory or field. Effects were measured in the offspring which received selenium exposure via maternal transfer. Larval mortality and teratogenic deformities such as skeletal, craniofacial, and fin deformities, and various forms of edema that result in mortality are the most sensitive indicators of selenium toxicity in fish larvae. Recent research suggests the mode of action of selenium-induced toxicity in fish larvae is due to oxidative stress and appears to be related to glutathione homeostasis (See **Section 2.3** for more detail on this subject.). Linking the mode of action directly to the assessment endpoint used in the derivation of the tissue-based criterion provides a consistent concentration-response relationship among the studies used in the data set. Using the most sensitive assessment endpoint (based on the state of the science) reduces uncertainty in the ability of the criterion to protect aquatic life.

6.3.1.2 Egg Ovary Chronic Values

Chronic Values (CV) were based on the most direct representation of exposure to the effect in the offspring, that is, the concentration of selenium in the egg/ovary. One way to assess the precision of the chronic values used in the derivation of the criterion is to look at the reproducibility of tests used to calculate the CV for a taxon. This precision assessment can be done with two tests conducted with cutthroat trout and three tests conducted with bluegill sunfish. The two cutthroat trout studies (Rudolph et al. 2008 and Nautilus Environmental 2011) had very similar EC_{10} values (24.7 and 27.7 mg Se/kg egg dw, respectively) for the same endpoint (larval survival) using fish collected at the same site. Two of the three bluegill tests (Coyle et al. 1993 and Doroshov et al. 1992a) also had very similar EC_{10} values (26.3 and 22.6 mg Se/kg egg dw, respectively) for larval endpoints determined in laboratory exposures. An

EC_{10} of 14.7 mg Se/kg ovary dw was determined in the third bluegill test, a mesocosm exposure study reported by Hermanutz et al. (1992, 1996). Although the mesocosm study had a lower EC_{10} value when compared with Coyle et al. (1993) and Doroshov et al. (1992a), it was within a factor of 1.8 and 1.5, respectively. Delos (2001) found such differences to be typical when equivalent toxicity tests of the same species are compared. The relatively low variability between chronic toxicity tests conducted with the same species indicates precision in the CV estimates, which reduces the uncertainty the tissue-based criterion.

Most of the CVs were determined using an EC_{10} value and a few were estimated using the NOEC. EC_{10} values were considered more appropriate than EC_{20}, because selenium is a tissue-based criterion due to its nature of exposure and effects for this bioaccumulative chemical. See **Section 2.7.1** for a discussion of why EC_{10}s were favored over EC_{20}s. The use of EC_{10}s and NOECs increases the certainty that the criterion will be protective of aquatic life.

6.3.1.3 Whole Body and Muscle Chronic Values

Effect levels (EC_{10} or NOECs) were determined directly for whole body or muscle tissues when the selenium concentrations for these tissues were measured and reported in the tests. Effect levels were calculated directly using muscle tissue for five of the chronic toxicity tests: northern pike, cutthroat trout (Rudolph), bluegill (Doroshov and Hermanutz) and white sturgeon, while effect levels for three tests were calculated directly using whole body selenium concentrations: bluegill (Coyle and Hermanutz) and brown trout. For the other tests that did not have muscle or whole body selenium measurements, conversion factors (*CF*s) were used to convert the egg/ovary CV to a muscle or whole body CV. The direct calculation of the muscle and whole body CVs (when data were available) reduced uncertainty in these effect level estimates.

6.3.1.4 Conversion Factors

When muscle or whole body chronic values could not be determined directly using selenium concentrations measured and reported for the respective tissue, conversion factors (*CF*) were used to convert the egg/ovary chronic value to either a muscle or whole body chronic value.

To derive egg-ovary to whole-body *CF* values, EPA defined matched pairs of selenium measurements from the eggs or ovaries and from the whole-body measured from the same individual fish or from matched composite samples. If multiple measurements from both eggs

and ovaries of the same individual or matched composite sample were available, the average value was used. Similar pairings were done for egg-ovary to muscle *CF* values.

After the data sets of the pairings were compiled, EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using ordinary least squares (OLS) linear regression of the matched pairs of measurements. If the regression resulted in a significant fit ($P \leq 0.05$) with a positive slope, EPA calculated the ratio of the egg-ovary to whole body (or muscle) selenium concentration of each matched pair and used the median ratio as the *CF* value for the species. A detailed comparison of the advantages and disadvantages of the median ratio and least squares regression approaches to calculating *CF*s, along with a comparison of *CF*s calculated from median ratios, OLS regression following log transformation, and total least squares (TLS) regression following log transformation is in Appendix N. Table N-3 provides a comparison of the median-based and regression-based *CF*s when they are used to convert an egg-ovary selenium concentration to muscle or whole body. Generally, the median-based and TLS-based *CF*s were similar for both tissue types and this similarity resulted in similar criterion element values (bottom row of Table N-3). The muscle criterion element value for the data set that contained directly calculated CVs and converted CVs was similar whether median or TLS *CF*s were used, 11.3 and 10.2, respectively. The whole body criterion element value was also similar using these two approaches, 8.5 and 9.4, respectively. The median-based *CF* approach was considered to be better than the regression-based *CF* approaches at reducing uncertainty. A detailed comparison and rationale for the median approach is discussed in appendix N.

EPA had sufficient egg-ovary and whole-body selenium measurements to directly calculate egg-ovary to whole body *CF* values for 13 species of fish. Similarly, there were sufficient egg-ovary and muscle selenium measurements to directly calculate egg-ovary to whole body *CF* values for 16 species of fish. To derive *CF* values for additional fish species, EPA used a taxonomic-relatedness approach (most similar taxon) approach to estimate *CF*. This approach is consistent to that done for TTF estimates, and is described in **Section 3.2.2**, and in greater detail in Appendix B.

The variability of *CF*s between fish species and within fish species was fairly low. EPA derived 13 *CF* values directly from matched pairs of egg-ovary and whole-body selenium measurements and an additional seven *CF* values by multiplying EO/M and M/WB conversion

factors (**Table 3.12**). Excluding mountain whitefish (*CF* = 7.4), *CF*s for 19 of the 20 species ranged from 1.20 to 3.11, a 2.6-fold difference. CF variability within each species was also low for 11 of the 13 species for which egg-ovary to whole-body CFs were determined directly and a standard deviation calculated (**Table 3.12**). The two species with relatively high standard deviations contained data that were potentially anomalous. When the potentially anomalous data were removed the standard deviations for these two species were reduced considerably (see footnote to **Table 3.12**).

6.3.2 Trophic Transfer Factors

A Trophic Transfer Factor (*TTF*) represents the transfer of selenium from one trophic level to the next higher trophic level. *TTF*s are used in the translation of the tissue criterion element concentration to a water element value. For a description of how *TTF*s are used in translation, see **Section 3.2.1**, Translation from Fish Tissue Concentration to Water Column Concentration.

Similar to CFs, EPA calculated *TTF*s from field data using the median-ratio approach after first performing OLS regression of matched pairs of selenium measurements for the two taxa representing successive trophic levels to determine if the relationship is significant ($P \leq 0.05$) and has a positive slope. EPA also evaluated using only OLS regression results to calculate *TTF* values. OLS regression was performed using matched concentrations of selenium in the food of a particular species or taxonomic group with the concentration of selenium in the organism's tissue, and then the slope of the regression was used as the *TTF* for that species or taxonomic group. An advantage of the regression approach is that it estimates the quantitative relationship of selenium across a range of environmental concentrations in a manner that allows statistical assessment. Disadvantages of this regression approach include the assumption that the underlying data are normally distributed; the possibility that one or a few very high or low values can have a disproportionate influence on the slope of the fitted line; and the fact that the bioaccumulation model does not account for a non-zero y-intercept. Constraining the y-intercept to zero (also known as regression through the origin or RTO) eliminates the added complexity of a non-zero y-intercept. However, RTO further increases the disproportionate influence of one or a few high values on the slope of the fitted line. Furthermore, RTO does not provide a straightforward way of evaluating goodness of fit (Gordon 1981).

The median-ratio approach, following confirmation of a significant ($P \leq 0.05$) relationship and positive slope, was considered to be more appropriate for deriving *TTF*s from field data that the OLS regression approach. Requiring a significant positive OLS linear regression coefficient confirms the relationship between selenium in organisms and the food they ingest is adequately represented by the available data. Using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias from measurements taken from aquatic systems with very high or very low selenium concentrations. Some aquatic organisms exhibit selenium bioaccumulation inversely related to water concentration (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). This inverse relationship is likely due to saturation uptake kinetics of specific transport mechanisms that regulate metals bioaccumulation within certain ranges (U.S. EPA 2007). EPA evaluated the effect of very high and very low selenium concentrations on the calculation of *TTF* values using the hybrid approach (use of median ratios for matched data with significant relationship and positive slope) described above by excluding selenium measurements above various minimum and/or below various maximum selenium concentrations. EPA found that using the median ratio effectively attenuates any effects of selenium concentration on the calculation of *TTF* values using the hybrid approach described above without the need to introduce additional arbitrary exclusion criteria.

*TTF*s were also determined using physiological coefficients (see **Section 3.2.2.1**, Derivation of Trophic Transfer Factors (TFF) Values. However, if a *TTF* value could be calculated from both physiological coefficients and field data, EPA used the *TTF* value calculated from the substantially larger number of field measurements to minimize statistical uncertainty.

*TTF*s were calculated for 32 fish species, and ranged from 0.68 to 2.67 (**Table 3.11**). The majority of fish *TTF*s fell within a relatively narrow range, with an interquartile range ($25^{th} – 75^{th}$ centile) of 1.03 to 1.42. Variability of *TTF*s among the 13 invertebrate taxa was higher, ranging from 0.74 to 4.58 (**Table 3.10**). Much of the variability among invertebrate *TTF*s was related to taxonomic groups. The two bivalve *TTF*s ranged from 4.00 to 4.58. The five insect *TTF*s ranged from 1.48 to 2.88, the five crustacean *TTF*s ranged from 0.74 to 1.89, and the *TTF* for blackworms was 1.29.

EPA translated the tissue criterion element concentration to water element values at field sites that had selenium measurements in the required water, particulates, invertebrates, and fish.

For species without sufficient data to directly calculate a *TTF* value at these sites, EPA estimated the *TTF* value by sequentially considering higher taxonomic classifications until one or more taxa for which a calculated *TTF* value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a *TTF* value available, EPA used the median *TTF* from the matching species.

6.3.3 Enrichment Factors

Enrichment factors are discussed in **Section 3.2.2.3** and Appendix H. This factor, describing how the bottom of the food chain takes up selenium, is the most variable between sites. Variability among *EF*s is the main reason that fish BAFs vary so much between sites, and this variability is the reason the national criterion for selenium needed to be tiered, with tissue having priority over water, to increase certainty that the criterion is protective as intended. The range of site *EF* values shown in Appendix H spans more than a 100-fold range.

The *EF* value measured at a particular site is also likely to be the site's most uncertain parameter, being a ratio of measurements of algae, detritus, and sediment, which may vary within a site in uncertain ways, and measurements of water, which vary over time. The approach for setting site *EF*s was designed to reduce uncertainty. As described in Appendix H, EPA calculated *EF* values by searching its database of selenium measurements and identifying all the selenium measurements from algae, detritus, or sediment. EPA then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water concentration was available for any given particulate measurement, the median water concentration was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median of those ratios. Selenium concentrations between particulate and water concentrations were higher for algae and detritus than for sediment. To reduce uncertainty in *EF* values associated with sediments, at least two particulate selenium measurements with corresponding water column measurements were required, and sediment measurements were used only if there was at least one other measurement from either algae or detritus. The geometric mean of the algae, detritus, and sediment ratios was then calculated and used as the site *EF*. Because there were at most only 3 possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric

mean in order to reduce the potential for one of the values to have excessive influence on the final site EF value. Sites with insufficient data to fulfill these data requirements were not used.

Had EPA increased the data requirements for setting a site *EF*, then the database would be restricted to a smaller number of sites. Because the variability between sites is high, reducing the number of sites in the database would decrease the confidence in the representativeness of the few sites retained (that is, it would increase the potential for sampling error in attempting to characterize the nation's waters). The *EF* determination process thus involved a balance between having enough information to reasonably characterize each site, and having enough sites to represent the range of the nation's waters.

Inclusion of selenium speciation information (such as selenate and selenite concentrations) was infeasible. Very few sites would have the requisite information, thereby increasing the uncertainty in the representativeness of any possible derived national criterion. Likewise, inclusion of a sulfate relationship was not feasible on a national basis at this time, for lack of sulfate data at many sites in the database.

Because *EF* is the BAF component that varies the most between sites, it is the most important in determining what the water concentration would be for a site if its fish tissue concentrations were hypothesized to be at the level of the fish tissue criterion element value. That is, sites with higher *EF*s tabulated in Appendix H have lower translated water concentrations in **Table 3.13**. Despite uncertainties in this parameter, model-predicted versus observed fish-tissue concentrations in the vicinity of the water element criterion concentrations are relatively unbiased, as shown in the figures of Appendix I.

For particular sites, the appropriateness of the national criterion can be resolved by site specific criteria when necessary (e.g., when a permit limit for water is required), as recommended in Appendix K. When taking measurements of a site, uncertainty in particulate measurements (the numerator of the *EF*) can be bypassed by using site-specific fish BAFs, since they only consider water and fish tissue selenium measurements. On the other hand, uncertainty in characterizing time-variable water concentrations is a problem shared by *EF*s and BAFs. However, this uncertainty can be reduced by sampling in a spatially and temporally robust manner, appropriate for the site in question, and then using the mathematical modeling approach to derive a site specific criterion.

6.3.4 Water Values

Derivation of the water criterion element from the egg-ovary criterion element is described in **Sections 2.7.8** and **3.2**, and involves *EF*s, *TTF*s, and *CF*s. Uncertainties in predicted tissue-to-water ratios combine the uncertainties in the parameters from which they are predicted. The prediction model is linear in all respects. Potential nonlinearities are therefore an uncertainty. **Section 6.2.1** and Appendix I assesses the accuracy of the predictions. As shown in the figures of Appendix I, the predicted values perform reasonably well in the vicinity of the water criterion element concentrations.

Although an earlier published draft document weighted sites by the number of fish species sampled (between 1 to 6 species per site), that overweighting of sites with several measured species was removed from this draft by using only the most bioaccumulative fish species per site, thereby reducing uncertainty that the fish tissue criterion element will be exceeded when using only water column concentration data. Lentic and lotic sites were assessed separately, per **Section 3.2.4**. This increases the likelihood that the water criterion element concentration will be appropriate for the site of application.

To reduce the likelihood that the water criterion element concentration will be under-protective for any particular site of application, the 20th percentiles of translated water concentrations for all lentic and lotic sites, respectively, were used as the water criterion element concentrations. As described previously, these distributions represented the translated water concentrations for the most bioaccumulative fish species at each site, which further reduces the likelihood that the fish tissue criterion element would be exceeded if the water criterion element was being met. These water criterion elements should not be interpreted to be potentially under-protective in 20 percent of sites, because when applied to a site's 30-day once-in-three-year maximum concentration (which is higher than its median), the 20th percentile site would not attain. The actual percentage of sites protected would thus be greater than 80 percent, but the exact percentage is uncertain.

6.4 Protection of Threatened or Endangered Species

The chronic toxicity dataset for selenium contains toxicity data for two Federally-listed endangered species, *Cyprinodon macularius* (desert pupfish) and *Oncorhynchus mykiss* (listed as steelhead, indicating anadromous individuals, but herein called rainbow trout, implying non-anadromous individuals). The dataset also contains toxicity data for *Acipenser transmontanus*

(white sturgeon), which is listed as endangered in specific locations, such as the Kootenai River white sturgeon in Idaho and Montana. The white sturgeon also serves as a surrogate for other sturgeon listed as threatened or endangered (e.g., pallid and shovelnose sturgeon). The *Acipenser* GMCV of 15.6 mg/kg dw egg is the lowest value in the dataset and therefore provides protection for other potentially sensitive sturgeon. The white sturgeon chronic value is greater than the chronic egg-ovary criterion element value.

Desert pupfish, *Cyprinodon macularius*, with a chronic value estimated to be ≥27 mg Se/kg dw egg, is not among the most sensitive species. Its chronic value of ≥27 mg Se/kg dw egg is substantially above the chronic egg-ovary criterion element value of 15.1 mg Se/kg dw.

Oncorhynchus mykiss has an SMCV of 24.5 mg Se/kg dw egg, whose genus is the fourth most sensitive species in the dataset. The dataset contains multiple studies with cutthroat trout (*Oncorhynchus clarkii*) some subspecies of which are Federally listed as threatened. The SMCV for cutthroat trout is 26.2 mg Se/kg dw egg. Both of these chronic values for *Oncorhynchus* species are greater than the chronic egg-ovary criterion element.

The dataset also contains toxicity information for *Salvelinus malma* (Dolly Varden) which is not threatened or endangered, but is so closely related to the threatened *Salvelinus confluentus* (bull trout) that it can hybridize with that species, producing fertile offspring (Baxter et al. 1997). Dolly Varden is the least sensitive fish species for which information is available, with an SMCV of 56 mg Se/kg dw egg. *Salvelinus fontinalis*, brook trout, can also hybridize with bull trout, but the offspring are sterile, suggesting that it is less closely related. With the available study of brook trout, although in **Section 6.1.5** the NOEC is conservatively set to >20.5 mg Se/kg dw egg, which was the average concentration at the Holm et al. (2005) high-exposure site. The concentration-response information for the offspring of individual females, presented in Appendix C, suggests that its EC_{10} could be substantially higher, possibly as high as that for Dolly Varden.

The egg-ovary criterion element value of 15.1 mg Se/kg (dw) is below all of the above mentioned chronic egg-ovary values for threatened and endangered (or closely related) species. However, because other threatened or endangered species could be more sensitive, if relevant new information becomes available in the future, it should be considered in state- or site-specific criterion calculations.

The protectiveness of the whole body criterion element concertation of 8.5 mg/kg dw to threatened and endangered species is also supported by a recent non-reproductive study with two sturgeon species. De Riu et al. (2014) fed juvenile green and white sturgeon (~30 g body weight) diets containing a range of selenium concentrations (selenomethionine added to diet formulation; 2.2 mg/kg Se in control diet (no added Se) and 19.7, 40.1 and 77.7 mg/kg Se in the three treatment diets). Several endpoints were monitored over the 8-week exposure period including survival and percent body weight increase (% BWI). White sturgeon had no mortalities through the highest dietary treatment. Green sturgeon juveniles had 0%, 7.7% and 23.1% mortality with the three dietary treatments. TRAP analysis (threshold sigmoid nonlinear regression) of the green sturgeon survival data resulted in a whole body EC_{10} value of 28.93 mg/kg dw. EC_{10} values were lower for % BWI using TRAP. For % BWI, the whole body EC_{10} value for green sturgeon was 16.36 mg/kg dw, and 23.94 mg/kg dw for white sturgeon.

Also notable, the background concentrations of selenium in the juvenile green and white sturgeon were also elevated at 7.2, 6.5 and 7.1 mg/kg dw (green sturgeon whole body), and 4.8 7.3 and 5.6 mg/kg dw (white sturgeon whole body) at test initiation, and after four and eight weeks of exposure, respectively.

The De Riu et al. (2014) study suggests that green sturgeon may be more sensitive to selenium than white sturgeon and also that the EPA whole body concentration of 8.5 mg/kg dw will be protective, based on the survival and growth data and the observation in De Riu 2014 that the control whole body tissue concentrations (up to 7.2 mg/kg dw) are approaching the proposed criterion. This is important because white sturgeon, as well as juvenile green sturgeon (up to three to four years), spend most of their time in the coastal rivers and estuaries. All species in the Acipenseriformes (sturgeon and paddlefish) spawn in freshwaters (Bemis and Kynard 1997) or spend their entire life in freshwater. The white sturgeon's EC_{10} in the dataset provides surrogacy for the threatened and endangered species from this group. For more information on the De Riu et al. (2014) study, see Appendix E.

6.4.1 Special Consideration for Pacific Salmonid Juveniles

The current criterion is based on reproductive effects (larval mortality and/or deformities) for offspring of selenium-exposed adults, and the whole-body criterion element is derived from the egg-ovary element, with an implicit assumption of adult exposure to selenium. One peer-reviewer of the 2014 EPA External Peer Review Draft criterion document raised concerns

regarding the protection of anadromous salmonids, since there is at least some evidence (e.g., Hamilton et al. 1990) that juvenile growth may be comparable in sensitivity to reproductive effects endpoints used by EPA. Anadromous salmon species (e.g., Chinook salmon) in the Pacific Northwest are unique in that reproductively mature adults are not exposed to selenium in the freshwater environment due to their life history; young juvenile salmon leave freshwater streams and rivers as smolts and mature to adulthood in the marine environment until migration for spawning begins. Furthermore, they are semelparous, breeding only once in their lifetime and subsequently dying, so there is no potential selenium exposure following spawning in freshwater.

Juvenile salmon have evolved different strategies for growth and maturation to the smolt stage, and may spend from three months to two years in freshwater (depending on timing of egg hatching and other factors) before migrating to estuarine areas as smolts and into the ocean to feed and mature. Salmon remain in the ocean for one to six years (more commonly two to four years), with the exception of a small proportion of yearling males (called jack salmon), which mature in freshwater or return after two or three months in salt water (NOAA 2011).

The physiological and morphological changes that allow these species to adapt to marine conditions as juveniles are reversed in returning adults preparing to migrate up natal streams to spawn. One key change is the cessation of feeding prior to re-entry into freshwater. Since mature females are not feeding after returning to freshwater, it is not representative to predict reproductive effects for anadromous salmonid species based on egg-ovary selenium concentrations, because the exposure is wholly from selenium sources in the marine environment (Groot and Margolis 1991).

6.4.1.1 Selenium Toxicity to Juvenile Salmonids

Hamilton et al. (1990) assessed the toxicity of two organoselenium diets in 90-day partial life cycle tests in freshwater with two life stages of Chinook salmon (*Oncorhynchus tshawytscha*). The first diet consisted of fish meal made from low-selenium mosquitofish (collected from a reference site) fortified with selenomethionine (here termed the SeMet diet). The second diet contained fish meal made from high-selenium mosquitofish (*Gambusia affinis*) collected from the San Luis Drain (SLD), California (here termed the SLD diet). This waterbody is known to have high concentrations of selenium. A 90-day partial life cycle study was conducted with swim-up stage salmon larvae in a standardized fresh water that simulated dilution of San Luis Drain water.

Survival and growth (length and weight) were measured at 30, 60, and 90 days. Unexplained control mortality (33%) between day 60 and day 90 introduced an unacceptable level of uncertainty into the overall health of the fish. The 1985 Aquatic Life Guidelines (Stephan et al. 1985) and the Manual of Instructions for Preparing Aquatic Life Water Quality Criteria Documents (Stephan 1987) require that excessive control mortality be treated as an exclusionary threshold in data quality assessments for regulatory purposes such as deriving water quality criteria. Therefore the 90-day survival data from this study was not used quantitatively. At 60 days, larval control mortality was acceptable (1%), and 60-day larval survival was > 90% in all SLD and SeMet treatments (3.2 ppm – 18.2 ppm) except for the high Se treatment (35.4 ppm). Whole body selenium concentrations were measured at 60 days, were 10.4 and 13.3 mg/kg dw, respectively, for larvae fed the SeMet and SLD diets of 18.2 mg/kg dw (Hamilton et al. 1990).

Although survival was similar in response to the two diets, larval growth responses differed between the SLD and SeMet diets. The salmon fed the SeMet mosquitofish diet had significant reductions in both length and weight at 30, 60, and 90 days; but only at the two highest concentrations (18.2 and 35.4 ppm). The average length and weight of the larvae fed the SLD mosquitofish diet were significantly lower at all concentrations at 30, 60, and 90 days. The greater effect on growth parameters fed the SLD mosquitofish meal diet could have been caused by one or more of several factors: 1) additional forms of organic selenium (e.g., selenocysteine) present in the SLD mosquitofish, 2) additional toxic elements (e.g., heavy metals) that were accumulated by the SLD mosquitofish, and not present in the reference site mosquitofish, and 3) differential metabolic processing of the organoselenium contained in the proteins of the SLD mosquitofish and fed to the larval salmon, versus the larvae fed the diet containing the free amino acid selenomethionine (Hamilton et.al. 1990).

EPA performed a regression on the 60-day weight and whole body concentrations, and derived a whole body EC_{10} value of 7.355 mg/kg dw for the SeMet diet for reduced growth, and a whole body EC_{10} value of 11.14 µg/g dw for the SLD diet for reduced growth. These values are the only two available EC_{10} Species Mean Chronic Values (SMCVs) for non-reproductive endpoints for the genus *Oncorhynchus*, and the Genus Mean Chronic Value (GMCV) is 9.052 mg/kg dw. This is greater than the national whole body criterion element concentration of 8.5 mg/kg dw, which will thus be protective of this genus.

EPA recommends that states and tribes consider use of the whole-body criterion element for juvenile (smolt) anadromous Pacific salmon species as the primary criterion element over the other elements due to the unique life history of these species, specifically, the lack of exposure to adult salmonids from selenium in freshwater prior to reproduction. The hierarchal structure of the egg-ovary tissue over the other tissue criterion elements applies to all other species in the family Salmonidae. The egg-ovary criterion element, as well as the other fish tissue criterion elements and the water column criterion elements still apply, as applicable, to protect the remainder of the aquatic community in these waters.

6.5 Aquatic-Dependent Wildlife is Beyond the Scope of this Aquatic Criterion Derivation

AWQC that are developed by EPA typically focus directly on aquatic life, not aquatic-dependent wildlife such as birds. As presented by Campbell (2011), EPA recognizes that selenium effects on aquatic-dependent wildlife are also of concern but considers them beyond the scope of this national criterion update. In the interest of providing updated guidance to protect against the known risks of selenium exposure to fish, EPA decided to focus its analyses on updating the existing selenium criterion for freshwater aquatic life based on the latest scientific evidence.

In the future, EPA plans to consider the effects of selenium on aquatic-dependent wildlife, potentially in the form of criteria expanded to address aquatic-dependent wildlife. When translated to a water concentration, a criterion protective of aquatic-dependent wildlife may be more stringent or less stringent than the values provided for aquatic life in this criterion document. This is because data indicate that for most ecosystems, selenium concentrations are generally conserved or increase incrementally at each trophic level in a food web (after a substantial increase from water to trophic level 1 (e.g., algae). Certain specific ecosystems (e.g., estuarine and marine systems more commonly) with mollusk-based food-webs may create a pathway for more selenium to bioaccumulate, particularly in molluscivorous predators (certain fish and aquatic bird species), since the available data indicate that mollusks generally have a higher trophic transfer factor than other invertebrate taxa. This level of bioaccumulation is typically lower, and in contrast to other bioaccumulative chemicals such as mercury, which have much greater biomagnification.

As stated previously, the single largest step in tissue selenium accumulation in aquatic environments occurs at the base of the food web where algae and other microorganisms accumulate selenium from water (Orr et al. 2012; Stewart et al. 2010). Mollusks such as mussels and clams accumulate selenium to a much greater extent than planktonic crustaceans and insects due to higher ingestion rates of both particulate-bound (algae) and dissolved selenium from the water column through filter feeding, and these organisms have a lower selenium elimination rate (Luoma and Rainbow 2005). Thus, aquatic-dependent wildlife criteria for species that are primarily molluscivores may have concentrations of concern that are not protected by the 2016 selenium criterion elements found in this document. The criteria values for aquatic-dependent wildlife would be expected to depend on the aquatic systems, species, and food webs considered, as well as spatial and temporal considerations related to selenium exposure and breeding and nesting seasons. Where sensitive aquatic-dependent (e.g., bird) species are known to exist, states should consider developing site-specific criteria based on data for such species.

6.6 SUMMARY

EPA developed the 2016 national 304(a) Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater to be protective of most aquatic life genera in most waters of the United States, with an intended goal of protecting approximately 95% of aquatic genera in an ecosystem. This freshwater chronic selenium criterion applies only to aquatic life, and is not intended to address selenium toxicity to aquatic-dependent wildlife such as aquatic-dependent birds. This document provides guidance to States and Tribes authorized to adopt water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of selenium.

The 2016 selenium criterion is a chronic criterion that is composed of four elements. All elements are protective against chronic selenium effects. Two elements are based on the concentration of selenium in fish tissue and two elements are based on the concentration of selenium in the water-column. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column - element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent element to account for potential chronic effects from short-term exposures (one value for lentic and one value for lotic aquatic systems). The assessment of the available data for fish, invertebrates, and amphibians

indicates that a criterion value derived from fish is expected to be protective of the aquatic community, based on available data.

EPA recommends that states and tribes adopt into their water quality standards a selenium criterion that includes all four elements, and express the four elements as a single criterion composed of multiple parts, in a manner that explicitly affirms that the whole-body or muscle elements supersede the water column element, and the egg-ovary element supersedes any other element. The magnitude of the fish egg-ovary element is derived from analysis of the available toxicity data. The magnitudes of the fish whole-body element and fish muscle elements are derived from the egg-ovary element coupled with data on concentration ratios among tissues. The magnitudes of the water column elements are derived from the egg-ovary element coupled with bioaccumulation considerations. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements into the selenium criterion ensures protection when neither fish egg-ovary nor fish whole-body nor muscle tissue measurements are available. There are two specific circumstances where the fish tissue concentrations do not fully represent potential adverse effects on fish and the aquatic ecosystem: 1) “fishless” waters, because of the inability to collect sufficient fish tissue to measure selenium concentrations in fish in such waters, and 2) areas with new selenium inputs, because the fish tissue concentrations in such systems would not yet represent steady state conditions upon which the criterion is based.

To ensure that the contribution of short-term exposures to the bioaccumulation risks is accounted for in all situations, EPA is recommending that the intermittent exposure element be included in the selenium criterion, as noted above. EPA is not recommending a separate acute criterion element derived from the results of toxicity tests having water-only exposure because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. Application of the intermittent exposure criterion element values to single day, high exposure events will provide protection from the most important selenium toxicity effect, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high exposure events.

The egg/ovary-based tissue criterion element of 15.1 mg Se/kg dw is based on a genus sensitivity distribution that used the most sensitive assessment endpoint observed in toxicity

tests, reproductive effects, and included fish species known to be sensitive to selenium (i.e., species from Salmonidae and Centrarchidae), as well as three endangered species (desert pupfish, rainbow trout and white sturgeon).

With respect to the chronic water column criterion elements, EPA intends the lentic and lotic values of 1.5 and 3.1 µg/L, respectively, to be protective of most surface waters in the U.S. These water concentrations represent the 20^{th} percentile of the distribution of translated water column values from sites across the U.S. The intermittent exposure water column criterion element is derived from the chronic water column criterion element, which was derived from the tissue-based criterion.

EPA recognizes selenium bioaccumulation potential depends on the structure of the food web and several biogeochemical factors that characterize a particular aquatic system. Uncertainty in the translation of the egg-ovary criterion element to the water column element can be reduced by deriving a site-specific criterion that uses site-specific selenium data and information on food-web dynamics from a biological assessment of the aquatic system. Appendix K provides recommendations and examples for developing site-specific selenium criteria.

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Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016 (Appendices A-N)

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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3.0 Comparison of Median- and Regression-based Conversion Factors to Calculate Chronic Values for Muscle and Whole Body Tissues N-14

APPENDIX A: SELENIUM CHEMISTRY

Selenium in aquatic ecosystems exists in a broad range of oxidation states: (+ VI) in selenates ($HSeO_4^-$, SeO_4^{2-}) and selenic acid (H_2SeO_4), (+ IV) in selenites ($HSeO_3^-$, SeO_3^{2-}) and selenous acid (H_2SeO_3), 0 in elemental selenium, and (-II) in selenides (Se^{2-}, HSe^-), hydrogen selenide (H_2Se), and organic selenides (R_2Se). Selenium also shows some tendency to form catenated species like organic diselenides (RseSeR). Within the normal physiological pH range and the reduction potential range permitted by water, only Se, SeO_3^{2-}, $HSeO_3^-$, and SeO_4^{2-} can exist at thermodynamic equilibrium (Milne 1998). While ionic reactions are expected to be rapid in water, oxidation-reduction reactions may be slow, and the possibility exists for the formation of HSe^- in living systems and some environments where anoxic conditions arise. The parallel behavior of comparable species of sulfur and selenium in living systems has often been observed, but it is important to recognize that their chemical characteristics are different in many ways. For instance, selenate is comparable to chromate in oxidizing strength and far stronger than sulfate [$E^0(SeO_4^{2-}/H_2SeO_3)$ = 1.15 V; $E^0(Cr_2O_7^{2-}/Cr^{3+})$ = 1.33V; $E^0(SO_4^{2-}/H_2SO_3)$ = 0.200V (standard potentials in acid solution: Weast 1969)], whereas selenide is a much stronger reducing agent than sulfide [$E^0(Se/H_2Se)$ = -0.36 V; $E^0[S/H_2S$]= 0.14V)].

1.0 INORGANIC SELENIUM

Selenate usually predominates in well-aerated surface waters, especially those with alkaline conditions. In spite of its oxidizing strength, selenate (SeO_4^{2-}) exhibits considerable kinetic stability in the presence of reducing agents (Cotton and Wilkinson 1988). The radius of SeO_4^{2-} is comparable to that of SO_4^{2-} (Frausto da Silva and Williams 1991), and uptake by cells is expected to take place via the same ion channels or permeases for both anions. Competition between sulfate and selenate uptake has been observed in many species: algae (Riedel and Sanders 1996), aquatic plants (Bailey et al. 1995), crustaceans (Ogle and Knight 1996), fungi (Gharieb et al. 1995), HeLa cells (Yan and Frenkel 1994), and wheat (Richter and Bergmann 1993). Reduced selenate bioconcentration with increasing sulfate concentration has been demonstrated in *Daphnia magna* (Hansen et al. 1993). A significant inverse relationship was shown to exist between acute selenate toxicity to aquatic organisms and ambient sulfate concentrations (Brix et al. 2001a). Competition with selenate has also been observed for phosphate in green algae (Riedel and Sanders 1996), and with chromate and tungstate in anaerobic bacteria (Oremland et al. 1989).

Selenous acid species ($HSeO_3^-$ and SeO_3^{2-}) can predominate in solution under the moderately oxidizing conditions encountered in oxygenated waters. Between pH 3.5 and 9.0 biselenite ion is the predominant ion in water, and at pH values below 7.0, selenites are rapidly reduced to elemental selenium under mildly reducing conditions (Faust 1981), situations that are common in bottom sediments.

Most selenite salts are less soluble than the corresponding selenates. The extremely low solubility of ferric selenite $Fe_2(SeO_3)_3$ ($K_s = 2.0 \pm 1.7 \times 10^{-31}$), and of the basic ferric selenite $Fe_2(OH)_4SeO_3$ ($K_s = 10^{-61.7}$), is important to the environmental cycling of selenium. Selenites also form stable adsorption complexes with ferric oxides, forming complexes of even lower solubility than the ferric selenites. Under certain conditions, selenite (in contrast to selenate) seems to be completely adsorbed in high amounts by ferric hydroxide and, to a lesser extent, by aluminum hydroxide (Faust 1981). Coprecipitation techniques have been applied for preconcentration of selenium in natural waters, using iron (III) hydroxides, which coprecipitates selectively the selenite, but not the selenate, species in river and sea waters (Yoshii et al. 1977). Alum and iron coagulation precipitation can be used in water treatment processes to remove selenite (Clifford et al. 1986). The low levels of selenium in ocean waters have been attributed to the adsorption of selenite by the oxides of metals, such as iron and manganese (National Academy of Sciences 1976).

Relative to selenate, selenite is more reactive because of its polar character, resulting from the asymmetric electron density of the ion, its basicity (attraction to bond with proton), and its nucleophilicity (attraction to bond to a nucleus using the lone pair electrons of the ion). No evidence has yet been presented to show that $HSeO_3^-$ or SeO_3^{2-} is taken up intact into the cell interior. Evidence indicates that selenite is reduced rapidly, even before uptake in some cases, making it difficult to distinguish between uptake and metabolic processes (Milne 1998). Freshwater phytoplankton process selenate and selenite by different mechanisms, leading to different concentrations within the cell, and the concentrations attained are affected by various chemical and biological factors in the environment (Riedel et al. 1991). These authors suggested that selenate is transported into the cell by a biological process with low affinity, whereas selenite appears to be largely physically adsorbed. Contradictory evidence suggesting that selenite uptake is enzymatically mediated was found with marine phytoplankton (Baines and Fisher 2001). Experimental results supporting the hypothesis that separate accumulation mechanisms for selenate and selenite are present in *D. magna* have been published (Maier et al. 1993). However, while some organisms appear to absorb selenite nonspecifically, specific transport systems exist in other species. Sulfate competition is insignificant in the aquatic plant *Ruppia maritima* (Bailey et al. 1995), and specific uptake systems have been demonstrated in some soft line microorganisms (Heider and Boeck 1993). Selenite uptake in green algae, unlike selenate, is increased substantially at lower pH values, a property that represents another difference between these two anions (Riedel and Sanders 1996). The uptake of inorganic selenium species, selenate and selenite, by the green alga *Chlamydomonas reinhardtii* (Dang) was examined as a function of pH over the range 5 to 9, and in media with varying concentrations of major ions and nutrients using ^{75}Se as a radiotracer. Little difference was noted in the uptake of selenate as a function of pH, with the maximum uptake found at pH 8; however, selenite uptake increased

substantially at the lower pH values. Differences in speciation are suggested to be the cause of these differences. Selenate exists as the divalent ion SeO_4^{2-} over the range of pH tested; whereas monovalent biselenite ion $HSeO_3^-$ is prevalent at these pH values. At the low end of the pH range, neutral selenous acid may also play a role.

Elemental selenium is not measurably soluble in water. It has been reported that elemental selenium is slowly metabolized by several bacteria (Bacon and Ingledew 1989), and the translocation of elemental selenium into the soft tissue of the marine mollusk *Macoma balthica* has been reported (Luoma et al. 1992). The bioavailability of elemental selenium to *M. balthica* was assessed by feeding the organisms ^{75}Se-labeled sediments in which the elemental selenium was precipitated by microbial dissimilatory reduction. A 22% absorption efficiency of particulate elemental selenium was observed. In view of the insolubility of elemental selenium, uptake may be preceded by air oxidation, or in reducing environments thiols may facilitate the solubilization (Amaratunga and Milne 1994). Elemental selenium can be the dominant fraction in sediments (Zawislanski and McGrath 1998).

Selenium is reduced to hydrogen selenide, H_2Se, or other selenides at relatively low redox potentials. Hydrogen selenide by itself is not expected to exist in the aquatic environment since the Se^0/H_2Se couple falls even below the H^+/H_2 couple. Aqueous solutions of H_2Se are actually unstable in air due to its decomposition into elemental selenium and water. Under moderately reducing conditions, heavy metals are precipitated as the selenides, which have extremely low solubilities. The following are log K_s values of some heavy metal selenides of environmental interest: -11.5 (Mn^{2+}), -26.0 (Fe^{2+}), -60.8 (Cu^+), -48.1 (Cu^{2+}), -29.4 (Zn^{2+}), -35.2 (Cd^{2+}), and -64.5 (Hg^{2+}). The precipitation of selenium as heavy metal selenides can be an important factor affecting the cycling of the element in soils and natural waters.

2.0 ORGANOSELENIUM

Organic selenides (conventionally treated as Se(-II) species) in variable concentrations, usually in the form of free and combined selenomethionine and selenocysteine, are also present in natural surface waters (Fisher and Reinfelder 1991). Dissolved organic selenides may be an important source of selenium for phytoplankton cells, because they can account for ~80% of the dissolved selenium in open ocean surface waters, and for a significant fraction in many other environments as well (Cutter 1989; Cutter and Cutter 1995). Dissolved organoselenium levels of 14.2%, 65% and 66% were measured in samples (one meter depth) from Hyco Reservoir, NC; Robinson Impoundment, SC; and Catfish Lake, NC; respectively (Cutter 1986). The Hyco Reservoir organoselenium was identified as being protein bound.

Organoselenium concentrations were found to range from 10.4% (58.7 μg/L) to 53.7% (1.02 μg/L) of the total selenium present in Lake Creek and Benton Lake, MT surface waters (Zhang and Moore 1996). Organoselenium quite often is measured as the difference between total dissolved selenium

and the sum of selenite plus selenate, and is therefore not typically characterized. Much more work is needed in the area of specific identification and characterization of the nature of the organic selenides present in aquatic ecosystems. Organoselenium form(s) are much more bioavailable and probably play a very important role in selenium ecotoxic effects (e.g. Besser et al., 1993; Rosetta and Knight 1995).

3.0 DEPARTURE FROM THERMODYNAMIC EQUILIBRIUM

In the highly dynamic natural waters, there is often a departure from thermodynamic equilibrium. In the thermodynamic models, kinetic barriers to equilibrium and biological processes are not adequately considered, and the speciation of selenium in oxidized natural waters is not accurately predicted. Selenate is usually the predominate form in solution; however, selenite and organoselenium can both exist at concentrations higher than predicted (Faust 1981; Luoma et al. 1997). Bioaccumulation by microorganisms, bioproduction and release of organoselenium, and mineralization of particulate selenium forms contribute to the disequilibrium.

4.0 PHYSICAL DISTRIBUTION OF SPECIES IN SURFACE WATER

The physical distribution of various selenium species in surface waters is regulated by:

- sorption to or incorporation in suspended particulate matter (SPM), and
- complexation with inorganic and/or organic colloidal material, such as $(FeO\ OH)_n$ and humic substances (dissolved organic matter, DOM).

Both sorption to SPM and complexation with colloidal matter reduces the bioavailability of the selenium species. The average fraction of selenium associated with the suspended particulate phase (0.45μm filtration) as determined from eleven different studies of various surface waters was found to be 16% (0-39% range) of the total selenium, i.e., an average operationally defined dissolved selenium level of 84% (Table A-1). In the James River, VA, the dissolved inorganic and organic selenium was found to be 77% and 70% associated with colloidal matter, respectively (Takayangi and Wong 1984). A study of lake ecosystems in Finland (Wang et al. 1995) found that 52% of the dissolved selenium was associated with humic substances, and in a similar speciation study of Finnish stream waters, Lahermo et al. (1998) determined that 36% of the selenium was complexed with humic matter. Hence, in various waterbodies physical distribution as well as chemical speciation of selenium must be considered in relationship to bioavailability and aquatic toxicity.

Until recently, the organic selenium fraction has been routinely measured as the difference between total dissolved selenium and the sum of selenite and selenate. Unfortunately, the calculation of this important selenium fraction in water as the difference between the total and measurable inorganic fractions has not permitted this fraction to be fully characterized. New techniques are currently being

developed which should help the specific identification and characterization of the nature of the organic selenides present in aquatic systems. This work is particularly important because portions of the organic selenium fraction (e.g., selenomethionine) of total dissolved selenium in water have been shown to be much more bioavailable than the other forms of selenium, and therefore this work is also important for understanding the manifestation of selenium ecotoxic effects.

Table A-1. Suspended particulate and dissolved selenium as a function of total selenium in freshwater and marine aquatic ecosystems.

Reference	Waterbody	Particulate Se (% of Total)	Fraction dissolved, fd
Cutter 1989	Carquinez, CA	20 - 40	0.6 - 0.8
Cutter 1986	Hyco Reservoir, NC	0	1
Tanizaki et al. 1992	Japanese Rivers	16	0.84
Luoma et al. 1992	San Francisco Bay, CA	22 - 31	0.69-0.78
Cumbie and VanHorn, 1978	Belews Lake, NC	8	0.92
GLEC 1997	Unnamed Stream, Albright, WV	4	0.96
Wang et al. 1995	Finnish Lakes	10	0.9
Lahermo et al. 1998	Finnish Streams	8	0.92
Hamilton et al. 2001a,b	Adobe Creek, Fruita, CO	18	0.82
Hamilton et al. 2001a,b	North Pond, Fruita, CO	0	1
Hamilton et al. 2001a,b	Fish Ponds, Fruita, CO	7	0.93
Nakamoto and Hassler 1992	Merced River, CA	0	1
Nakamoto and Hassler 1992	Salt Slough, CA	4	0.96
Welsh and Maughan 1994	Cibola Lake, CA	39	0.62
Welsh and Maughan 1994	Hart Mine Marsh, Blythe, CA	6	0.94
Welsh and Maughan 1994	Colorado River, Blythe, CA	11	0.89
Welsh and Maughan 1994	Palo Verda Oxbow Lake, CA	33	0.67
Welsh and Maughan 1994	Palo Verda Outfall Drain, CA	0	1
Welsh and Maughan 1994	Pretty Water Lake, CA	21	0.79

APPENDIX B: CONVERSIONS

1.0 CONVERSION OF WET TO DRY TISSUE WEIGHT

1.1 Methodology

Conversion factors (*CF*) derived from selenium measurements were calculated using concentrations expressed as dry weights (µg/g dry weight). The majority of tissue and whole-body selenium concentrations were reported as dry weights. Measurements reported as wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type.

Species-specific percent moisture data for muscle tissue were available for bluegill (Gillespie and Baumann 1986; Nakamoto and Hassler 1992), rainbow trout (Seiler and Skorupa 2001), and for a composite average of nine fish species (May et al. 2000). Species specific percent moisture data for ovaries were available for bluegill (Gillespie and Baumann 1986; Nakamoto and Hassler 1992), fathead minnow (GEI Associates 2008; Rickwood et al. 2008), and rainbow trout (Seiler and Skorupa 2001). Species-specific % moisture data for whole-body tissues were available for bluegill (USGS NCBP).

Measurements reported as wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data were unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) were used. Table B-1a lists percent moisture by tissue type, species, data source, and the target species and study for which the % moisture data were used to convert from wet to dry weight. Table B-1b is a list of 38 freshwater fish species and their percent solids and moisture. Although these data were not needed for wet to dry weight conversion in any of the studies in this document, they are provided here as a potential resource.

Table B-1a. Percent moisture, by species and tissue type.

% Moisture Data Source		% Moisture by Tissue			Conversion Applied to	
Species	**Study**	**Whole-body**	**Muscle**	**Ovary**	**Species**	**Study**
Used in derivation of FCV						
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Brook trout	Holm et al. 2005
Fathead minnow	Average of GEI Assoc. 2008; Rickwood et al. 2008			75.30	Fathead minnow	Schultz and Hermanutz 1990
Bluegill	Average of Gillespie & Baumann 1986 and Nakamoto & Hassler 1992			76.00	Bluegill	Hermanutz et al. 1996
Avg of 9 spp	May et al. 2000		78.4		Striped bass	Coughlan and Velte 1989
Used in conversion of FCV in egg/ovary to whole-body Se concentrations						
Bluegill	USGS NCBP	74.80			Bluegill	Hermanutz et al. 1996
Bluegill	May et al. 2000		80.09		Bluegill	Hermanutz et al. 1996
Bluegill	Average of Gillespie & Baumann 1986 and Nakamoto & Hassler 1992			76.00	Bluegill	Hermanutz et al. 1996
Rainbow trout	May et al. 2000		77.54		Brook Trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Brook Trout	Holm et al. 2005
Rainbow trout	May et al. 2000		77.54		Rainbow Trout	Holm et al. 2005
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow Trout	Holm et al. 2005
Rainbow trout	May et al. 2000		77.54		Rainbow Trout	Casey & Siwik 2000
Rainbow trout	Seiler & Skorupa 2001			61.20	Rainbow Trout	Casey & Siwik 2000

Table B-1b. Percent solids and moisture for whole body fish tissues by species.
Data provided by GEI Consultants (GEI 2014).

Species	Average % solids	Count	Min	Max	Avg % moisture
Black bullhead	23.18	6	18.4	27	76.82
Blacknose dace	26.25	44	21.3	31.2	73.75
Bluntnose minnow	25.2	3	23.8	25.9	74.8
Brook stickleback	24.18	57	19.3	27.8	75.82
Carp	21.8	6	21.1	22.8	78.2
Central Stoneroller	25.38	174	17.2	33.7	74.62
Common carp	24.54	62	17.4	43	75.64
Creek chub	23.29	306	16.5	29.3	76.71
Fantail darter	27.71	15	19.5	72.3	72.29
Fathead minnow	23.36	298	15.3	100	76.64
Green sunfish	23.87	150	7.9	29	76.13
Greenside darter	25.55	11	21.7	27	74.45
Johnny darter	28.3	1	--	--	71.7
Largemouth bass	24.26	64	20.6	28.8	75.74
Log perch	23.05	2	22.3	23.8	76.95
Longnose dace	26.75	17	23.4	31.3	73.25
Mimic shiner	24.9	2	24	25.8	75.1
Mosquitofish	23.96	8	22.5	24	76.04
Northern hogsucker	23.93	113	17	39	76.07
Plains killifish	24.5	9	23.3	26.1	75.5
Rainbow darter	27.17	85	12	33.3	72.83
Red shiner	26.93	46	20.9	34.8	73.07
Redside shiner	24.44	8	21.8	26.9	75.56
River chub	24.8	4	22.9	27.3	75.2
River redhorse	20.8	1	--	--	79.2
Rock bass	25.05	24	21.2	29.3	74.95
Rosyface shiner	30.25	2	27.6	32.9	69.75
Rosyside shiner	24.54	5	23.1	25.7	75.46
Sand shiner	26.03	83	20.7	30.7	73.97
Sauger	23	1	--	--	77
Silver shiner	23.4	7	22.3	24.6	76.6
Smallmouth bass	25.78	12	22.7	28.1	74.22
Speckled dace	26.04	35	21	31.2	73.96
Striped shiner	22.9	64	18.2	28.8	77.1
Sunfish	23.2	1	-	-	76.8
Variegated darter	27.45	13	21.7	30.3	72.55
White sucker	22.63	246	16.5	28.4	77.37
Yellow perch	26.02	5	24	28.4	73.98
Grand total	24.85	1990			75.15

2.0 DERIVATION OF TISSUE CONVERSION FACTORS

2.1 Methodology

EPA used a mechanistic bioaccumulation modeling approach to derive a mathematical relationship between the concentration of selenium in water to the concentration of selenium in the eggs and ovaries of fish. This approach characterizes selenium bioaccumulation as a series of steps representing the phase transformation of selenium from dissolved to particulate form, and then the trophic transfer of selenium through aquatic food webs to invertebrates and fish. The final step in this process is the transfer of selenium into eggs and ovary tissue.

Equation 1 quantitatively models the transfer of selenium through each environmental compartment as a series of site-specific and species-specific parameters. The parameter *CF* in Equation 1 represents the species-specific proportion of selenium in egg or ovary tissue relative to the average concentration of selenium in all body tissues and is given as:

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \text{(Equation 1)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g dw)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (µg/g dw).

EPA derived species-specific conversion factor (*CF*) values using the same methods that were used to derive species-specific *TTF* values from field data. To derive whole-body to egg-ovary *CF* values, the EPA defined matched pairs of selenium measurements from the whole-body and from the eggs or ovaries measured from the same individual fish or from matched composite samples. Egg-ovary concentration was defined as a measurement from either the eggs or the ovaries. If multiple measurements from both eggs and ovaries of the same individual or matched composite sample were available, the average value was used. EPA first confirmed a statistical relationship between egg-ovary and whole body selenium for each species using ordinary least squares (OLS) linear regression. If the regression resulted in a statistically significant ($P<0.05$) positive slope, EPA calculated the ratio of the egg-ovary to whole body selenium concentration for each matched pair of measurements and used the median as the *CF* value for that species.

EPA derived *CF* values from selenium measurements in units of µg/g dry weight. The majority of tissue and whole body selenium concentrations were reported as dry weights. Measurements reported as

wet weight were converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data were unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) were used. A listing of percent moisture concentrations by species and target tissue are provided in Table B-1a.

For those species without sufficient data to directly calculate an egg-ovary to whole body *CF*, but which had sufficient data to calculate a conversion factor for either egg-ovary to muscle or whole body to muscle, EPA followed a two stage approach based on taxonomic similarity, similar to that described above. If a fish species had species specific egg-ovary to muscle conversion factor, but no whole body data with which to calculate an egg to whole body *CF*, then available data would be used to estimate a muscle to whole body conversion factor for that species based on taxonomic relatedness. The estimated muscle to whole body factor would be multiplied by the directly measured egg-ovary to muscle factor to estimate an egg-ovary to whole body *CF* for that species. For example, rainbow trout has a species specific egg-ovary to muscle conversion factor of 1.92, but does not have a species specific egg-ovary to whole body *CF*. Using the taxonomic approach described above, the most closely related taxa to rainbow trout with muscle to whole body conversion factors are in the class Actinopterygii. The median conversion factor for the 8 species within that class is 1.27. The final egg-ovary to whole body *CF* for rainbow trout is 2.44 (Table B-6), or 1.92 x 1.27.

The EPA developed species-specific egg-ovary to muscle and muscle to whole-body correction factors following the procedure described for whole-body to egg-ovary conversion factors. The EPA obtained matched pairs of selenium measurements in the whole-body and muscle filets and matched pairs of selenium measurements in muscle filets and whole-body from published scientific literature. EPA first confirmed a statistical relationship between the two tissue types for each species using OLS linear regression. If the regression resulted in a significant fit with a positive slope, the EPA calculated the ratio of each matched pair of measurements and then calculated the median ratio.

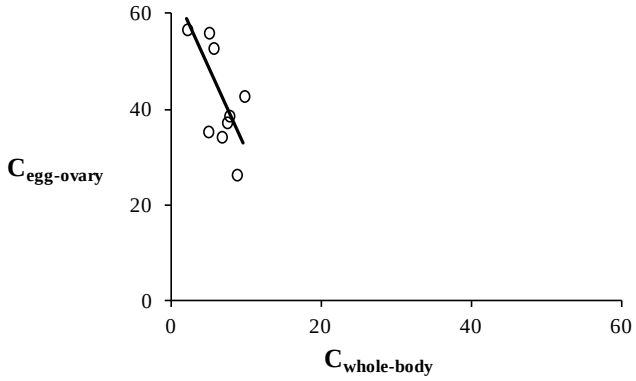
2.2 CF values calculated directly from whole-body and egg-ovary selenium measurements

$C_{whole\text{-}body}$ = Selenium concentration in all tissues (µg/g dw)
C_{egg} = Selenium concentration in eggs (µg/g dw)
C_{ovary} = Selenium concentration in ovary tissue (µg/g dw)
$C_{egg\text{-}ovary}$ = Average selenium concentration in eggs and ovaries $\left(\frac{C_{egg} + C_{ovary}}{2}\right)$

Ratio = $\frac{C_{egg-ovary}}{C_{whole-body}}$

Black bullhead (*Ameiurus melas*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	5.30	-	64.30	64.30	12.13
Osmundson et al. 2007	4.80	-	35.40	35.40	7.38
Osmundson et al. 2007	5.50	-	52.80	52.80	9.60
Osmundson et al. 2007	4.90	-	56.00	56.00	11.43
Osmundson et al. 2007	9.60	-	42.80	42.80	4.46
Osmundson et al. 2007	7.60	-	38.70	38.70	5.09
Osmundson et al. 2007	7.30	-	37.30	37.30	5.11
Osmundson et al. 2007	6.60	-	34.30	34.30	5.20
Osmundson et al. 2007	8.60	-	26.40	26.40	3.07
Osmundson et al. 2007	2.00	-	56.70	56.70	28.35
Osmundson et al. 2007	5.30	-	64.30	64.30	12.13



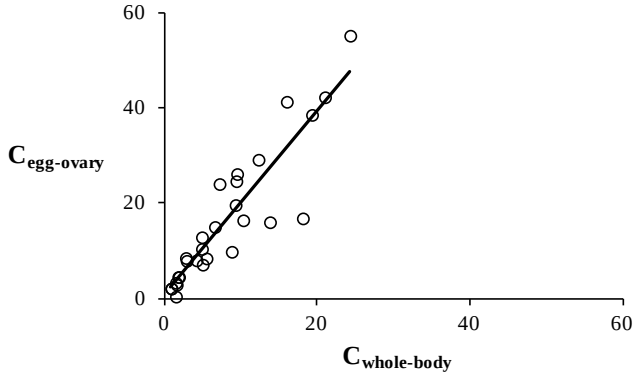
Median ratio: 6.29

R^2: 0.37
F: 4.67
df: 8
P: 0.046

Not used because negative slope.

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Coyle et al. 1993	0.90	1.90	2.10	2.00	2.22
Coyle et al. 1993	2.90	7.30	8.30	7.80	2.69
Coyle et al. 1993	4.90	13.00	12.50	12.75	2.60
Coyle et al. 1993	7.20	22.80	25.00	23.90	3.32
Coyle et al. 1993	16.00	41.30	41.00	41.15	2.57
Doroshov et al. 1992	1.60	2.80	-	2.80	1.75
Doroshov et al. 1992	5.50	8.30	-	8.30	1.51
Doroshov et al. 1992	9.30	19.50	-	19.50	2.10
Doroshov et al. 1992	19.30	38.40	-	38.40	1.99
Hermanutz et al. 1996	1.50	-	0.30	0.30	0.20
Hermanutz et al. 1996	18.10	-	16.70	16.70	0.92
Hermanutz et al. 1996	1.90	-	4.40	4.40	2.32
Hermanutz et al. 1996	2.80	-	8.40	8.40	3.00
Hermanutz et al. 1996	12.30	-	29.00	29.00	2.36
Hermanutz et al. 1996	9.40	-	24.50	24.50	2.61
Hermanutz et al. 1996	1.50	-	3.20	3.20	2.13
Hermanutz et al. 1996	4.90	-	10.30	10.30	2.10
Hermanutz et al. 1996	21.00	-	42.10	42.10	2.00
Hermanutz et al. 1996	24.30	-	55.00	55.00	2.26
Hermanutz et al. 1996	5.00	-	7.00	7.00	1.40
Hermanutz et al. 1996	9.50	-	26.00	26.00	2.74
Hermanutz et al. 1996	6.60	-	14.90	14.90	2.26
Hermanutz et al. 1996	1.80	-	4.40	4.40	2.44
Hermanutz et al. 1996	4.20	-	7.90	7.90	1.88
Hermanutz et al. 1996	10.30	-	16.30	16.30	1.58
Hermanutz et al. 1996	13.80	-	15.90	15.90	1.15
Osmundson et al. 2007	8.80	-	9.70	9.70	1.10

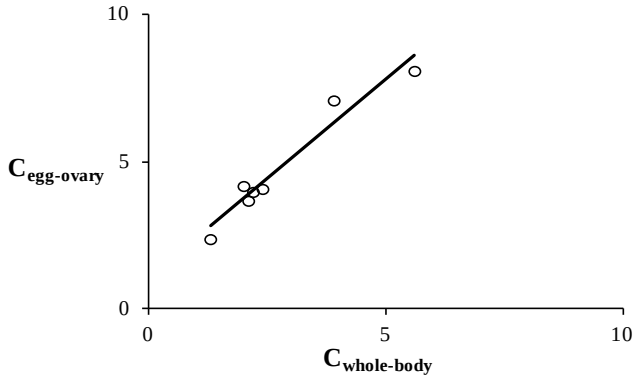


Median ratio: 2.13

R^2: 0.82
F: 110.9
df: 25
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	1.30	-	2.40	2.40	1.85
Osmundson et al. 2007	2.00	-	4.20	4.20	2.10
Osmundson et al. 2007	2.10	-	3.70	3.70	1.76
Osmundson et al. 2007	2.20	-	4.00	4.00	1.82
Osmundson et al. 2007	2.40	-	4.10	4.10	1.71
Osmundson et al. 2007	3.90	-	7.10	7.10	1.82
Osmundson et al. 2007	5.60	-	8.10	8.10	1.45



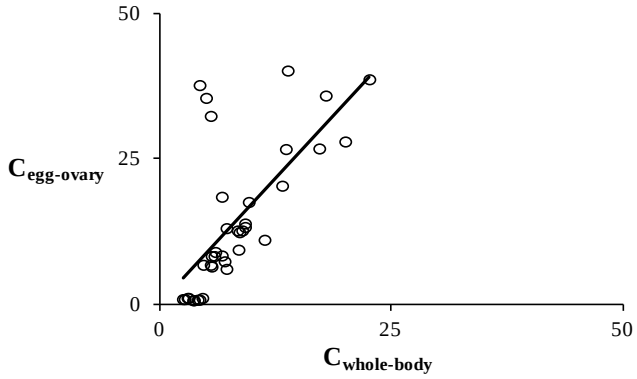
Median ratio: 1.82

R^2: 0.95
F: 88.9
df: 5
P: <0.001

Brown trout (*Salmo trutta*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Formation 2011 Saratoga fish hatchery	3.60	0.80	-	0.80	0.22
Formation 2011 Saratoga fish hatchery	4.10	0.90	-	0.90	0.22
Formation 2011 Saratoga fish hatchery	3.70	0.80	-	0.80	0.22
Formation 2011 Saratoga fish hatchery	4.30	0.90	-	0.90	0.21
Formation 2011 Saratoga fish hatchery	3.00	1.20	-	1.20	0.40
Formation 2011 Saratoga fish hatchery	3.10	1.20	-	1.20	0.39
Formation 2011 Saratoga fish hatchery	2.70	1.00	-	1.00	0.37
Formation 2011 Saratoga fish hatchery	2.50	1.00	-	1.00	0.40
Formation 2011 Saratoga fish hatchery	8.90	12.80	-	12.80	1.44
Formation 2011	13.80	40.30	-	40.30	2.92
Formation 2011	17.90	36.00	-	36.00	2.01
Formation 2011	13.60	26.80	-	26.80	1.97
Formation 2011	17.20	26.90	-	26.90	1.56
Formation 2011	6.70	18.60	-	18.60	2.78
Formation 2011	9.60	17.70	-	17.70	1.84
Formation 2011	22.60	38.80	-	38.80	1.72
Formation 2011	7.20	13.20	-	13.20	1.83
Formation 2011	9.20	13.40	-	13.40	1.46

Brown trout (*Salmo trutta*)					
Formation 2011	13.20	20.50	-	20.50	1.55
Formation 2011	8.60	12.50	-	12.50	1.45
Formation 2011	11.30	11.20	-	11.20	0.99
Formation 2011	20.00	28.10	-	28.10	1.41
Formation 2011	8.40	12.80	-	12.80	1.52
Formation 2011	5.60	8.40	-	8.40	1.50
Formation 2011	6.70	8.50	-	8.50	1.27
Formation 2011	5.90	8.40	-	8.40	1.42
Formation 2011	6.00	9.10	-	9.10	1.52
Formation 2011	7.00	7.50	-	7.50	1.07
Formation 2011	5.60	6.60	-	6.60	1.18
Formation 2011	4.70	6.90	-	6.90	1.47
Formation 2011	7.20	6.20	-	6.20	0.86
Formation 2011	9.20	14.00	-	14.00	1.52
Formation 2011	5.50	6.90	-	6.90	1.25
Formation 2011	8.50	9.50	-	9.50	1.12
Osmundson et al. 2007	4.60	-	1.20	1.20	0.26
Osmundson et al. 2007	4.30	-	37.80	37.80	8.79
Osmundson et al. 2007	5.00	-	35.60	35.60	7.12
Osmundson et al. 2007	5.50	-	32.50	32.50	5.91

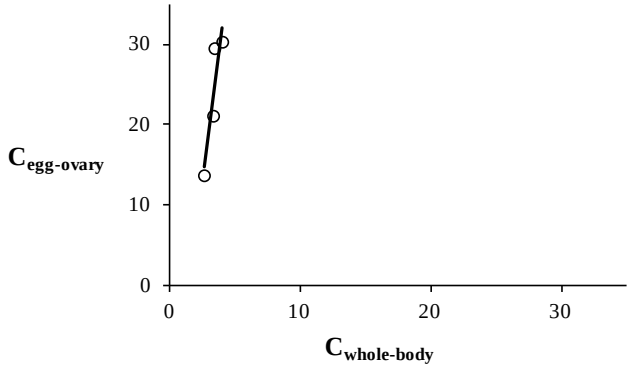


Median ratio: 1.45

R^2: 0.47
F: 31.3
df: 36
P: <0.001

Channel catfish (*Ictalurus punctatus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.40	-	29.50	29.50	8.68
Osmundson et al. 2007	3.30	-	21.10	21.10	6.39
Osmundson et al. 2007	2.60	-	13.70	13.70	5.27
Osmundson et al. 2007	4.00	-	30.30	30.30	7.58



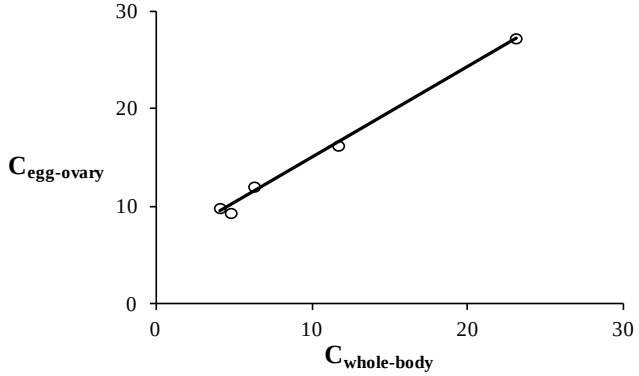
Median ratio: 6.98

R^2: 0.82
F: 9.1
df: 2
P: 0.099

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	6.30	-	12.10	12.10	1.92
Osmundson et al. 2007	4.80	-	9.40	9.40	1.96
Osmundson et al. 2007	11.70	-	16.30	16.30	1.39
Osmundson et al. 2007	23.10	-	27.30	27.30	1.18
Osmundson et al. 2007	4.10	-	9.90	9.90	2.41

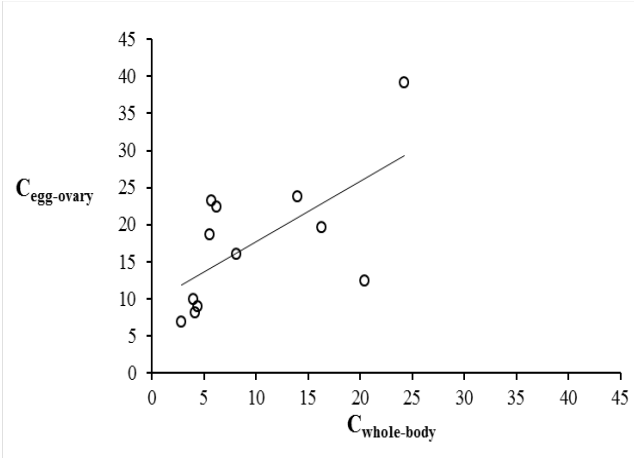


Median ratio: 1.92

R^2: 0.96
F: 584.8
df: 3
P: <0.001

Creek chub (*Semotilus atromaculatus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
GEI 2014	2.89	6.86	-	6.86	2.37
GEI 2014	4	9.94	-	9.94	2.49
GEI 2014	4.14	8.1	-	8.1	1.96
GEI 2014	4.46	8.98	-	8.98	2.01
GEI 2014	5.57	18.63	-	18.63	3.34
GEI 2014	6.23	22.35	-	22.35	3.59
GEI 2014	24.26	39.07	-	39.07	1.61
GEI 2014	20.49	12.38	-	12.38	0.60
GEI 2014	16.33	19.59	-	19.59	1.20
GEI 2014	14.03	23.78	-	23.78	1.69
GEI 2014	5.71	23.21	-	23.21	4.06
GEI 2014	8.17	16.03	-	16.03	1.96



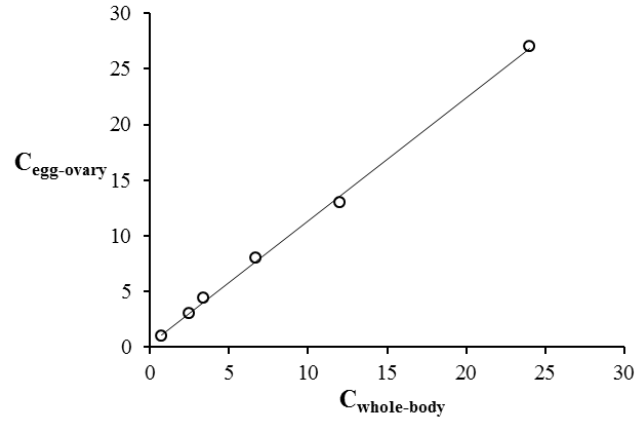
Median ratio: 1.99

R^2: 0.82
F: 7.09
df: 10
P: 0.012

Desert pupfish (*Cyprinodon macularius*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Besser et al. 2012	0.75	1	-	1	1.33
Besser et al. 2012	2.5	3	-	3	1.20
Besser et al. 2012	3.4	4.4	-	4.4	1.29
Besser et al. 2012	6.7	8	-	8	1.19
Besser et al. 2012	12	13	-	13	1.08
Besser et al. 2012	24	27	-	27	1.13

Desert pupfish (*Cyprinodon macularius*)



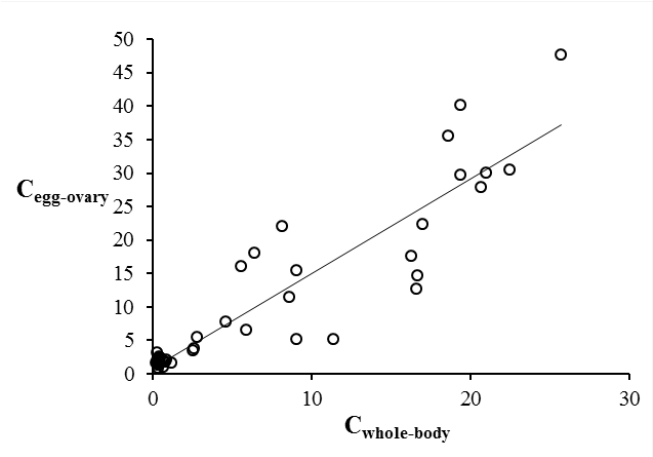
Median ratio: 1.20

R^2: 1.00
F: 194.3
df: 4
P: <0.001

Cutthroat trout (*Oncorhynchus clarkii*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Hardy 2005	0.70	1.00	-	1.00	1.43
Hardy 2005	2.60	3.80	-	3.80	1.46
Hardy 2005	2.80	5.50	-	5.50	1.96
Hardy 2005	6.40	18.00	-	18.00	2.81
Hardy 2005	1.20	1.60	-	1.60	1.33
Hardy 2005	4.60	7.80	-	7.80	1.70
Hardy 2005	5.90	6.60	-	6.60	1.12
Hardy 2005	9.10	5.10	-	5.10	0.56
Hardy 2005	11.40	5.20	-	5.20	0.46
Hardy 2005	5.60	16.00	-	16.00	2.86
Formation 2012	2.56	3.43	-	3.43	1.34
Formation 2012	16.3	17.6	-	17.6	1.08
Formation 2012	20.7	27.9	-	27.9	1.35
Formation 2012	19.4	29.7	-	29.7	1.53
Formation 2012	17	22.3	-	22.3	1.31
Formation 2012	16.7	14.6	-	14.6	0.87
Formation 2012	25.7	47.6	-	47.6	1.85
Formation 2012	8.17	22	-	22	2.69
Formation 2012	9.07	15.4	-	15.4	1.70
Formation 2012	8.63	11.4	-	11.4	1.32
Formation 2012	16.6	12.7	-	12.7	0.77
Formation 2012	19.4	40.1	-	40.1	2.07
Formation 2012	21	30	-	30	1.43
Formation 2012	18.6	35.6	-	35.6	1.91
Formation 2012	22.5	30.5	-	30.5	1.36
Formation 2012 Henry Lake fish hatchery	0.4	1.65	-	1.65	4.13

Cutthroat trout (*Oncorhynchus clarkii*)					
Formation 2012 Henry Lake fish hatchery	0.45	2.03	-	2.03	4.51
Formation 2012 Henry Lake fish hatchery	0.44	2.48	-	2.48	5.64
Formation 2012 Henry Lake fish hatchery	0.36	1.36	-	1.36	3.78
Formation 2012 Henry Lake fish hatchery	0.5	2.33	-	2.33	4.66
Formation 2012 Henry Lake fish hatchery	0.36	0.83	-	0.83	2.31
Formation 2012 Henry Lake fish hatchery	0.44	2.26	-	2.26	5.14
Formation 2012 Henry Lake fish hatchery	0.28	1.87	-	1.87	6.68
Formation 2012 Henry Lake fish hatchery	0.44	1.98	-	1.98	4.50
Formation 2012 Henry Lake fish hatchery	0.43	1.34	-	1.34	3.12
Formation 2012 Henry Lake fish hatchery	0.31	3.23	-	3.23	10.42
Formation 2012 Henry Lake fish hatchery	0.23	1.58	-	1.58	6.87
Formation 2012 Henry Lake fish hatchery	0.72	1.93	-	1.93	2.68
Formation 2012 Henry Lake fish hatchery	0.73	1.79	-	1.79	2.45
Formation 2012 Henry Lake fish hatchery	0.91	2.06	-	2.06	2.26
Formation 2012 Henry Lake fish hatchery	0.85	1.74	-	1.74	2.05



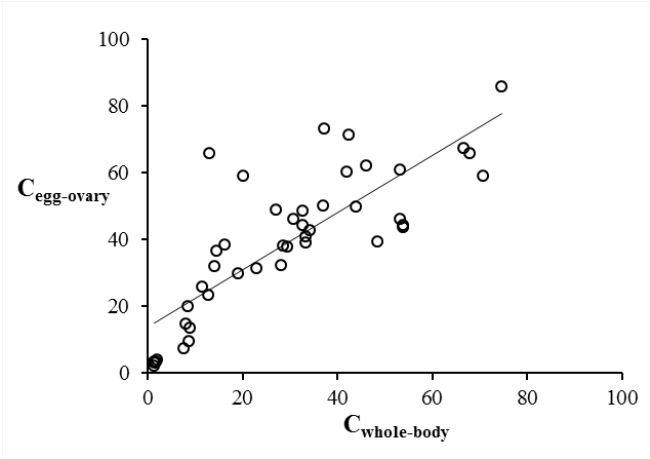
Median ratio: 1.96

R^2: 0.83
F: 194.3
df: 39
P: <0.001

Fathead minnow (*Pimephales promelas*)					
Study	**$C_{whole\text{-}body}$**	**C_{egg}**	**C_{ovary}**	**$C_{egg\text{-}ovary}$**	**Ratio**
GEI 2014	2.04	3.81	-	3.81	1.87
GEI 2014	1.39	2.23	-	2.23	1.60
GEI 2014	1.85	3.31	-	3.31	1.79
GEI 2014	1.32	3.43	-	3.43	2.60
GEI 2014	1.55	3.08	-	3.08	1.99
GEI 2014	37.13	50.06	-	50.06	1.35
GEI 2014	29.54	37.77	-	37.77	1.28
GEI 2014	33.32	40.82	-	40.82	1.23
GEI 2014	28.26	32.23	-	32.23	1.14
GEI 2014	30.74	46.21	-	46.21	1.50

Fathead minnow (*Pimephales promelas*)					
GEI 2014	53.17	60.84	-	60.84	1.14
GEI 2014	48.52	39.28	-	39.28	0.81
GEI 2014	53.81	44.28	-	44.28	0.82
GEI 2014	53.2	46.21	-	46.21	0.87
GEI 2014	54.01	43.51	-	43.51	0.81
GEI 2014	12.93	23.18	-	23.18	1.79
GEI 2014	8.19	14.67	-	14.67	1.79
GEI 2014	14.25	32.04	-	32.04	2.25
GEI 2014	8.65	19.95	-	19.95	2.31
GEI 2014	16.33	38.51	-	38.51	2.36
GEI 2014	7.69	7.39	-	7.39	0.96
GEI 2014	19.05	29.69	-	29.69	1.56
GEI 2014	8.78	9.55	-	9.55	1.09
GEI 2014	14.68	36.58	-	36.58	2.49
GEI 2014	9.02	13.63	-	13.63	1.51
GEI 2014	46.17	61.99	-	61.99	1.34
GEI 2014	41.97	60.07	-	60.07	1.43
GEI 2014	34.33	42.74	-	42.74	1.24
GEI 2014	33.4	38.89	-	38.89	1.16
GEI 2014	42.53	71.24	-	71.24	1.68
GEI 2014	74.56	85.87	-	85.87	1.15
GEI 2014	67.94	65.85	-	65.85	0.97
GEI 2014	70.85	58.91	-	58.91	0.83
GEI 2014	43.93	49.67	-	49.67	1.13
GEI 2014	66.57	67.39	-	67.39	1.01
GEI 2014	20.21	58.91	-	58.91	2.91
GEI 2014	13.08	65.85	-	65.85	5.03
GEI 2014	23.02	31.38	-	31.38	1.36
GEI 2014	11.55	25.72	-	25.72	2.23
GEI 2014	32.8	48.52	-	48.52	1.48
GEI 2014	27.17	48.9	-	48.9	1.80
GEI 2014	28.54	38.04	-	38.04	1.33
GEI 2014	37.2	73.16	-	73.16	1.97
GEI 2014	32.79	44.28	-	44.28	1.35
GEI 2014	46.17	61.99	-	61.99	1.87

Fathead minnow (*Pimephales promelas*)

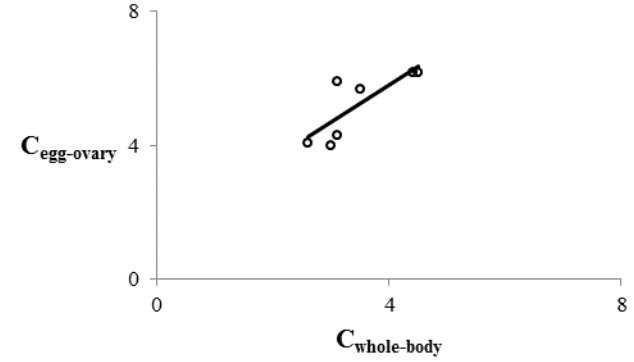


Median ratio: 1.40

R^2: 0.86
F: 81.4
df: 42
P: <0.001

Flannelmouth sucker (*Catostomus latipinnis*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.00	-	4.00	4.00	1.33
Osmundson et al. 2007	2.60	-	4.10	4.10	1.58
Osmundson et al. 2007	3.10	-	5.90	5.90	1.90
Osmundson et al. 2007	3.10	-	4.30	4.30	1.39
Osmundson et al. 2007	3.50	-	5.70	5.70	1.63
Osmundson et al. 2007	4.40	-	6.20	6.20	1.41
Osmundson et al. 2007	4.50	-	6.20	6.20	1.38



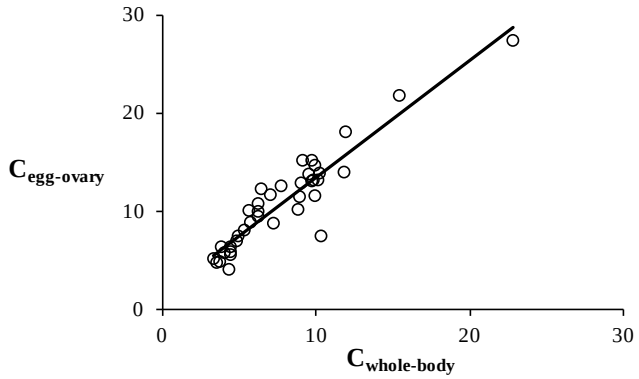
Median ratio: 1.41

R^2: 0.65
F: 9.2
df: 5
P: 0.021

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	22.80	-	27.40	27.40	1.20
Osmundson et al. 2007	8.80	-	10.20	10.20	1.16
Osmundson et al. 2007	15.40	-	21.80	21.80	1.42
Osmundson et al. 2007	4.80	-	7.00	7.00	1.46
Osmundson et al. 2007	5.70	-	8.90	8.90	1.56
Osmundson et al. 2007	4.40	-	6.40	6.40	1.45
Osmundson et al. 2007	3.80	-	6.40	6.40	1.68
Osmundson et al. 2007	11.90	-	18.10	18.10	1.52
Osmundson et al. 2007	6.40	-	12.30	12.30	1.92
Osmundson et al. 2007	9.50	-	13.80	13.80	1.45
Osmundson et al. 2007	9.10	-	15.20	15.20	1.67
Osmundson et al. 2007	6.20	-	10.80	10.80	1.74
Osmundson et al. 2007	7.00	-	11.70	11.70	1.67
Osmundson et al. 2007	7.70	-	12.60	12.60	1.64
Osmundson et al. 2007	6.20	-	10.00	10.00	1.61
Osmundson et al. 2007	10.20	-	13.90	13.90	1.36
Osmundson et al. 2007	9.70	-	15.20	15.20	1.57
Osmundson et al. 2007	9.90	-	14.70	14.70	1.48
Osmundson et al. 2007	7.20	-	8.80	8.80	1.22
Osmundson et al. 2007	9.00	-	12.90	12.90	1.43
Osmundson et al. 2007	9.70	-	13.10	13.10	1.35
Osmundson et al. 2007	8.90	-	11.50	11.50	1.29
Osmundson et al. 2007	9.80	-	13.20	13.20	1.35
Osmundson et al. 2007	9.90	-	11.60	11.60	1.17
Osmundson et al. 2007	10.30	-	7.50	7.50	0.73
Osmundson et al. 2007	5.30	-	8.10	8.10	1.53
Osmundson et al. 2007	10.10	-	13.20	13.20	1.31
Osmundson et al. 2007	11.80	-	14.00	14.00	1.19
Osmundson et al. 2007	3.30	-	5.20	5.20	1.58
Osmundson et al. 2007	4.00	-	5.80	5.80	1.45
Osmundson et al. 2007	4.30	-	4.10	4.10	0.95
Osmundson et al. 2007	3.70	-	4.90	4.90	1.32
Osmundson et al. 2007	6.20	-	9.50	9.50	1.53
Osmundson et al. 2007	3.50	-	4.80	4.80	1.37
Osmundson et al. 2007	4.40	-	5.60	5.60	1.27
Osmundson et al. 2007	5.60	-	10.10	10.10	1.80
Osmundson et al. 2007	4.90	-	7.50	7.50	1.53
Osmundson et al. 2007	4.40	-	5.90	5.90	1.34

Green sunfish (*Lepomis cyanellus*)

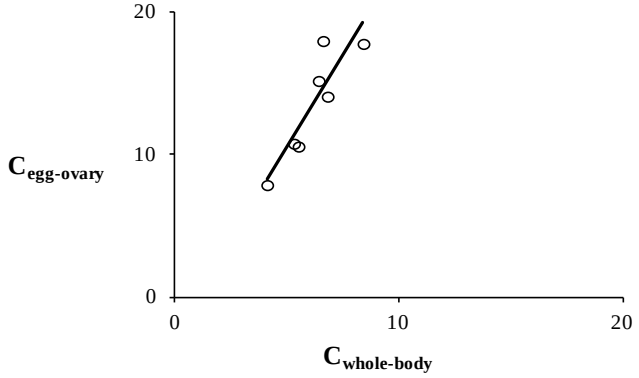


Median ratio: 1.45

R^2: 0.87
F: 240.0
df: 36
P: < 0.001

Roundtail chub (*Gila robusta*)

Study	$C_{whole\text{-}body}$	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	4.10	-	7.90	7.90	1.93
Osmundson et al. 2007	5.30	-	10.80	10.80	2.04
Osmundson et al. 2007	6.40	-	15.20	15.20	2.38
Osmundson et al. 2007	6.80	-	14.10	14.10	2.07
Osmundson et al. 2007	5.50	-	10.60	10.60	1.93
Osmundson et al. 2007	6.60	-	18.00	18.00	2.73
Osmundson et al. 2007	8.40	-	17.80	17.80	2.12

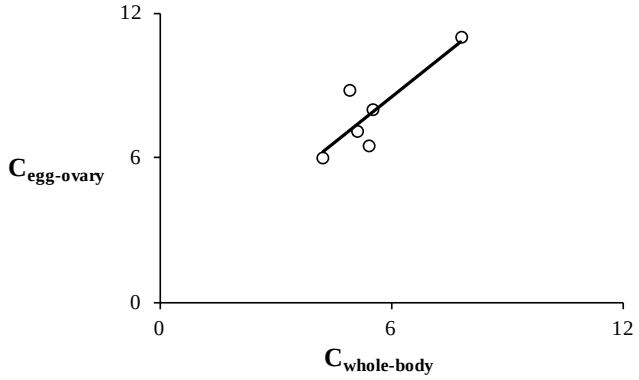


Median ratio: 2.07

R^2: 0.80
F: 20.4
df: 5
P: 0.004

Smallmouth bass (*Micropterus dolomieu*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	4.20	-	6.00	6.00	1.43
Osmundson et al. 2007	5.50	-	8.00	8.00	1.45
Osmundson et al. 2007	5.40	-	6.50	6.50	1.20
Osmundson et al. 2007	7.80	-	11.00	11.00	1.41
Osmundson et al. 2007	5.10	-	7.10	7.10	1.39
Osmundson et al. 2007	4.90	-	8.80	8.80	1.80



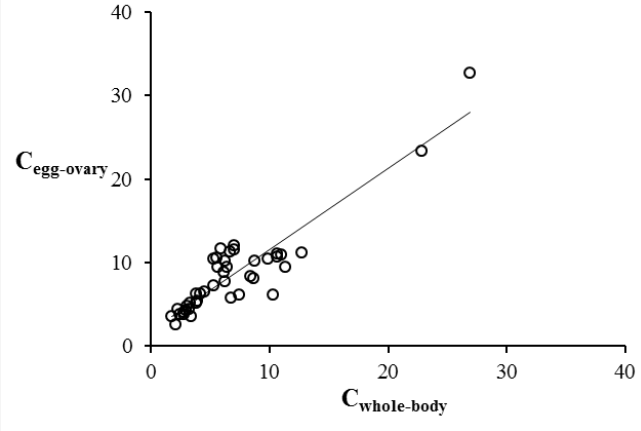
Median ratio: 1.42

R2: 0.73
F: 10.6
df: 4
P: 0.026

White sucker (*Catostomus commersonii*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.80	-	6.20	6.20	1.63
Osmundson et al. 2007	4.20	-	6.20	6.20	1.48
Osmundson et al. 2007	3.30	-	5.20	5.20	1.58
Osmundson et al. 2007	4.50	-	6.50	6.50	1.44
Osmundson et al. 2007	6.30	-	7.70	7.70	1.22
Osmundson et al. 2007	6.80	-	5.80	5.80	0.85
Osmundson et al. 2007	11.00	-	10.90	10.90	0.99
Osmundson et al. 2007	12.70	-	11.20	11.20	0.88
Osmundson et al. 2007	5.70	-	9.40	9.40	1.65
Osmundson et al. 2007	3.90	-	5.40	5.40	1.38
Osmundson et al. 2007	3.80	-	5.10	5.10	1.34
Osmundson et al. 2007	9.90	-	10.40	10.40	1.05
Osmundson et al. 2007	5.30	-	10.40	10.40	1.96
Osmundson et al. 2007	10.70	-	11.00	11.00	1.03
Osmundson et al. 2007	5.90	-	11.70	11.70	1.98
Osmundson et al. 2007	7.00	-	11.60	11.60	1.66
Osmundson et al. 2007	6.40	-	9.40	9.40	1.47
Osmundson et al. 2007	6.30	-	10.20	10.20	1.62
Osmundson et al. 2007	5.30	-	7.30	7.30	1.38
Osmundson et al. 2007	6.20	-	8.90	8.90	1.44

White sucker (*Catostomus commersonii*)					
Osmundson et al. 2007	5.60	-	10.50	10.50	1.88
Osmundson et al. 2007	8.80	-	10.20	10.20	1.16
Osmundson et al. 2007	8.70	-	8.10	8.10	0.93
Osmundson et al. 2007	11.40	-	9.50	9.50	0.83
Osmundson et al. 2007	10.70	-	10.70	10.70	1.00
Osmundson et al. 2007	8.40	-	8.30	8.30	0.99
Osmundson et al. 2007	7.00	-	12.00	12.00	1.71
Osmundson et al. 2007	7.50	-	6.10	6.10	0.81
Osmundson et al. 2007	10.30	-	6.10	6.10	0.59
Osmundson et al. 2007	6.70	-	11.30	11.30	1.69
Osmundson et al. 2007	2.10	-	2.60	2.60	1.24
Osmundson et al. 2007	1.80	-	3.60	3.60	2.00
Osmundson et al. 2007	3.20	-	4.40	4.40	1.38
Osmundson et al. 2007	2.30	-	4.40	4.40	1.91
Osmundson et al. 2007	3.10	-	4.80	4.80	1.55
Osmundson et al. 2007	3.00	-	4.30	4.30	1.43
Osmundson et al. 2007	2.80	-	4.10	4.10	1.46
Osmundson et al. 2007	2.50	-	3.80	3.80	1.52
Osmundson et al. 2007	3.40	-	3.60	3.60	1.06
Osmundson et al. 2007	2.80	-	3.80	3.80	1.36
GEI 2014	26.9	-	32.7	32.7	1.22
GEI 2014	22.9	-	23.3	23.3	1.02



Median ratio: 1.38

R^2: 0.83
F: 200.4
df: 40
P: < 0.001

Table B-2. Summary of egg-ovary to whole body conversion factors (*CF*) from matched pairs of whole-body and egg-ovary measurements.

Common name	Scientific name	Median ratio (*CF*)
Bluegill	*Lepomis macrochirus*	2.13
Bluehead sucker	*Catostomus discobolus*	1.82
Brown trout	*Salmo trutta*	1.45
Common carp	*Cyprinus carpio*	1.92
Creek chub	*Semotilus atromaculatus*	1.99
Cutthroat trout	*Oncorhynchus clarkii*	1.96
Desert pupfish	*Cyprinodon macularius*	1.20
Fathead minnow	*Pimephales promelas*	1.40
Flannelmouth sucker	*Catostomus latipinnis*	1.41
Green sunfish	*Lepomis cyanellus*	1.45
Roundtail chub	*Gila robusta*	2.07
Smallmouth bass	*Micropterus dolomieu*	1.42
White sucker	*Catostomus commersonii*	1.38

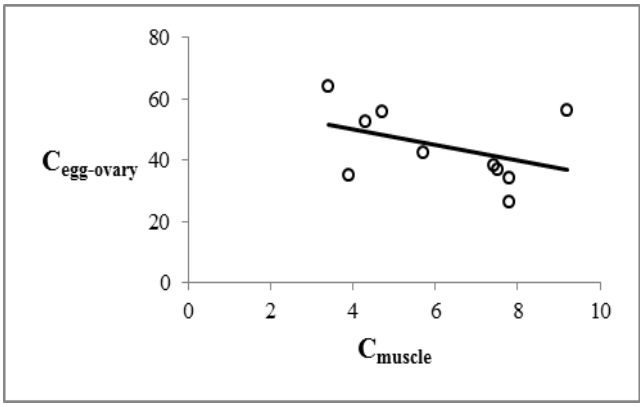
2.3 Muscle to egg-ovary conversion factors

C_{muscle} = Selenium concentration in muscle tissue only (µg/g dw)
C_{egg} = Selenium concentration in eggs (µg/g dw)
C_{ovary} = Selenium concentration in ovary tissue (µg/g dw)
$C_{egg\text{-}ovary}$ = Average selenium concentration in eggs and ovaries $\left(\frac{C_{egg} + C_{o\,\mathrm{var}\,y}}{2}\right)$

Ratio = $\frac{C_{egg-o\,\mathrm{var}\,y}}{C_{muscle}}$

Black bullhead (*Ameiurus melas*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.40	-	64.30	64.30	18.91
Osmundson et al. 2007	3.90	-	35.40	35.40	9.08
Osmundson et al. 2007	4.30	-	52.80	52.80	12.28
Osmundson et al. 2007	4.70	-	56.00	56.00	11.91
Osmundson et al. 2007	5.70	-	42.80	42.80	7.51
Osmundson et al. 2007	7.40	-	38.70	38.70	5.23
Osmundson et al. 2007	7.50	-	37.30	37.30	4.97
Osmundson et al. 2007	7.80	-	34.30	34.30	4.40
Osmundson et al. 2007	7.80	-	26.40	26.40	3.38
Osmundson et al. 2007	9.20	-	56.70	56.70	6.16



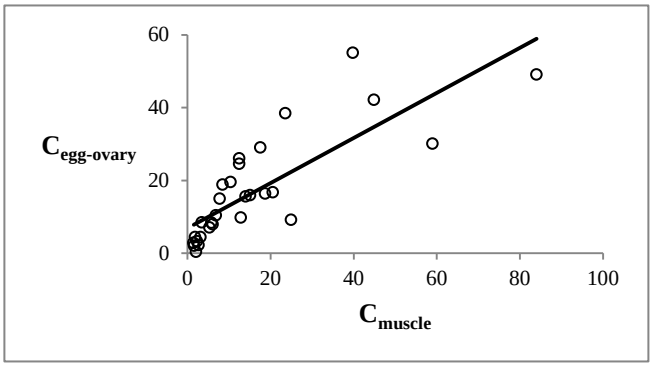
Median ratio: 6.84

R^2: 0.17
F: 1.65
df: 8
P: 0.250

Not used because P > 0.05 and negative slope.

Bluegill (*Lepomis macrochirus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Bryson et al. 1984	84.0	-	49.0	49.0	0.58
Bryson et al. 1985a (pt. 1)	59.0	-	30.0	30.0	0.51
Bryson et al. 1985a (pt. 1)	2.7	-	2.2	2.2	0.81
Bryson et al. 1985a (pt. 2)	25.0	-	9.1	9.1	0.36
Doroshov et al. 1992	1.5	2.8	-	2.8	1.87
Doroshov et al. 1992	5.8	8.3	-	8.3	1.43
Doroshov et al. 1992	10.4	19.5	-	19.5	1.88
Doroshov et al. 1992	23.6	38.4	-	38.4	1.63
Hermanutz et al. 1996	1.6	-	2.0	2.0	1.25
Hermanutz et al. 1996	8.5	-	18.8	18.8	2.21
Hermanutz et al. 1996	14	-	15.5	15.5	1.11
Hermanutz et al. 1996	2.1	-	0.3	0.3	0.14
Hermanutz et al. 1996	20.6	-	16.7	16.7	0.81
Hermanutz et al. 1996	1.9	-	4.4	4.4	2.32
Hermanutz et al. 1996	3.5	-	8.4	8.4	2.40
Hermanutz et al. 1996	17.6	-	29.0	29.0	1.65
Hermanutz et al. 1996	12.5	-	24.5	24.5	1.96
Hermanutz et al. 1996	2.3	-	3.2	3.2	1.39
Hermanutz et al. 1996	6.9	-	10.3	10.3	1.49
Hermanutz et al. 1996	44.9	-	42.1	42.1	0.94
Hermanutz et al. 1996	39.8	-	55.0	55.0	1.38
Hermanutz et al. 1996	5.3	-	7.0	7.0	1.32
Hermanutz et al. 1996	12.5	-	26.0	26.0	2.08
Hermanutz et al. 1996	7.8	-	14.9	14.9	1.91
Hermanutz et al. 1996	3.2	-	4.4	4.4	1.38
Hermanutz et al. 1996	6.1	-	7.9	7.9	1.30
Hermanutz et al. 1996	18.7	-	16.3	16.3	0.87
Hermanutz et al. 1996	15.1	-	15.9	15.9	1.05
Osmundson et al. 2007	12.9	-	9.7	9.7	0.75

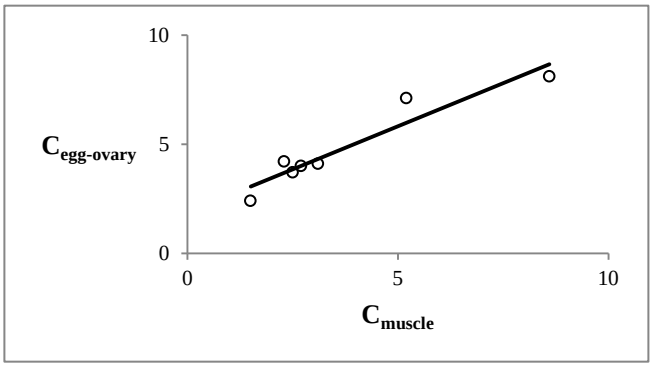


Median ratio: 1.38

R^2: 0.65
F: 50.37
df: 27
P: <0.001

Bluehead sucker (*Catostomus discobolus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	1.5	-	2.4	2.4	1.60
Osmundson et al. 2007	2.3	-	4.2	4.2	1.83
Osmundson et al. 2007	2.5	-	3.7	3.7	1.48
Osmundson et al. 2007	2.7	-	4	4	1.48
Osmundson et al. 2007	3.1	-	4.1	4.1	1.32
Osmundson et al. 2007	5.2	-	7.1	7.1	1.37
Osmundson et al. 2007	8.6	-	8.1	8.1	0.94



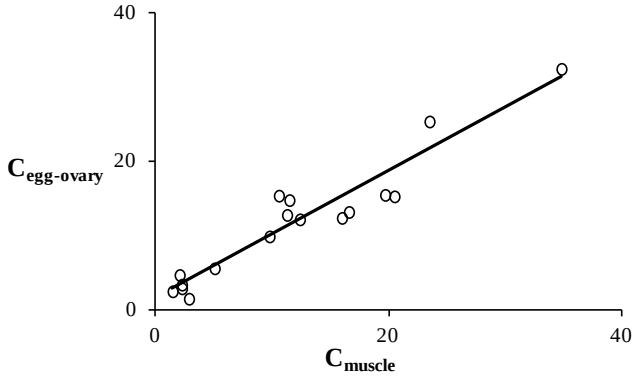
Median ratio: 1.48

R^2: 0.91
F: 47.70
df: 5
P: <0.001

Brook trout (*Salvelinus fontinalis*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Holm et al. 2005	2.80	1.50	-	1.50	0.54
Holm et al. 2005	1.40	2.50	-	2.50	1.79
Holm et al. 2005	2.20	3.40	-	3.40	1.55
Holm et al. 2005	2.00	4.70	-	4.70	2.35
Holm et al. 2005	2.20	2.90	-	2.90	1.32
Holm et al. 2005	5.00	5.60	-	5.60	1.12
Holm et al. 2005	9.70	9.90	-	9.90	1.02
Holm et al. 2005	10.50	15.40	-	15.40	1.47
Holm et al. 2005	11.20	12.80	-	12.80	1.14
Holm et al. 2005	11.40	14.80	-	14.80	1.30
Holm et al. 2005	12.30	12.20	-	12.20	0.99
Holm et al. 2005	15.90	12.40	-	12.40	0.78
Holm et al. 2005	16.50	13.20	-	13.20	0.80
Holm et al. 2005	19.60	15.50	-	15.50	0.79
Holm et al. 2005	20.40	15.30	-	15.30	0.75
Holm et al. 2005	23.40	25.40	-	25.40	1.09
Holm et al. 2005	34.70	32.50	-	32.50	0.94

Brook trout (*Salvelinus fontinalis*)

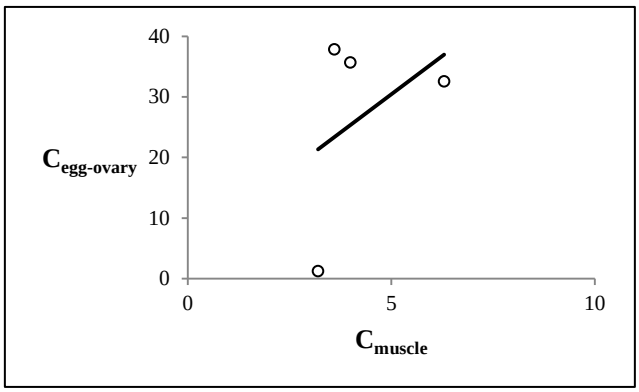


Median ratio: 1.09

R^2: 0.91
F: 152.3
df: 15
P: < 0.001

Brown trout (*Salmo trutta*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.2	-	1.2	1.2	0.38
Osmundson et al. 2007	3.6	-	37.8	37.8	10.50
Osmundson et al. 2007	4	-	35.6	35.6	8.90
Osmundson et al. 2007	6.3	-	32.5	32.5	5.16



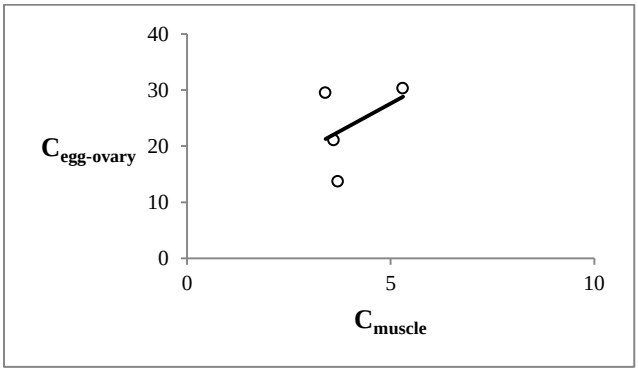
Median ratio: 7.03

R^2: 0.17
F: 0.40
df: 2
P: 0.71

Not used because P > 0.05.

Channel catfish (*Ictaluris punctatus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	3.4	-	29.5	29.5	8.68
Osmundson et al. 2007	3.6	-	21.1	21.1	5.86
Osmundson et al. 2007	3.7	-	13.7	13.7	3.70
Osmundson et al. 2007	5.3	-	30.3	30.3	5.72



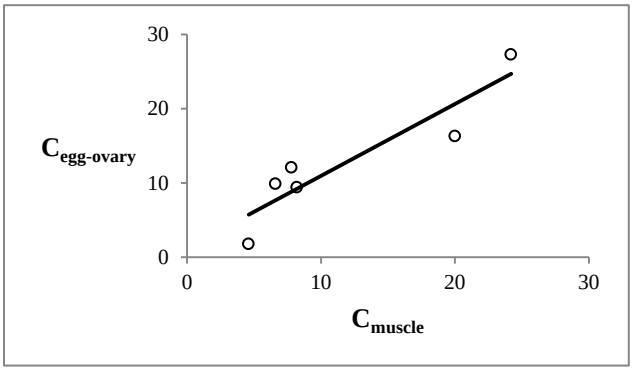
Median ratio: 5.79

R^2: 0.20
F: 0.49
df: 2
P: 0.67

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Garcia-Hernandez 2000	4.6	-	1.8	1.8	0.39
Osmundson et al. 2007	7.8	-	12.1	12.1	1.55
Osmundson et al. 2007	8.2	-	9.4	9.4	1.15
Osmundson et al. 2007	20	-	16.3	16.3	0.82
Osmundson et al. 2007	24.2	-	27.3	27.3	1.13
Osmundson et al. 2007	6.6	-	9.9	9.9	1.50



Median ratio: 1.14

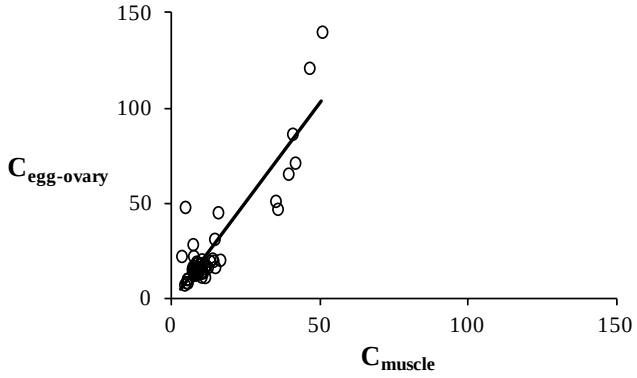
R^2: 0.84
F: 21.7
df: 4
P: 0.007

Cutthroat trout (*Oncorhynchus clarkii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Golder 2005	6.80	-	28.20	28.20	4.15
Golder 2005	4.20	-	47.80	47.80	11.38
Golder 2005	3.00	-	22.00	22.00	7.33
Golder 2005	4.90	-	9.80	9.80	2.00
Golder 2005	4.50	-	8.20	8.20	1.82
Golder 2005	4.00	-	7.00	7.00	1.75
Golder 2005	5.00	-	10.00	10.00	2.00
Golder 2005	5.00	-	10.00	10.00	2.00
Golder 2005	5.00	-	8.00	8.00	1.60
Golder 2005	8.40	-	16.20	16.20	1.93
Golder 2005	8.30	-	18.30	18.30	2.20
Golder 2005	7.00	-	14.30	14.30	2.04
Golder 2005	6.60	-	14.30	14.30	2.17
Golder 2005	8.40	-	14.70	14.70	1.75
Golder 2005	9.80	-	16.40	16.40	1.67
Golder 2005	8.50	-	15.90	15.90	1.87
Golder 2005	16.00	-	20.00	20.00	1.25
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	8.00	-	19.00	19.00	2.38
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	9.00	-	16.00	16.00	1.78
Golder 2005	7.00	-	13.00	13.00	1.86
Golder 2005	7.00	-	14.00	14.00	2.00
Golder 2005	8.00	-	14.00	14.00	1.75
Golder 2005	9.80	-	20.20	20.20	2.06
Golder 2005	7.00	-	22.00	22.00	3.14
Golder 2005	9.00	-	16.00	16.00	1.78
Golder 2005	7.00	-	12.00	12.00	1.71
Golder 2005	8.00	-	13.00	13.00	1.63
Golder 2005	10.00	-	14.00	14.00	1.40
Kennedy et al. 2000	41.30	75.40	66.80	71.10	1.72
Kennedy et al. 2000	15.30	58.40	31.60	45.00	2.94
Kennedy et al. 2000	14.10	30.60	31.40	31.00	2.20
Kennedy et al. 2000	12.50	20.20	18.50	19.35	1.55
Kennedy et al. 2000	13.70	19.40	19.50	19.45	1.42
Kennedy et al. 2000	14.30	16.20	16.20	16.20	1.13
Kennedy et al. 2000	9.50	16.10	19.30	17.70	1.86
Kennedy et al. 2000	9.40	14.40	22.00	18.20	1.94
Kennedy et al. 2000	8.70	13.20	17.00	15.10	1.74
Kennedy et al. 2000	9.50	12.60	13.60	13.10	1.38
Kennedy et al. 2000	10.20	12.30	14.50	13.40	1.31

Cutthroat trout (*Oncorhynchus clarkii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Kennedy et al. 2000	10.70	10.50	20.60	15.55	1.45
Kennedy et al. 2000	6.60	9.90	21.50	15.70	2.38
Kennedy et al. 2000	9.70	9.10	13.20	11.15	1.15
Kennedy et al. 2000	10.90	8.50	13.40	10.95	1.00
Kennedy et al. 2000	6.90	13.20	20.30	16.75	2.43
Rudolph et al. 2007	7.70	13.90	-	13.90	1.81
Rudolph et al. 2007	8.20	12.50	-	12.50	1.52
Rudolph et al. 2007	8.00	15.00	-	15.00	1.88
Rudolph et al. 2007	8.10	14.90	-	14.90	1.84
Rudolph et al. 2007	6.60	15.20	-	15.20	2.30
Rudolph et al. 2007	8.50	12.90	-	12.90	1.52
Rudolph et al. 2007	7.20	12.30	-	12.30	1.71
Rudolph et al. 2007	7.30	16.70	-	16.70	2.29
Rudolph et al. 2007	7.60	13.10	-	13.10	1.72
Rudolph et al. 2007	8.70	15.60	-	15.60	1.79
Rudolph et al. 2007	8.20	13.90	-	13.90	1.70
Rudolph et al. 2007	7.90	15.10	-	15.10	1.91
Rudolph et al. 2007	7.60	12.30	-	12.30	1.62
Rudolph et al. 2007	11.80	16.10	-	16.10	1.36
Rudolph et al. 2007	40.40	86.30	-	86.30	2.14
Rudolph et al. 2007	46.10	121.00	-	121.00	2.62
Rudolph et al. 2007	50.40	140.00	-	140.00	2.78
Rudolph et al. 2007	34.70	51.00	-	51.00	1.47
Rudolph et al. 2007	39.00	65.30	-	65.30	1.67
Rudolph et al. 2007	35.40	46.80	-	46.80	1.32
Rudolph et al. 2007	11.30	16.90	-	16.90	1.50
Rudolph et al. 2007	13.40	20.60	-	20.60	1.54

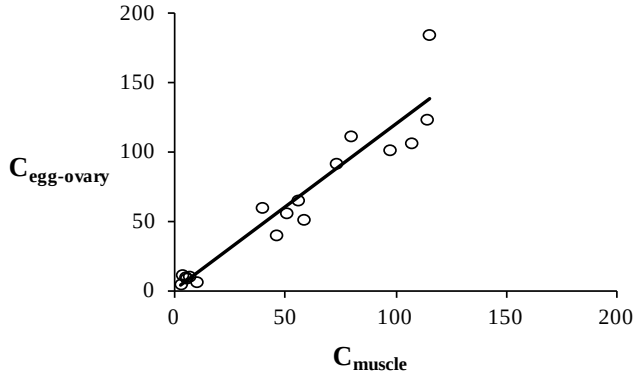


Median ratio: 1.81

R^2: 0.82
F: 308.3
df: 67
P: < 0.001

Dolly Varden (*Salvelinus malma*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Golder 2009	73.00	92.30	-	92.30	1.26
Golder 2009	45.90	40.70	-	40.70	0.89
Golder 2009	107.00	107.00	-	107.00	1.00
Golder 2009	97.20	102.00	-	102.00	1.05
Golder 2009	114.00	124.00	-	124.00	1.09
Golder 2009	115.00	185.00	-	185.00	1.61
Golder 2009	79.60	112.00	-	112.00	1.41
Golder 2009	9.90	7.00	-	7.00	0.71
Golder 2009	3.40	12.10	-	12.10	3.56
Golder 2009	5.30	9.60	-	9.60	1.81
Golder 2009	2.80	5.40	-	5.40	1.93
Golder 2009	4.90	10.50	-	10.50	2.14
Golder 2009	6.60	11.00	-	11.00	1.67
Golder 2009	55.70	65.80	-	65.80	1.18
Golder 2009	58.30	51.90	-	51.90	0.89
Golder 2009	39.50	60.50	-	60.50	1.53
Golder 2009	50.50	56.60	-	56.60	1.12

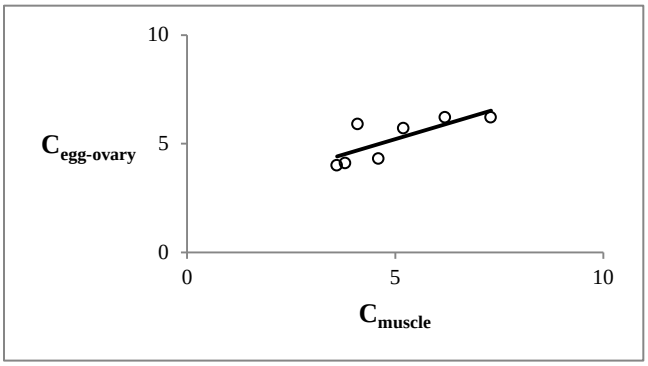


Median ratio: 1.26

R^2: 0.90
F: 140.3
df: 15
P: < 0. 001

Flannelmouth sucker (*Catostomus latipinnis*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.6	-	4.0	4.0	1.11
Osmundson et al. 2007	3.8	-	4.1	4.1	1.08
Osmundson et al. 2007	4.1	-	5.9	5.9	1.44
Osmundson et al. 2007	4.6	-	4.3	4.3	0.93
Osmundson et al. 2007	5.2	-	5.7	5.7	1.10
Osmundson et al. 2007	6.2	-	6.2	6.2	1.00
Osmundson et al. 2007	7.3	-	6.2	6.2	0.85



Median ratio: 1.08

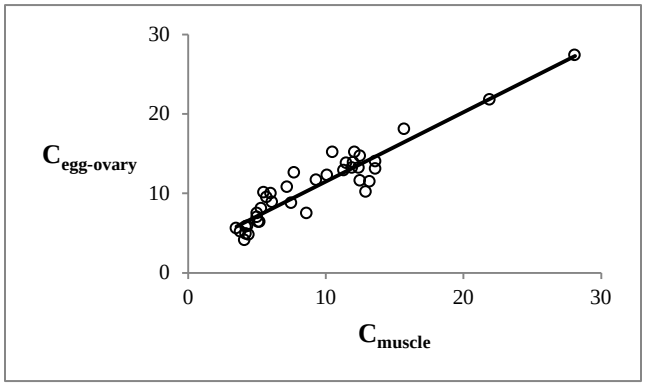
R^2: 0.58
F: 6.92
df: 5
P: 0.036

Green sunfish (*Lepomis cyanellus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	28.1	-	27.4	27.4	0.98
Osmundson et al. 2007	12.9	-	10.2	10.2	0.79
Osmundson et al. 2007	21.9	-	21.8	21.8	1.00
Osmundson et al. 2007	5	-	7	7	1.40
Osmundson et al. 2007	6.1	-	8.9	8.9	1.46
Osmundson et al. 2007	5.2	-	6.4	6.4	1.23
Osmundson et al. 2007	5.1	-	6.4	6.4	1.25
Osmundson et al. 2007	15.7	-	18.1	18.1	1.15
Osmundson et al. 2007	10.1	-	12.3	12.3	1.22
Osmundson et al. 2007	11.5	-	13.8	13.8	1.20
Osmundson et al. 2007	10.5	-	15.2	15.2	1.45
Osmundson et al. 2007	7.2	-	10.8	10.8	1.50
Osmundson et al. 2007	9.3	-	11.7	11.7	1.26
Osmundson et al. 2007	7.7	-	12.6	12.6	1.64
Osmundson et al. 2007	6	-	10	10	1.67
Osmundson et al. 2007	12	-	13.9	13.9	1.16
Osmundson et al. 2007	12.1	-	15.2	15.2	1.26
Osmundson et al. 2007	12.5	-	14.7	14.7	1.18
Osmundson et al. 2007	7.5	-	8.8	8.8	1.17
Osmundson et al. 2007	11.3	-	12.9	12.9	1.14

Green sunfish (*Lepomis cyanellus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	13.6	-	13.1	13.1	0.96
Osmundson et al. 2007	13.2	-	11.5	11.5	0.87
Osmundson et al. 2007	12.4	-	13.2	13.2	1.06
Osmundson et al. 2007	12.5	-	11.6	11.6	0.93
Osmundson et al. 2007	8.6	-	7.5	7.5	0.87
Osmundson et al. 2007	5.3	-	8.1	8.1	1.53
Osmundson et al. 2007	11.9	-	13.2	13.2	1.11
Osmundson et al. 2007	13.6	-	14	14	1.03
Osmundson et al. 2007	3.8	-	5.2	5.2	1.37
Osmundson et al. 2007	4.2	-	5.8	5.8	1.38
Osmundson et al. 2007	4.1	-	4.1	4.1	1.00
Osmundson et al. 2007	4.2	-	4.9	4.9	1.17
Osmundson et al. 2007	5.7	-	9.5	9.5	1.67
Osmundson et al. 2007	4.4	-	4.8	4.8	1.09
Osmundson et al. 2007	3.5	-	5.6	5.6	1.60
Osmundson et al. 2007	5.5	-	10.1	10.1	1.84
Osmundson et al. 2007	5	-	7.5	7.5	1.50
Osmundson et al. 2007	4.3	-	5.9	5.9	1.37

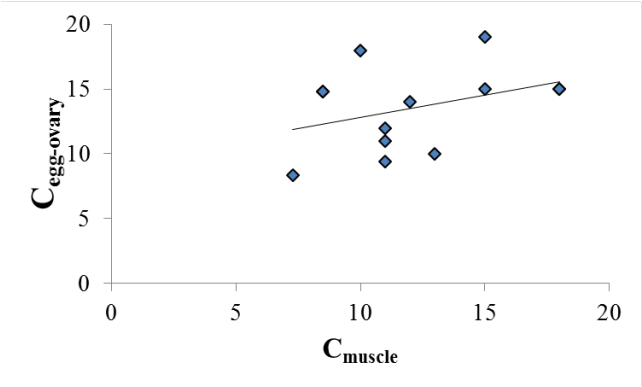


Median ratio: 1.21

R^2: 0.89
F: 281.4
df: 36
P: <0.001

Largemouth bass (*Micropterus salmoides*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Carolina Power & Light 1997	8.48	-	14.79	14.79	1.74
Carolina Power & Light 1997	8.48	-	14.79	14.79	1.74
Carolina Power & Light 1997	7.29	-	8.35	8.35	1.15
Carolina Power & Light 1997	15	-	19	19	1.27
Carolina Power & Light 1997	15	-	15	15	1.00
Carolina Power & Light 1997	12	-	14	14	1.17
Carolina Power & Light 1997	10	-	18	18	1.80
Carolina Power & Light 1997	18	-	15	15	0.83
Carolina Power & Light 1997	18	-	15	15	0.83
Carolina Power & Light 1997	11	-	12	12	1.09
Carolina Power & Light 1997	11	-	9.4	9.4	0.85
Carolina Power & Light 1997	13	-	10	10	0.77
Carolina Power & Light 1997	11	-	11	11	1.00

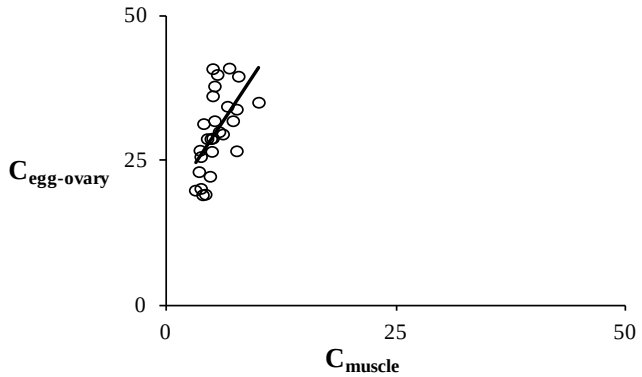


Median ratio: 1.09

R^2: 0.14
F: 1.74
df: 11
P: 0.22
Not used because P>0.05

Mountain whitefish (*Prosopium williamsoni*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Golder 2005	3.60	-	26.90	26.90	7.47
Golder 2005	3.70	-	25.80	25.80	6.97
Golder 2005	3.10	-	20.00	20.00	6.45
Golder 2005	4.20	-	19.30	19.30	4.60
Golder 2005	3.90	-	19.20	19.20	4.92
Golder 2005	3.50	-	23.20	23.20	6.63
Golder 2005	5.20	-	38.00	38.00	7.31
Golder 2005	5.00	-	41.00	41.00	8.20
Golder 2005	5.20	-	32.00	32.00	6.15
Golder 2005	7.60	-	34.00	34.00	4.47
Golder 2005	7.20	-	32.00	32.00	4.44
Golder 2005	5.50	-	40.00	40.00	7.27
Golder 2005	7.80	-	39.70	39.70	5.09
Golder 2005	3.70	-	20.30	20.30	5.49
Golder 2005	4.70	-	22.40	22.40	4.77
Golder 2005	4.40	-	28.90	28.90	6.57
Golder 2005	5.70	-	30.10	30.10	5.28
Golder 2005	4.00	-	31.50	31.50	7.88
Golder 2005	10.00	-	35.20	35.20	3.52
Golder 2005	4.90	-	26.70	26.70	5.45
Golder 2005	7.60	-	26.80	26.80	3.53
Golder 2005	6.10	-	29.70	29.70	4.87
Golder 2005	6.80	-	41.10	41.10	6.04
Golder 2005	5.00	-	29.00	29.00	5.80
Golder 2005	6.60	-	34.50	34.50	5.23
Golder 2005	5.00	-	36.30	36.30	7.26
Golder 2005	4.80	-	28.90	28.90	6.02

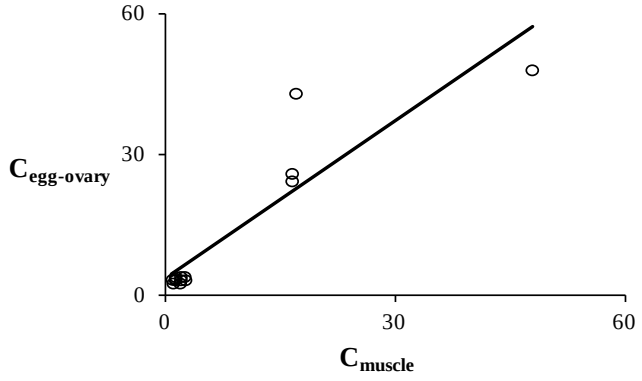


Median ratio: 5.80

R^2: 0.33
F: 12.4
df: 25
P: <0.001

Northern pike (*Esox lucius*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Muscatello et al. 2006	0.90	3.50	-	3.50	3.89
Muscatello et al. 2006	1.90	2.70	-	2.70	1.42
Muscatello et al. 2006	2.60	3.40	-	3.40	1.31
Muscatello et al. 2006	1.30	3.70	-	3.70	2.85
Muscatello et al. 2006	1.00	2.70	-	2.70	2.70
Muscatello et al. 2006	17.00	43.20	-	43.20	2.54
Muscatello et al. 2006	16.50	24.50	-	24.50	1.48
Muscatello et al. 2006	16.50	26.10	-	26.10	1.58
Muscatello et al. 2006	2.00	3.40	-	3.40	1.70
Muscatello et al. 2006	2.00	4.10	-	4.10	2.05
Muscatello et al. 2006	1.30	4.10	-	4.10	3.15
Muscatello et al. 2006	2.50	4.10	-	4.10	1.64
Muscatello et al. 2006	1.30	3.40	-	3.40	2.62
Muscatello et al. 2006	47.80	48.20	-	48.20	1.01



Median ratio: 1.88

R^2: 0.83
F: 58.9
df: 12
P: <0.001

Rainbow trout (*Oncorhynchus mykiss*)

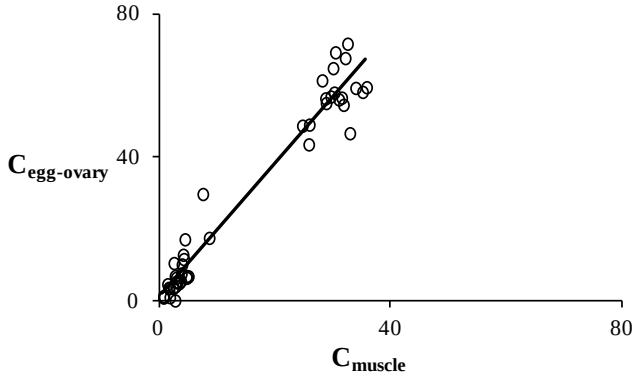
Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.10	11.60	-	11.60	2.83
Casey and Siwik 2000	3.80	10.10	-	10.10	2.66
Casey and Siwik 2000	2.60	0.10	-	0.10	0.04
Casey and Siwik 2000	3.30	4.90	-	4.90	1.48
Casey and Siwik 2000	2.30	3.60	-	3.60	1.57
Casey and Siwik 2000	2.80	5.30	-	5.30	1.89
Casey and Siwik 2000	2.30	3.70	-	3.70	1.61
Casey and Siwik 2000	2.80	6.40	-	6.40	2.29
Casey and Siwik 2000	3.00	5.20	-	5.20	1.73
Casey and Siwik 2000	4.90	6.80	-	6.80	1.39
Casey and Siwik 2000	1.50	3.60	-	3.60	2.40
Casey and Siwik 2000	2.60	6.90	-	6.90	2.65

Rainbow trout (*Oncorhynchus mykiss*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.60	6.90	-	6.90	1.50
Casey and Siwik 2000	4.60	6.40	-	6.40	1.39
Casey and Siwik 2000	3.60	5.50	-	5.50	1.53
Casey and Siwik 2000	2.40	10.50	-	10.50	4.38
Casey and Siwik 2000	3.70	7.60	-	7.60	2.05
Casey and Siwik 2000	2.70	4.10	-	4.10	1.52
Casey and Siwik 2000	0.70	1.10	-	1.10	1.57
Casey and Siwik 2000	0.60	0.90	-	0.90	1.50
Casey and Siwik 2000	0.60	1.30	-	1.30	2.17
Casey and Siwik 2000	28.60	56.30	-	56.30	1.97
Casey and Siwik 2000	30.90	56.00	-	56.00	1.81
Casey and Siwik 2000	32.40	71.50	-	71.50	2.21
Casey and Siwik 2000	28.00	61.30	-	61.30	2.19
Casey and Siwik 2000	31.70	54.50	-	54.50	1.72
Casey and Siwik 2000	29.50	56.80	-	56.80	1.93
Casey and Siwik 2000	30.10	57.90	-	57.90	1.92
Casey and Siwik 2000	29.90	64.70	-	64.70	2.16
Casey and Siwik 2000	32.80	46.60	-	46.60	1.42
Casey and Siwik 2000	31.40	56.50	-	56.50	1.80
Casey and Siwik 2000	32.00	67.50	-	67.50	2.11
Casey and Siwik 2000	35.70	59.40	-	59.40	1.66
Casey and Siwik 2000	24.60	48.70	-	48.70	1.98
Casey and Siwik 2000	30.30	69.10	-	69.10	2.28
Casey and Siwik 2000	25.70	43.50	-	43.50	1.69
Casey and Siwik 2000	35.00	58.10	-	58.10	1.66
Casey and Siwik 2000	33.80	59.20	-	59.20	1.75
Casey and Siwik 2000	28.70	55.00	-	55.00	1.92
Casey and Siwik 2000	25.80	49.00	-	49.00	1.90
Holm et al. 2005	1.70	1.00	-	1.00	0.59
Holm et al. 2005	1.60	3.50	-	3.50	2.19
Holm et al. 2005	1.30	4.60	-	4.60	3.54
Holm et al. 2005	4.00	12.80	-	12.80	3.20
Holm et al. 2005	4.30	17.10	-	17.10	3.98
Holm et al. 2005	8.50	17.50	-	17.50	2.06
Holm et al. 2005	7.40	29.70	-	29.70	4.01

Rainbow trout (*Oncorhynchus mykiss*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio



Median ratio: 1.92

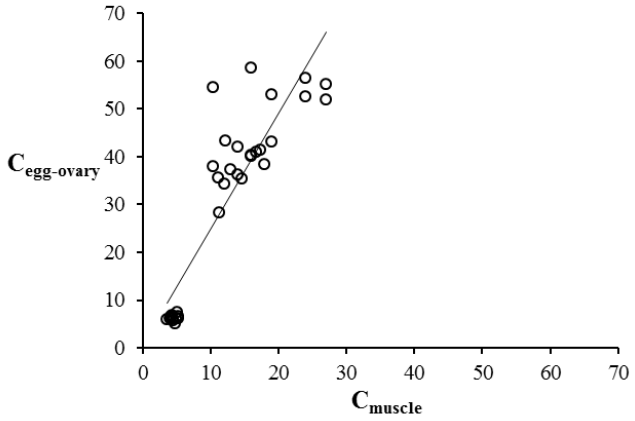
R^2: 0.96
F: 990.0
df: 45
P: <0. 001

Razorback sucker (*Xyrauchen texanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Hamilton et al. 2001a	5	7.5	-	7.5	1.50
Hamilton et al. 2001a	4	6.1	-	6.1	1.53
Hamilton et al. 2001a	4.2	6.6	-	6.6	1.57
Hamilton et al. 2001a	4.4	6.2	-	6.2	1.41
Hamilton et al. 2001a	4.5	5.8	-	5.8	1.29
Hamilton et al. 2001a	4.3	6.8	-	6.8	1.58
Hamilton et al. 2001a	11.1	35.5	-	35.5	3.20
Hamilton et al. 2001a	12.2	43.4	-	43.4	3.56
Hamilton et al. 2001a	10.4	54.5	-	54.5	5.24
Hamilton et al. 2001a	11.3	28.2	-	28.2	2.50
Hamilton et al. 2001a	10.4	38	-	38	3.65
Hamilton et al. 2001a	17.3	41.3	-	41.3	2.39
Hamilton et al. 2001a	13	37.2	-	37.2	2.86
Hamilton et al. 2001a	16.7	40.9	-	40.9	2.45
Hamilton et al. 2001a	14.6	35.3	-	35.3	2.42
Hamilton et al. 2001a	12.1	34.3	-	34.3	2.83
Hamilton et al. 2001b	4.7	5	-	5	1.06
Hamilton et al. 2001b	5.3	6.2	-	6.2	1.17
Hamilton et al. 2001b	3.6	5.9	-	5.9	1.64
Hamilton et al. 2001b	5.3	6.5	-	6.5	1.23
Hamilton et al. 2001b	4.1	6.35	-	6.35	1.55
Hamilton et al. 2001b	4.9	6.1	-	6.1	1.24
Hamilton et al. 2001b	16	40.1	-	40.1	2.51
Hamilton et al. 2001b	18	38.4	-	38.4	2.13
Hamilton et al. 2001b	16	40.2	-	40.2	2.51
Hamilton et al. 2001b	19	43.1	-	43.1	2.27

Razorback sucker (*Xyrauchen texanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Hamilton et al. 2001b	14	41.9	-	41.9	2.99
Hamilton et al. 2001b	14	36.2	-	36.2	2.59
Hamilton et al. 2001b	24	56.5	-	56.5	2.35
Hamilton et al. 2001b	27	51.8	-	51.8	1.92
Hamilton et al. 2001b	24	52.6	-	52.6	2.19
Hamilton et al. 2001b	27	55.1	-	55.1	2.04
Hamilton et al. 2001b	19	53	-	53	2.79
Hamilton et al. 2001b	16	58.5	-	58.5	3.66
Waddell and May 1995 [a]	4.40	3.70	-	3.70	×
Waddell and May 1995 [a]	7.10	4.70	-	4.70	×
Waddell and May 1995 [a]	32.00	10.60	-	10.60	×



Median ratio: 2.31

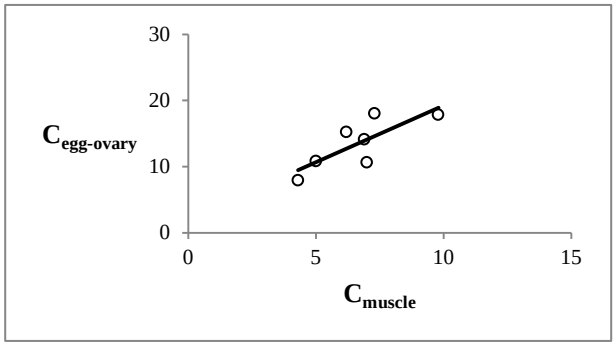
R^2: 0.80
F: 125.6
df: 32
P: <0.001

[a] Data from this study were excluded because results were atypical.

Roundtail chub (*Gila robusta*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	4.3	-	7.9	7.9	1.84
Osmundson et al. 2007	5	-	10.8	10.8	2.16
Osmundson et al. 2007	6.2	-	15.2	15.2	2.45
Osmundson et al. 2007	6.9	-		14.1	2.04
Osmundson et al. 2007	7	-	10.6	10.6	1.51
Osmundson et al. 2007	7.3	-	18	18	2.47
Osmundson et al. 2007	9.8	-	17.8	17.8	1.82

Roundtail chub (*Gila robusta*)

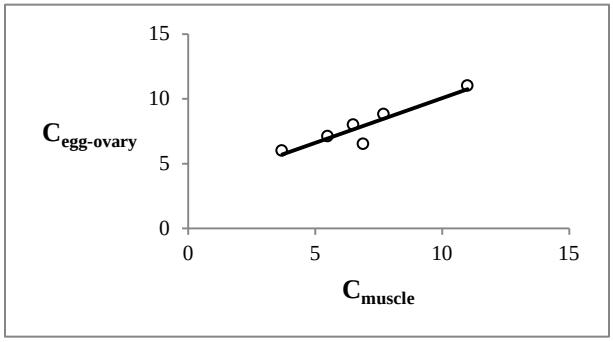


Median ratio: 2.04

R^2: 0.62
F: 8.27
df: 5
P: 0.026

Smallmouth bass (*Micropterus dolomieu*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	Ratio
Osmundson et al. 2007	3.7	-	6.0	6.0	1.62
Osmundson et al. 2007	6.5	-	8.0	8.0	1.23
Osmundson et al. 2007	6.9	-	6.5	6.5	0.94
Osmundson et al. 2007	11			11	1.00
Osmundson et al. 2007	5.5	-	7.1	7.1	1.29
Osmundson et al. 2007	7.7	-	8.8	8.8	1.14

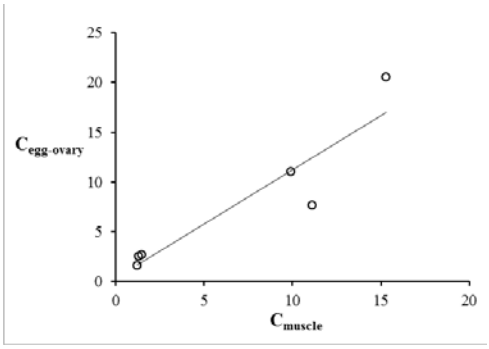


Median ratio: 1.19

R^2: 0.85
F: 23.5
df: 4
P: 0.006

White Sturgeon (*Acipenser transmontanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Linville 2006	1.28	2.46	-	2.46	2.46
Linville 2006	1.22	1.61	-	1.61	1.61
Linville 2006	1.48	2.68	-	2.68	2.68
Linville 2006	9.93	11	-	11	11
Linville 2006	15.3	20.5	-	20.5	20.5
Linville 2006	11.1	7.61	-	7.61	7.61



Median ratio: 1.33

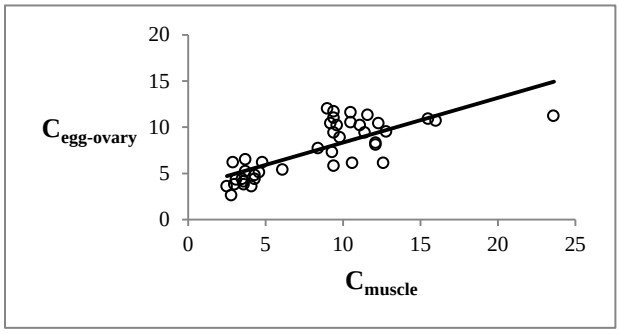
R^2: 0.86
F: 24.96
df: 4
P: 0.006

White Sucker (*Catostomus commersonii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	2.9	-	6.2	6.2	2.14
Osmundson et al. 2007	4.8	-	6.2	6.2	1.29
Osmundson et al. 2007	3.7	-	5.2	5.2	1.41
Osmundson et al. 2007	3.7	-	6.5	6.5	1.76
Osmundson et al. 2007	8.4	-	7.7	7.7	0.92
Osmundson et al. 2007	9.4	-	5.8	5.8	0.62
Osmundson et al. 2007	15.5	-	10.9	10.9	0.70
Osmundson et al. 2007	23.6	-	11.2	11.2	0.47
Osmundson et al. 2007	9.4	-	9.4	9.4	1.00
Osmundson et al. 2007	6.1	-	5.4	5.4	0.89
Osmundson et al. 2007	4.6	-	5.1	5.1	1.11
Osmundson et al. 2007	12.3	-	10.4	10.4	0.85
Osmundson et al. 2007	9.2	-	10.4	10.4	1.13
Osmundson et al. 2007	9.4	-	11	11	1.17
Osmundson et al. 2007	9.4	-	11.7	11.7	1.24
Osmundson et al. 2007	10.5	-	11.6	11.6	1.10
Osmundson et al. 2007	11.4	-	9.4	9.4	0.82
Osmundson et al. 2007	9.6	-	10.2	10.2	1.06
Osmundson et al. 2007	9.3	-	7.3	7.3	0.78
Osmundson et al. 2007	9.8	-	8.9	8.9	0.91
Osmundson et al. 2007	10.5	-	10.5	10.5	1.00

White Sucker (*Catostomus commersonii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Osmundson et al. 2007	11.1	-	10.2	10.2	0.92
Osmundson et al. 2007	12.1	-	8.1	8.1	0.67
Osmundson et al. 2007	12.8	-	9.5	9.5	0.74
Osmundson et al. 2007	16.0	-	10.7	10.7	0.67
Osmundson et al. 2007	12.1	-	8.3	8.3	0.69
Osmundson et al. 2007	9.0	-	12	12	1.33
Osmundson et al. 2007	10.6	-	6.1	6.1	0.58
Osmundson et al. 2007	12.6	-	6.1	6.1	0.48
Osmundson et al. 2007	11.6	-	11.3	11.3	0.97
Osmundson et al. 2007	2.8	-	2.6	2.6	0.93
Osmundson et al. 2007	2.5	-	3.6	3.6	1.44
Osmundson et al. 2007	4.3	-	4.4	4.4	1.02
Osmundson et al. 2007	3.5	-	4.4	4.4	1.26
Osmundson et al. 2007	4.3	-	4.8	4.8	1.12
Osmundson et al. 2007	3.1	-	4.3	4.3	1.39
Osmundson et al. 2007	3.6	-	4.1	4.1	1.14
Osmundson et al. 2007	3.0	-	3.8	3.8	1.27
Osmundson et al. 2007	4.1	-	3.6	3.6	0.88
Osmundson et al. 2007	3.6	-	3.8	3.8	1.06



Median ratio: 1.00

R^2: 0.59
F: 53.92
df: 38
P: < 0.001

Table B-3. Summary of egg-ovary to muscle conversion factors.

Common name	Scientific name	Median ratio
Bluegill	*Lepomis macrochirus*	1.38
Bluehead sucker	*Catostomus discobolus*	1.48
Brook trout	*Salvelinus fontinalis*	1.09
Common carp	*Cyprinus carpio*	1.14
Cutthroat trout	*Oncorhynchus clarkii*	1.81
Dolly Varden	*Salvelinus malma*	1.26
Flannelmouth sucker	*Catostomus latipinnis*	1.08
Green sunfish	*Lepomis cyanellus*	1.21
Mountain whitefish	*Prosopium williamsoni*	5.80
Northern pike	*Esox lucius*	1.88
Rainbow trout	*Oncorhynchus mykiss*	1.92
Razorback sucker	*Xyrauchen texanus*	2.31
Roundtail chub	*Gila robusta*	2.04
Smallmouth bass	*Micropterus dolomieu*	1.19
White sturgeon	*Acipenser transmontanus*	1.33
White sucker	*Catostomus commersonii*	1.00

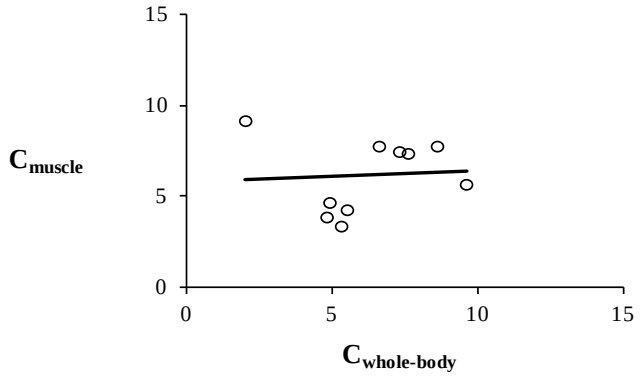
2.4 Muscle to whole-body conversion factors

$C_{whole\text{-}body}$ = Selenium concentration in all tissues (µg/g dw)
C_{muscle} = Selenium concentration in muscle tissue only (µg/g dw)

$$\text{Ratio} = \frac{C_{muscle}}{C_{whole-body}}$$

Black bullhead (*Ameiurus melas*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	5.30	3.40	0.64
Osmundson et al. 2007	4.80	3.90	0.81
Osmundson et al. 2007	5.50	4.30	0.78
Osmundson et al. 2007	4.90	4.70	0.96
Osmundson et al. 2007	9.60	5.70	0.59
Osmundson et al. 2007	7.60	7.40	0.97
Osmundson et al. 2007	7.30	7.50	1.03
Osmundson et al. 2007	6.60	7.80	1.18
Osmundson et al. 2007	8.60	7.80	0.91
Osmundson et al. 2007	2.00	9.20	4.60



Median ratio: 0.93

R^2: 0.00
F: 0.03
df: 8
P: 0.973

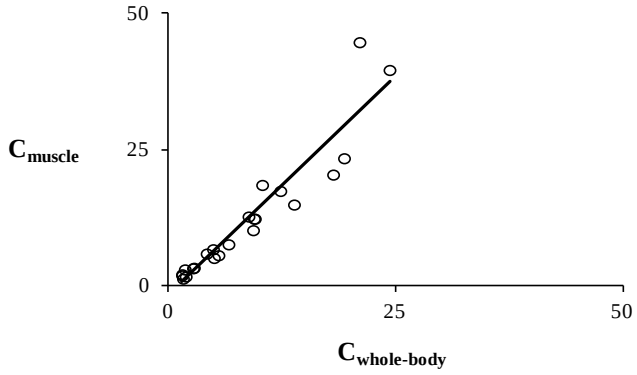
Not used because P > 0.05.

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Doroshov et al. 1992	1.60	1.50	0.94
Doroshov et al. 1992	5.50	5.80	1.05
Doroshov et al. 1992	9.30	10.40	1.12
Doroshov et al. 1992	19.30	23.60	1.22
Hermanutz et al. 1996	1.50	2.10	1.40
Hermanutz et al. 1996	18.10	20.60	1.14
Hermanutz et al. 1996	1.90	1.90	1.00
Hermanutz et al. 1996	2.80	3.50	1.25

Bluegill (*Lepomis macrochirus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Hermanutz et al. 1996	12.30	17.60	1.43
Hermanutz et al. 1996	9.40	12.50	1.33
Hermanutz et al. 1996	1.50	2.30	1.53
Hermanutz et al. 1996	4.90	6.90	1.41
Hermanutz et al. 1996	21.00	44.90	2.14
Hermanutz et al. 1996	24.30	39.80	1.64
Hermanutz et al. 1996	2.70	3.40	1.26
Hermanutz et al. 1996	5.00	5.30	1.06
Hermanutz et al. 1996	9.50	12.50	1.32
Hermanutz et al. 1996	6.60	7.80	1.18
Hermanutz et al. 1996	1.80	3.20	1.78
Hermanutz et al. 1996	4.20	6.10	1.45
Hermanutz et al. 1996	10.30	18.70	1.82
Hermanutz et al. 1996	13.80	15.10	1.09
Osmundson et al. 2007	8.80	12.90	1.47

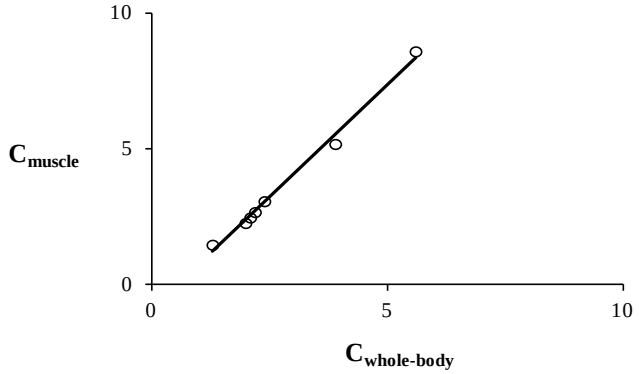


Median ratio: 1.32

R^2: 0.89
F: 172.2
df: 21
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	1.30	1.50	1.15
Osmundson et al. 2007	2.00	2.30	1.15
Osmundson et al. 2007	2.10	2.50	1.19
Osmundson et al. 2007	2.20	2.70	1.23
Osmundson et al. 2007	2.40	3.10	1.29
Osmundson et al. 2007	3.90	5.20	1.33
Osmundson et al. 2007	5.60	8.60	1.54

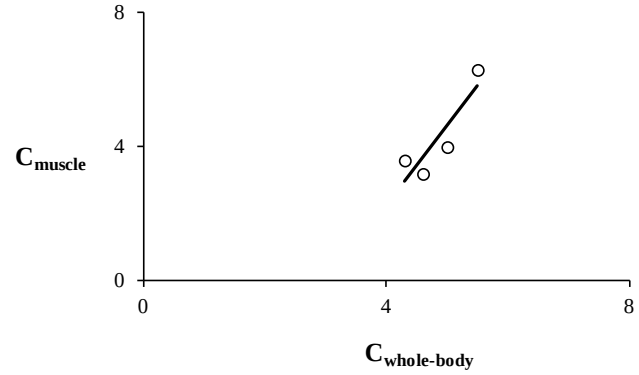


Median ratio: 1.23

R^2: 0.99
F: 682.9
df: 5
P: <0.001

Brown trout (*Salmo trutta*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.60	3.20	0.70
Osmundson et al. 2007	4.30	3.60	0.84
Osmundson et al. 2007	5.00	4.00	0.80
Osmundson et al. 2007	5.50	6.30	1.15



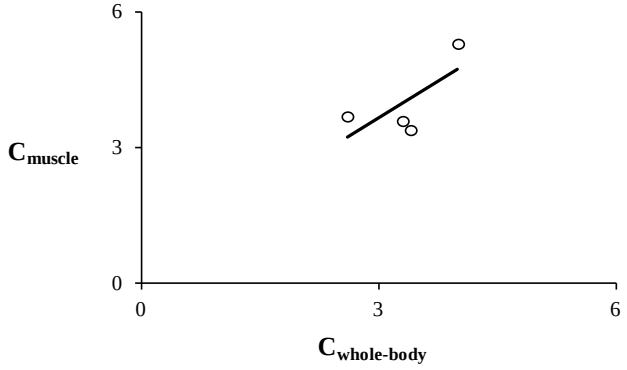
Median ratio: 0.82

R^2: 0.78
F: 7.2
df: 2
P: 0.122

Not used because P > 0.05.

Channel catfish (*Ictalurus punctatus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.40	3.40	1.00
Osmundson et al. 2007	3.30	3.60	1.09
Osmundson et al. 2007	2.60	3.70	1.42
Osmundson et al. 2007	4.00	5.30	1.33



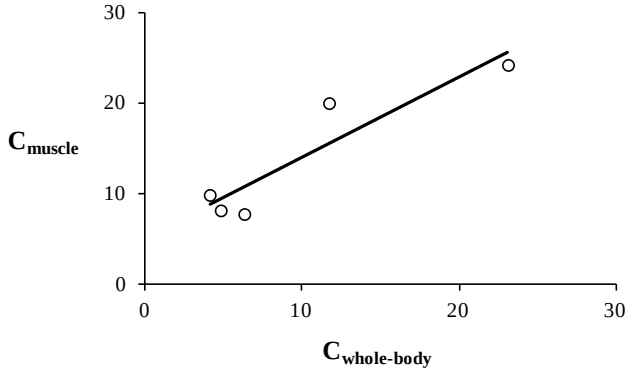
Median ratio: 1.21

R^2: 0.49
F: 2.0
df: 2
P: 0.338

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	6.30	7.80	1.24
Osmundson et al. 2007	4.80	8.20	1.71
Osmundson et al. 2007	11.70	20.00	1.71
Osmundson et al. 2007	23.10	24.20	1.05
Osmundson et al. 2007	4.10	6.60	1.61

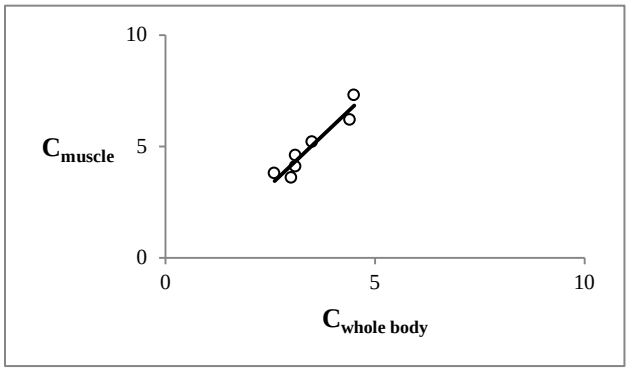


Median ratio: 1.61

R^2: 0.85
F: 17.6
df: 3
P: 0.017

Flannelmouth sucker (*Catostomus latipinnis*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.0	3.6	1.20
Osmundson et al. 2007	2.6	3.8	1.46
Osmundson et al. 2007	3.1	4.1	1.32
Osmundson et al. 2007	3.1	4.6	1.48
Osmundson et al. 2007	3.5	5.2	1.49
Osmundson et al. 2007	4.4	6.2	1.41
Osmundson et al. 2007	4.5	7.3	1.62



Median ratio: 1.46

R^2: 0.91
F: 50.1
df: 5
P: <0.001

Green sunfish (*Lepomis cyanellus*)

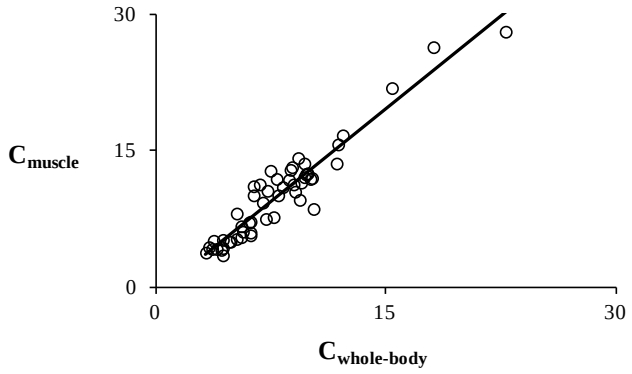
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	22.80	28.10	1.23
Osmundson et al. 2007	8.80	12.90	1.47
Osmundson et al. 2007	15.40	21.90	1.42
Osmundson et al. 2007	4.80	5.00	1.04
Osmundson et al. 2007	5.70	6.10	1.07
Osmundson et al. 2007	4.40	5.20	1.18
Osmundson et al. 2007	3.80	5.10	1.34
Osmundson et al. 2007	11.90	15.70	1.32
Osmundson et al. 2007	6.40	10.10	1.58
Osmundson et al. 2007	9.50	11.50	1.21
Osmundson et al. 2007	9.10	10.50	1.15
Osmundson et al. 2007	6.20	7.20	1.16
Osmundson et al. 2007	7.00	9.30	1.33
Osmundson et al. 2007	7.70	7.70	1.00
Osmundson et al. 2007	6.20	6.00	0.97
Osmundson et al. 2007	10.20	12.00	1.18
Osmundson et al. 2007	9.70	12.10	1.25
Osmundson et al. 2007	9.90	12.50	1.26
Osmundson et al. 2007	7.20	7.50	1.04
Osmundson et al. 2007	9.00	11.30	1.26

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	9.70	13.60	1.40
Osmundson et al. 2007	8.90	13.20	1.48
Osmundson et al. 2007	9.80	12.40	1.27
Osmundson et al. 2007	9.90	12.50	1.26
Osmundson et al. 2007	10.30	8.60	0.83
Osmundson et al. 2007	5.30	5.30	1.00
Osmundson et al. 2007	10.10	11.90	1.18
Osmundson et al. 2007	11.80	13.60	1.15
Osmundson et al. 2007	3.30	3.80	1.15
Osmundson et al. 2007	4.00	4.20	1.05
Osmundson et al. 2007	4.30	4.10	0.95
Osmundson et al. 2007	3.70	4.20	1.14
Osmundson et al. 2007	6.20	5.70	0.92
Osmundson et al. 2007	3.50	4.40	1.26
Osmundson et al. 2007	4.40	3.50	0.80
Osmundson et al. 2007	5.60	5.50	0.98
Osmundson et al. 2007	4.90	5.00	1.02
Osmundson et al. 2007	4.40	4.30	0.98
Osmundson et al. 2007	8.00	10.10	1.26
Osmundson et al. 2007	7.90	11.90	1.51
Osmundson et al. 2007	6.40	11.10	1.73
Osmundson et al. 2007	8.70	11.80	1.36
Osmundson et al. 2007	8.30	11.00	1.33
Osmundson et al. 2007	6.10	7.10	1.16
Osmundson et al. 2007	5.60	6.70	1.20
Osmundson et al. 2007	18.10	26.40	1.46
Osmundson et al. 2007	9.40	9.60	1.02
Osmundson et al. 2007	12.20	16.70	1.37
Osmundson et al. 2007	5.30	8.10	1.53
Osmundson et al. 2007	7.30	10.60	1.45
Osmundson et al. 2007	9.30	14.20	1.53
Osmundson et al. 2007	6.80	11.30	1.66
Osmundson et al. 2007	7.50	12.80	1.71

Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio

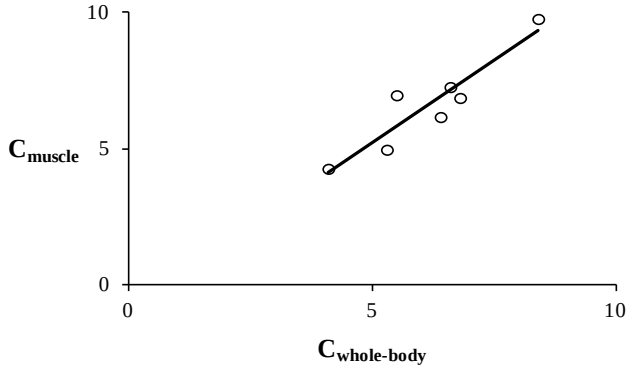


Median ratio: 1.23

R^2: 0.91
F: 501.6
df: 51
P: < 0.001

Roundtail chub (*Gila robusta*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.10	4.30	1.05
Osmundson et al. 2007	5.30	5.00	0.94
Osmundson et al. 2007	6.40	6.20	0.97
Osmundson et al. 2007	6.80	6.90	1.01
Osmundson et al. 2007	5.50	7.00	1.27
Osmundson et al. 2007	6.60	7.30	1.11
Osmundson et al. 2007	8.40	9.80	1.17

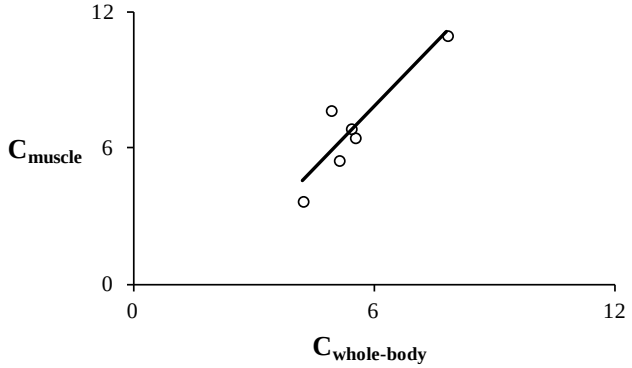


Median ratio: 1.05

R^2: 0.86
F: 29.6
df: 5
P: 0.002

Smallmouth bass (*Micropterus dolomieu*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	4.20	3.70	0.88
Osmundson et al. 2007	5.50	6.50	1.18
Osmundson et al. 2007	5.40	6.90	1.28
Osmundson et al. 2007	7.80	11.0	1.41
Osmundson et al. 2007	5.10	7.10	1.08
Osmundson et al. 2007	4.90	8.80	1.57



Median ratio: 1.23

R^2: 0.83
F: 20.2
df: 4
P: 0.008

White sucker (*Catostomus commersonii*)

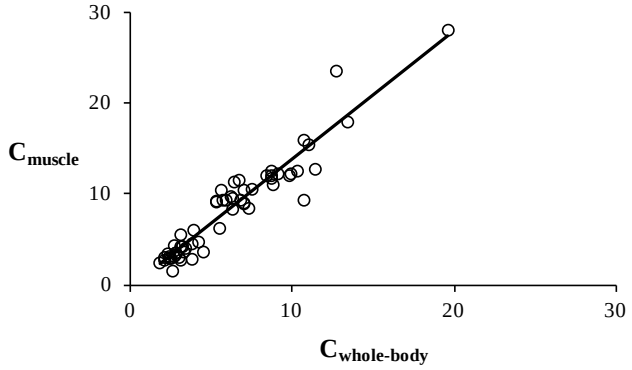
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.80	2.90	0.76
Osmundson et al. 2007	4.20	4.80	1.14
Osmundson et al. 2007	3.30	3.70	1.12
Osmundson et al. 2007	4.50	3.70	0.82
Osmundson et al. 2007	6.30	8.40	1.33
Osmundson et al. 2007	6.80	9.40	1.38
Osmundson et al. 2007	11.00	15.50	1.41
Osmundson et al. 2007	12.70	23.60	1.86
Osmundson et al. 2007	5.70	9.40	1.65
Osmundson et al. 2007	3.90	6.10	1.56
Osmundson et al. 2007	3.80	4.60	1.21
Osmundson et al. 2007	9.90	12.30	1.24
Osmundson et al. 2007	5.30	9.20	1.74
Osmundson et al. 2007	10.70	9.40	0.88
Osmundson et al. 2007	5.90	9.40	1.59
Osmundson et al. 2007	7.00	10.50	1.50
Osmundson et al. 2007	6.40	11.40	1.78
Osmundson et al. 2007	6.30	9.60	1.52
Osmundson et al. 2007	5.30	9.30	1.75
Osmundson et al. 2007	6.20	9.80	1.58

White sucker (*Catostomus commersonii*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	5.60	10.50	1.88
Osmundson et al. 2007	8.80	11.10	1.26
Osmundson et al. 2007	8.70	12.10	1.39
Osmundson et al. 2007	11.40	12.80	1.12
Osmundson et al. 2007	10.70	16.00	1.50
Osmundson et al. 2007	8.40	12.10	1.44
Osmundson et al. 2007	7.00	9.00	1.29
Osmundson et al. 2007	7.50	10.60	1.41
Osmundson et al. 2007	10.30	12.60	1.22
Osmundson et al. 2007	6.70	11.60	1.73
Osmundson et al. 2007	2.10	2.80	1.33
Osmundson et al. 2007	1.80	2.50	1.39
Osmundson et al. 2007	3.20	4.30	1.34
Osmundson et al. 2007	2.30	3.50	1.52
Osmundson et al. 2007	3.10	4.30	1.39
Osmundson et al. 2007	3.00	3.10	1.03
Osmundson et al. 2007	2.80	3.60	1.29
Osmundson et al. 2007	2.50	3.00	1.20
Osmundson et al. 2007	3.40	4.10	1.21
Osmundson et al. 2007	2.80	3.60	1.29
Osmundson et al. 2007	3.10	5.60	1.81
Osmundson et al. 2007	5.50	6.30	1.15
Osmundson et al. 2007	7.00	9.10	1.30
Osmundson et al. 2007	7.30	8.50	1.16
Osmundson et al. 2007	2.40	3.00	1.25
Osmundson et al. 2007	2.70	4.40	1.63
Osmundson et al. 2007	2.70	3.20	1.19
Osmundson et al. 2007	2.60	1.60	0.62
Osmundson et al. 2007	19.60	28.10	1.43
Osmundson et al. 2007	9.80	12.10	1.23
Osmundson et al. 2007	8.70	11.80	1.36
Osmundson et al. 2007	8.70	12.60	1.45
Osmundson et al. 2007	9.10	12.30	1.35
Osmundson et al. 2007	13.40	18.00	1.34
Osmundson et al. 2007	3.10	2.80	0.90
Osmundson et al. 2007	2.40	3.20	1.33
Osmundson et al. 2007	2.10	3.10	1.48
Osmundson et al. 2007	3.20	4.30	1.34
Osmundson et al. 2007	2.80	3.40	1.21

White sucker (*Catostomus commersonii*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio



Median ratio: 1.34

R^2: 0.91
F: 561.3
df: 57
P: < 0.001

Table B-4. Muscle to whole-body correction factor.

Common name	Scientific name	Median ratio
Bluegill	*Lepomis macrochirus*	1.32
Bluehead sucker	*Catostomus discobolus*	1.23
Common carp	*Cyprinus carpio*	1.61
Flannelmouth sucker	*Catostomus latipinnis*	1.46
Green sunfish	*Lepomis cyanellus*	1.23
Roundtail chub	*Gila robusta*	1.05
Smallmouth bass	*Micropterus dolomieu*	1.23
White sucker	*Catostomus commersonii*	1.34

Table B-5. Directly calculated final egg-ovary to whole body conversion factors (*CF*).

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Species				
Bluegill	2.13			2.13
Bluehead sucker	1.82			1.82
Brook trout		1.09	1.27	1.38
Brown trout	1.45			1.45
Common carp	1.92			1.92
Creek chub	1.99			1.99
Cutthroat trout	1.96			1.96

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Desert pupfish	1.20			1.20
Dolly Varden		1.26	1.27	1.61
Fathead minnow	1.40			1.40
Flannelmouth sucker	1.41			1.41
Green sunfish	1.45			1.45
Mountain whitefish		5.80	1.27	7.39
Northern pike		1.88	1.27	2.39
Rainbow trout		1.92	1.27	2.44
Razorback sucker		2.31	1.34	3.11
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sturgeon		1.33	1.27	1.69
White sucker	1.38			1.38
Genus				
Catostomus				1.41
Gila				2.07
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				1.96
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				1.95
Salmonidae				1.71
Order				
Cyprinodontiformes				1.20
Perciformes				1.45

Common name	Median ratio ($C_{egg\text{-}ovary}$/ $C_{whole\text{-}body}$)	Median ratio ($C_{egg\text{-}ovary}$/ C_{muscle})	Muscle to whole-body correction factor	Final *CF* values
Class				
Actinopterygii				1.45

Table B-6. All EPA-derived egg-ovary to whole body (CF), egg-ovary to muscle, and muscle to whole body conversion factors directly calculated or estimated using taxonomic classification. (See main text for explanation of the taxonomic classification approach).

		Direct calculation			Values based on taxonomic classification								Final E-O / WB	
Common name	Scientific name	E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
alligator gar	Atractosteus spatula				Lepistosteiformes	Lepisosteidae	Atractosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bigmouth buffalo	Ictiobus cyprinellus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
black bullhead	Ameiurus melas				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
black crappie	Pomoxis nigromaculatus				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
black redhorse	Moxostoma duquesnei				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
blacknose dace	Rhinichthys atratulus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
blue catfish	Ictalurus furcatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bluegill	Lepomis macrochirus	2.13	1.38	1.32	Perciformes	Centrarchidae	Lepomis	2.13	Exact match	1.38	Exact match	1.32	Exact match	2.13	Exact match
bluehead sucker	Catostomus discobolus	1.82	1.48	1.23	Cypriniformes	Catostomidae	Catostomus	1.82	Exact match	1.48	Exact match	1.23	Exact match	1.82	Exact match
brassy minnow	Hybognathus hankinsoni				Cypriniformes	Cyprinidae	Hybognathus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
brook stickleback	Culaea inconstans				Gasterosteiformes	Gasterosteidae	Culaea	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brook trout	Salvelinus fontinalis		1.09		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.09	Exact match	1.27	All fish	1.38	E-O/WB * M/WB
brown bullhead	Ameiurus nebulosus				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brown trout	Salmo trutta	1.45			Salmoniformes	Salmonidae	Salmo	1.45	Exact match	1.81	Family Salmonidae	1.27	All fish	1.45	Exact match
burbot	Lota lota				Gadiformes	Lotidae	Lota	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bullhead					Siluriformes	Ictaluridae		1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
chain pickerel	Esox				Esociformes	Esocidae	Esox	1.45	All fish	1.88	Genus Esox	1.27	All fish	2.39	E-O/WB * M/WB
channel catfish	Ictalurus punctatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
common carp	Cyprinus carpio	1.92	1.14	1.61	Cypriniformes	Cyprinidae	Cyprinus	1.92	Exact match	1.14	Exact match	1.61	Exact match	1.92	Exact match
common snook	Centropomus undecimalis				Perciformes	Centropomidae	Centropomus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
crappie	Pomoxis sp.				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
creek chub	Semotilus atromaculatus	1.99			Cypriniformes	Cyprinidae	Semotilus	1.99	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.99	Family Cyprinidae
cutthroat trout	Oncorhynchus clarkii	1.96	1.81		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Exact match	1.81	Exact match	1.27	All fish	1.96	Exact match

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
desert pupfish	Cyprinodon macularius	1.20			Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.20	Exact match	1.35	All fish	1.27	All fish	1.20	Exact match
Dolly Varden	Salvelinus malma		1.26		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.26	Exact match	1.27	All fish	1.61	E-O/WB * M/WB
fathead minnow	Pimephales promelas	1.40			Cypriniformes	Cyprinidae	Pimephales	1.40	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.40	Family Cyprinidae
flannelmouth sucker	Catostomus latipinnis	1.41	1.08	1.46	Cypriniformes	Catostomidae	Catostomus	1.41	Exact match	1.08	Exact match	1.46	Exact match	1.41	Exact match
flathead catfish	Pylodictis				Siluriformes	Ictaluridae	Pylodictus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
flathead chub	Platygobio gracilis				Cypriniformes	Cyprinidae	Platygobio	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
freshwater drum	Aplodinotus grunniens				Perciformes	Sciaenidae	Aplodinotus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
goldeye	Hiodon alosoides				Hiodontiformes	Hiodontidae	Hiodon	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
gizzard shad	Dorosoma cepedianum				Clupeiformes	Clupeidae	Dorosoma	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
green sunfish	Lepomis cyanellus	1.45	1.21	1.23	Perciformes	Centrarchidae	Lepomis	1.45	Exact match	1.21	Exact match	1.23	Exact match	1.45	Exact match
iowa darter	Etheostoma exile				Perciformes	Percidae	Etheostoma	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
Japanese medaka	Oryzias latipes				Beloniformes	Adrianichthyidae	Oryzias	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
kokanee salmon	Oncorhynchus nerka				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
largemouth bass	Micropterus salmoides				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
largescale sucker	Catostomus macrocheilus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
longnose dace	Rhinichthys cataractae				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
longnose sucker	Catostomus catostomus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
mosquitofish	Gambusia sp.				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
mottled sculpin	Cottus bairdi				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
mountain whitefish	Prosopium williamsoni		5.80		Salmoniformes	Salmonidae	Prosopium	1.71	Family Salmonidae	5.80	Exact match	1.27	All fish	7.39	E-O/WB * M/WB
ninespine stickleback	Pungitius pungitius				Gasterosteiformes	Gasterosteidae	Pungitius	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
northern pike	Esox lucius		1.88		Esociformes	Esocidae	Esox	1.45	All fish	1.88	Exact match	1.27	All fish	2.39	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
northern pikeminnow	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern plains killifish	Fundulus kansae				Cyprinodontiformes	Fundulidae	Fundulus	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
northern redbelly dace	Chrosomus eos				Cypriniformes	Cyprinidae	Chrosomus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern squawfish	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
quillback	Carpiodes cyprinus				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rainbow trout	Oncorhynchus mykiss		1.92		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.92	Exact match	1.27	All fish	2.44	E-O/WB * M/WB
razorback sucker	Xyrauchen texanus		2.31		Cypriniformes	Catostomidae	Xyrauchen	1.41	Family Catostomidae	2.31	Exact match	1.34	Family Catostomidae	3.11	E-O/WB * M/WB
red shiner	Cyprinella lutrensis				Cypriniformes	Cyprinidae	Cyprinella	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
redbreast sunfish	Lepomis auritus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redear sunfish	Lepomis microlophus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redside shiner	Richardsonius balteatus				Cypriniformes	Cyprinidae	Richardsonius	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
river carpsucker	Carpiodes carpio				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
river redhorse	Moxostoma carinatum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rock bass	Ambloplites rupestris				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
roundtail chub	Gila robusta	2.07	2.04	1.05	Cypriniformes	Cyprinidae	Gila	2.07	Exact match	2.04	Exact match	1.05	Exact match	2.07	Exact match
sacramento perch	Archoplites interruptus				Perciformes	Centrarchidae	Archoplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
sacramento pikeminnow	Ptychocheilus grandis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sailfin molly	Poecilia latipinna				Cyprinodontiformes	Poeciliidae	Poecilia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
sand shiner	Notropis stramineus				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sauger	Sander canadensis				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
sculpin	Cottus sp.				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
shadow bass	Ambloplites ariommus				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
shorthead redhorse	Moxostoma macrolepidotum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
silver carp	Hypophthalmichthys molitrix				Cypriniformes	Cyprinidae	Hypophthalmichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
smallmouth bass	Micropterus dolomieu	1.42	1.19	1.23	Perciformes	Centrarchidae	Micropterus	1.42	Exact match	1.19	Exact match	1.23	Exact match	1.42	Exact match
smallmouth buffalo	Ictiobus bubalus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
speckled dace	Rhinichthys osculus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spottail shiner	Notropis hudsonius				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spotted bass	Micropterus punctulatus				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
spotted gar	Lepisosteus oculatus				Lepistosteiformes	Lepisosteidae	Lepisosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
stonecat	Noturus flavus				Siluriformes	Ictaluridae	Noturus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
striped bass	Morone saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
striped mullet	Mugil cephalus				Mugiliformes	Mugilidae	Mugil	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
sucker					Cypriniformes	Catostomidae		1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
tilapia					Perciformes	Cichlidae		1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
trout species	Oncorhynchus sp.				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
tui chub	Gila bicolor				Cypriniformes	Cyprinidae	Gila	2.07	Genus Gila	2.04	Genus Gila	1.05	Genus Gila	2.07	Genus Gila
utah sucker	Catostomus ardens				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
walleye	Sander vitreus				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
western mosquitofish	Gambusia affinis				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
white bass	Morone chrysops				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
white crappie	Pomoxis annularis				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
white sturgeon	Acipenser transmontanus		1.33		Acipenseriformes	Acipenseridae	Acipenser	1.45	All fish	1.33	Exact match	1.27	All fish	1.69	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
white sucker	Catostomus commersonii	1.38	1.00	1.34	Cypriniformes	Catostomidae	Catostomus	1.38	Exact match	1.00	Exact match	1.34	Exact match	1.38	Exact match
wiper	Morone chrysops x Moron saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
yellow bullhead	Ameiurus natalis				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
yellow perch	Perca flavescens				Perciformes	Percidae	Perca	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes

3.0 DERIVATION OF TROPHIC TRANSFER FACTOR VALUES

3.1 Methodology

Taxa specific trophic transfer factors (TTF) to quantify the degree of biomagnification across a given trophic level were calculated from either physiological parameters measured in laboratory studies or from field measurements of paired selenium concentrations in consumer species and their food. TTFs from both approaches were used to calculate translated water concentrations; however, when TTF data of similar quality are available from both approached, as was the case with bluegill, field-derived TTF data are used.

Physiological data consisted of assimilation efficiencies (AE), measured as either a percentage or a proportion, ingestion rates (IR), measured as grams of Se per grams of food consumed per day, and efflux rate constant (k_e), measured as 1/day. All available data were collected for a particular species, and then the TTF for that species was calculated using the equation:

$$\text{TTF} = \frac{AE \; x \; IR}{k_e}$$

Where AE, IR, and K_e were estimated as the median value of all available data for that parameter for that species.

The majority of TTF were calculated using paired whole-body Se measurements from organisms collected at the same site in the field. TTFs for trophic level 2 organisms were determined using the equation:

$$TTF^{TL2} = \frac{C_{tissue}^{TL2}}{C_{food}^{TL2}}$$

Where C_{food}^{TL2} equals the average Se concentration in particulate matter, defined as the average of C_{algae}, $C_{detritus}$, and $C_{sediment}$. Of the three types of particulate matter potentially assumed by TL2 organisms (e.g., the majority of invertebrates), $C_{sediment}$ correlated relatively poorly to C_{tissue}^{TL2}, when compared to C_{algae} and $C_{detritus}$. In order to minimize potentially erroneous TTF calculations based solely on sediment Se concentrations, while note completely discounting the importance of organic matter in sediments as a potential food source, $C_{sediment}$ was included in $C_{particulate}$ calculations only when either C_{algae} or $C_{detritus}$ data were also available.

TTFs for trophic level 3 organisms were determined using the equation:

$$TTF^{TL3} = \frac{C_{tissue}^{TL3}}{C_{food}^{TL3}}$$

Where C_{food}^{TL3} equals the average whole-body Se concentration in invertebrates collected at the same site as their potential predator species. The majority of trophic level 3 organisms are fish species, but damselflies and dragonflies of the order Odonata are also trophic level 3 organisms, and TTF^{TL3} values were calculated for those species as well.

For all field derived data used to determine TTFs, EPA first confirmed a statistical relationship between whole-body selenium concentrations for each species and its food using OLS linear regression. If the regression resulted in a statistically significant ($P<0.05$) positive slope, EPA calculated the TTF as the median ratio of the paired concentration data.

3.2 TTF values from physiological coefficients

AE (%) = Assimilation efficiency
IR ($g\ g^{-1}\ d^{-1}$) = Ingestion rate
k_e (d^{-1}) = Efflux rate constant
TTF = $\frac{AE\ x\ IR}{K_e}$

3.2.1 Invertebrates

Baltic macoma (*Macoma balthica*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
22.5				Luoma et al. 1992
91.0				Luoma et al. 1992
84.0				Luoma et al. 1992
95.0				Luoma et al. 1992
78.0		0.03		Reinfelder et al. 1997
74.0		0.03		Reinfelder et al. 1997
92.3				Schleckat et al. 2002
58.0				Schleckat et al. 2002
85.8				Schleckat et al. 2002
64.9				Schleckat et al. 2002
90.4				Schleckat et al. 2002
Median Values and TTF				
84.0	0.27[a]	0.03	7.56	

[a] Value taken from *Mytilus edulis*

Short-necked clam (*Ruditapes philippinarum*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
70.0		0.013		Zhang et al. 1990
52.0		0.013		Zhang et al. 1990
Median Values and TTF				
61.0	0.27[a]	0.013	12.67	

[a] Value taken from *Mytilus edulis*

Quahog (*Mercenaria mercenaria*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
100.1				Reinfelder and Fisher 1994
92.0		0.01		Reinfelder et al. 1997
Median Values and TTF				
96.1	0.27[a]	0.01	25.93	

[a] Value taken from *Mytilus edulis*

Eastern Oyster (*Crassostrea virginica*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.005		Okazaki and Panietz 1981
105.4				Reinfelder and Fisher 1994
70.0		0.070		Reinfelder et al. 1997
Median Values and TTF				
87.7	0.27a	0.038	6.31	

[a] Value taken from *Mytilus edulis*

Common mussel (*Mytilus edulis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
86.0		0.02		Reinfelder et al. 1997
75.0		0.05		Reinfelder et al. 1997
60.7				Wang and Fisher 1996
48.0				Wang and Fisher 1996
13.7				Wang and Fisher 1996
55.1				Wang and Fisher 1996
55.8				Wang and Fisher 1996
71.9				Wang and Fisher 1996
71.5				Wang and Fisher 1996
27.9				Wang and Fisher 1996
84.4				Wang and Fisher 1996
81.0				Wang and Fisher 1996
79.4				Wang and Fisher 1996

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
63.0		0.037		Wang and Fisher 1996
61.5		0.05		Wang and Fisher 1996
69.0		0.027		Wang and Fisher 1996
81.0		0.022		Wang and Fisher 1997
82.0		0.020		Wang and Fisher 1997
72.0		0.018		Wang and Fisher 1997
78.0		0.055		Wang et al. 1995
76.0		0.065		Wang et al. 1995
71.0		0.058		Wang et al. 1995
33.9				Wang et al. 1996
27.5				Wang et al. 1996
				Wang et al. 1996
				Wang et al. 1996
	0.27	0.022		Wang et al. 1996
		0.026		Wang et al. 1996
		0.019		Wang et al. 1996
Median Values and TTF				
71.3	0.27	0.026	7.30	

Asian clam (*Corbicula fluminea*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.05	0.006		Lee et al. 2006
Median Values and TTF				
55.0	0.05	0.006	4.58	

Zebra mussel (*Dreissena polymorpha*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
18.0				Roditi and Fisher 1999
24.0				Roditi and Fisher 1999
46.0				Roditi and Fisher 1999
40.0				Roditi and Fisher 1999
41.0				Roditi and Fisher 1999
7.7				Roditi and Fisher 1999
23.0				Roditi and Fisher 1999
28.0				Roditi and Fisher 1999
	0.40			Roditi and Fisher 1999
		0.026		Roditi and Fisher 1999
Median Values and TTF				
26.0	0.40	0.026	4.00	

Water flea (*Daphnia magna*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
	0.08			Goulet et al. 2007
	0.34			Goulet et al. 2007
57.9				Yu and Wang 2002b
43.0				Yu and Wang 2002b
39.8				Yu and Wang 2002b
33.0				Yu and Wang 2002b
41.4				Yu and Wang 2002b
41.5				Yu and Wang 2002b
38.0				Yu and Wang 2002b
24.5				Yu and Wang 2002b
		0.101		Yu and Wang 2002b
		0.12		Yu and Wang 2002b
		0.131		Yu and Wang 2002b
		0.134		Yu and Wang 2002b
		0.108		Yu and Wang 2002b
		0.112		Yu and Wang 2002b
Median Values and TTF				
40.6	0.21	0.12	0.74	

Copepod (*Temora longicornis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.42	0.115		Wang and Fisher 1998
Median Values and TTF				
55.0	0.42	0.115	2.01	

Copepod (Small, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.35	

Copepod (Large, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
52.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.41	

Blackworm (*Lumbriculus variegatus*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.009		Riedel and Cole 2001
		0.006		Riedel and Cole 2001
24.0	0.067	0.013		Riedel and Cole 2001
9.0	0.067	0.009		Riedel and Cole 2001
Median Values and TTF				
16.5	0.067	0.0086	1.29	

Mayfly (*Centroptilum triangulifer*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
38.0	0.72	0.25		Riedel and Cole 2001
40.0	0.72	0.19		Riedel and Cole 2001
Median Values and TTF				
39.0	0.72	0.22	1.28	

a – not used because field TTF data available

3.2.2 Vertebrates

Bluegill (*Lepomis macrochirus*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
34.0				Besser et al. 1993
22.0				Besser et al. 1993
24.0				Besser et al. 1993
36.0				Besser et al. 1993
30.0				Besser et al. 1993
32.0				Besser et al. 1993
43.0				Besser et al. 1993
40.0				Besser et al. 1993
37.0		0.041		Besser et al. 1993
		0.031		Besser et al. 1993
		0.034		Besser et al. 1993
36.0		0.031		Besser et al. 1993
		0.038		Besser et al. 1993
		0.038		Besser et al. 1993
	0.008			Whitledge and Haywood 2000
	0.042			Whitledge and Haywood 2000
Median Values and TTF				
35.0	0.025	0.036	1.156[a]	

[a] Not used because of availability of acceptable field-based TTF data

Fathead Minnow (*Pimephales promelas*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0				Presser and Luoma 2010
	0.050			Bertram and Brooks 1986
		0.029		Bertram and Brooks 1986
		0.019		Bertram and Brooks 1986
		0.3		Bertram and Brooks 1986
		0.014		Bertram and Brooks 1986
		0.013		Bertram and Brooks 1986
		0.016		Bertram and Brooks 1986
		0.012		Bertram and Brooks 1986
		0.026		Bertram and Brooks 1986
		0.018		Bertram and Brooks 1986
		0.025		Bertram and Brooks 1986
Median Values and TTF				
50.0	0.050	0.0185	1.35	

Striped Bass (*Morone saxatilis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
33	0.17	0.09		Baines et al. 2002
42	0.5	0.08		Baines et al. 2002
	0.12			Buckel and Stoner 2004
	0.16			Buckel and Stoner 2004
	0.11			Buckel and Stoner 2004
	0.08			Buckel and Stoner 2004
Median Values and TTF				
37.5	0.335	0.085	1.48	

TTF calculated from only Baines et al. (2002) because it had complete data.

3.3 TTF values from field data

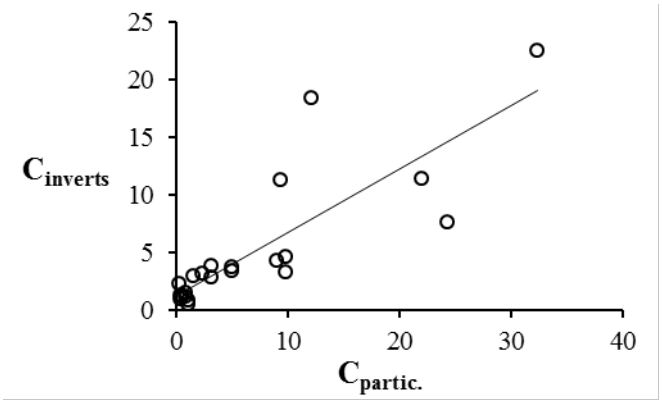
3.3.1 Invertebrates

C_{alg} = Selenium concentration in algae (mg/kg)
C_{det} = Selenium concentration in detritus (mg/kg)
C_{sed} = Selenium concentration in sediment (mg/kg)
C_{invert} = Selenium concentration in invertebrate tissue (mg/kg)
C_{part} = Average selenium concentration in particulate material $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$
Ratio = $\frac{C_{invert}}{C_{part}}$

Scuds (Amphipoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	18.40	1.52
Birkner 1978	20	3.00		41.00	22.00	11.40	0.52
Birkner 1978	7	0.18		2.80	1.49	2.90	1.95
Birkner 1978	19	16.80		1.20	9.00	4.30	0.48
Birkner 1978	30	17.30		47.30	32.30	22.50	0.70
Birkner 1978	3	0.10		0.30	0.20	2.30	11.50
Birkner 1978	22	4.60		44.00	24.30	7.60	0.31
Birkner 1978	23	7.80		10.80	9.30	11.30	1.22
Lambing et al. 1994	S46	2.30			2.30	3.20	1.39
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.44	0.40
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.86	0.79
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.60	0.47
Saiki et al. 1993	GT5	4.50	14.95		9.73	3.30	0.34
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.40	0.69
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.70	0.76
Saiki et al. 1993	SJR2	1.25	5.00		3.13	3.80	1.22
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.80	0.90
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.10	1.30
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.89	2.47
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42

Scuds (Amphipoda)

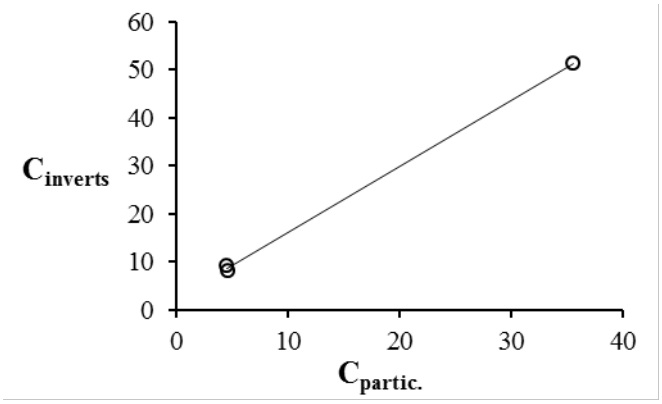


Median ratio: 1.22

R^2: 0.69
F: 46.9
df: 21
P: < 0.001

Earthworms and Leeches (Annelida)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Lemly 1985	Badin Lake	8.20		0.91	4.56	8.10	1.78
Lemly 1985	Belews Lake	62.70		8.27	35.49	51.15	1.44
Lemly 1985	High Rock Lake	8.25		0.79	4.52	9.05	2.00



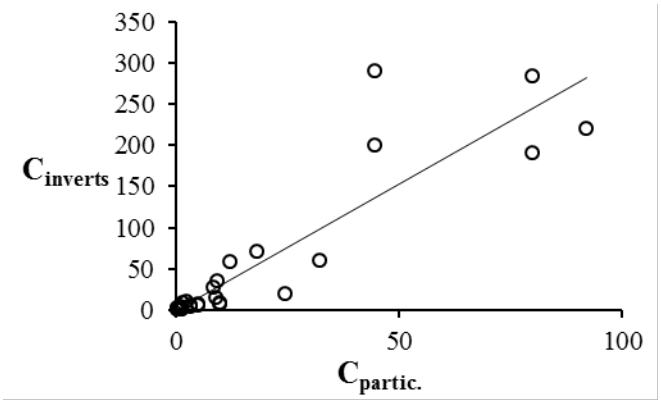
Median ratio: 1.78

R^2: 1.00
F: 2426
df: 1
P: < 0.001

Midges (Chironomidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	58.20	4.81
Birkner 1978	19	16.80		1.20	9.00	15.30	1.70
Birkner 1978	30	17.30		47.30	32.30	59.30	1.84
Birkner 1978	3	0.10		0.30	0.20	2.50	12.50
Birkner 1978	22	4.60		44.00	24.30	18.80	0.77
Birkner 1978	27	10.35		6.50	8.43	26.70	3.17
Birkner 1978	12	2.30		0.30	1.30	7.70	5.92
Birkner 1978	23	7.80		10.80	9.30	34.20	3.68
Grasso et al. 1995	17	1.87		0.40	1.14	2.07	1.82
Lambing et al. 1994	S46	2.30			2.30	9.70	4.22
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	71.00	3.91
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	200.0	4.48
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	290.0	6.49
Saiki and Lowe 1987	Kesterson Pond 8	136.5	92.00	6.05	92.00	220.0	2.39
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	190.0	2.38
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	284.0	3.55
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.74	4.18
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.30	3.13
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	3.00	3.37
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.30	1.46
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.58	0.53
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.00	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	8.90	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	7.20	0.74
Saiki et al. 1993	GT4	1.39	8.40		4.90	5.40	1.10
Saiki et al. 1993	GT4	1.39	8.40		4.90	6.90	1.41
Saiki et al. 1993	SJR2	1.25	5.00		3.13	6.00	1.92
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.10	1.31
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.47	1.31
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.00	2.78
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.53	1.16
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.84	1.85

Midges (Chironomidae)

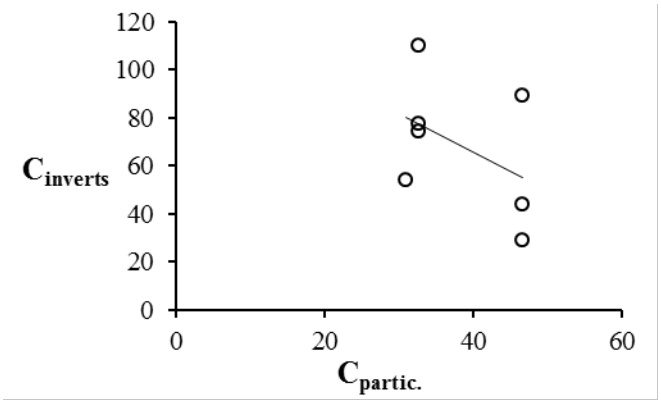


Median ratio: 1.90

R^2: 0.82
F: 144.0
df: 32
P: < 0.001

Beetles (Coleoptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	77.60	2.38
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	74.10	2.27
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	110.00	3.37
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	54.00	1.75
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	89.10	1.92
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	28.80	0.62
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	43.70	0.94



Median ratio: 1.92

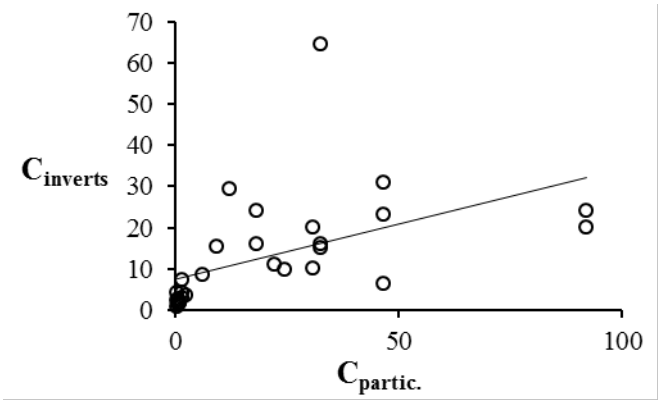
R^2: 0.20
F: 1.24
df: 5
P: 0.36
Not used because P > 0.05 and negative slope.

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	18	7.60		4.30	5.95	8.40	1.41
Birkner 1978	29	8.80		15.40	12.10	29.40	2.43

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	4.20	2.82
Birkner 1978	3	0.10		0.30	0.20	4.20	21.00
Birkner 1978	22	4.60		44.00	24.30	9.90	0.41
Birkner 1978	12	2.30		0.30	1.30	7.30	5.62
Birkner 1978	23	7.80		10.80	9.30	15.50	1.67
Lambing et al. 1994	S46	2.30			2.30	3.40	1.48
Rinella et al. 1994	G	0.84		0.50	0.67	1.38	2.06
Rinella et al. 1994	A	2.21		0.40	1.31	2.98	2.28
Rinella et al. 1994	Q	1.42		0.50	0.96	2.00	2.08
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	24.00	1.32
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	16.00	0.88
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	20.00	0.22
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	24.00	0.26
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	2.15	5.17
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	0.87	2.10
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.76	1.98
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.53	1.72
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.90	0.49
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	64.60	1.98
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.10	0.46
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	20.00	0.65
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	10.00	0.32
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	23.00	0.49
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	30.90	0.66
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	6.46	0.14
Rinella and Schuler 1992	18	0.59			0.59	2.70	4.58



Median ratio: 1.48

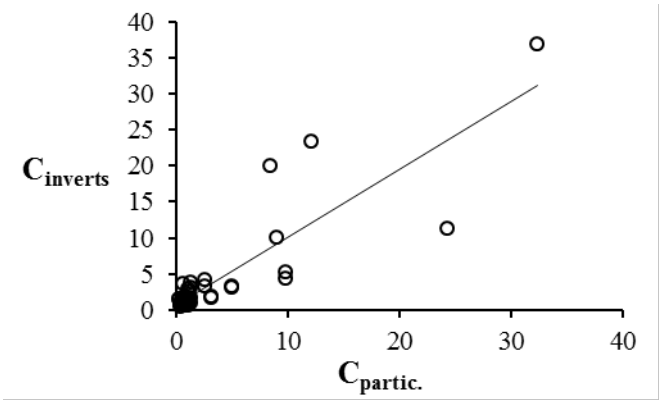
R^2: 0.25
F: 9.17
df: 27
P: < 0.001

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	23.30	1.93
Birkner 1978	19	16.80		1.20	9.00	10.10	1.12
Birkner 1978	30	17.30		47.30	32.30	36.80	1.14
Birkner 1978	22	4.60		44.00	24.30	11.30	0.47
Birkner 1978	27	10.35		6.50	8.43	20.00	2.37
Butler et al. 1993	SP2	1.60		0.50	1.05	2.60	2.48
Butler et al. 1993	SP2	1.60		0.50	1.05	2.90	2.76
Butler et al. 1995	AK	0.45		0.20	0.33	0.76	2.34
Butler et al. 1995	AK	0.45		0.20	0.33	0.79	2.43
Butler et al. 1995	DD	0.88		0.70	0.79	0.62	0.78
Butler et al. 1995	DD	0.88		0.70	0.79	1.10	1.39
Butler et al. 1995	HD1	0.59			0.59	0.86	1.46
Butler et al. 1995	HD1	0.59			0.59	0.79	1.34
Butler et al. 1995	HD2	0.45		0.20	0.32	0.96	2.98
Butler et al. 1995	HD2	0.45		0.20	0.32	1.00	3.10
Butler et al. 1995	ME2	1.11		1.10	1.10	1.10	1.00
Butler et al. 1995	ME2	1.11		1.10	1.10	1.40	1.27
Butler et al. 1995	ME4	1.04		0.50	0.77	1.30	1.69
Butler et al. 1995	ME4	1.04		0.50	0.77	1.80	2.35
Butler et al. 1995	ME3	0.82		0.40	0.61	1.40	2.30
Butler et al. 1995	ME3	0.82		0.40	0.61	3.70	6.07
Butler et al. 1995	NW	3.45		1.60	2.53	4.20	1.66
Butler et al. 1995	NW	3.45		1.60	2.53	3.30	1.31
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.40	6.83
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.50	7.32
Butler et al. 1997	CHK	1.19			1.19	0.90	0.76
Butler et al. 1997	MN2	0.79			0.79	0.83	1.06
Butler et al. 1997	MUD2	1.30			1.30	3.10	2.38
Butler et al. 1997	MUD2	1.30			1.30	3.80	2.92
Butler et al. 1997	TRH	1.25			1.25	0.98	0.78
Butler et al. 1997	TRH	1.25			1.25	1.60	1.28
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.67	0.62
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.83	0.76
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.20	0.53
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.40	0.45
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.10	0.63
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.20	0.65
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.70	0.54
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.90	0.61

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	SJR3	0.45	1.25		0.85	0.77	0.91
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.30	1.53
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.50	1.39
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.74	2.06
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.87	1.91
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.85	1.87

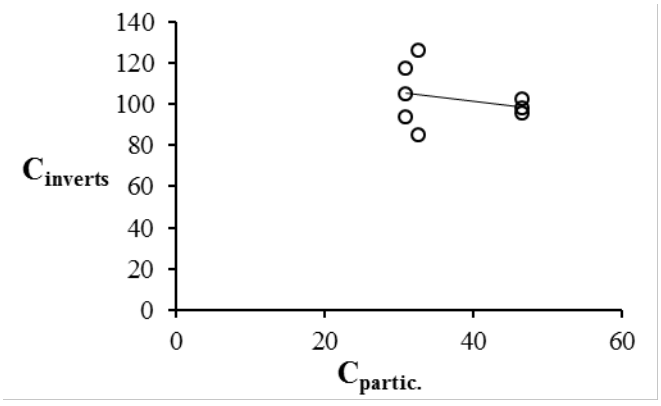


Median ratio: 1.46

R^2: 0.74
F: 130.8
df: 45
P: < 0.001

True flies (Diptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	126.00	3.87
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	85.10	2.61
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	117.00	3.79
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	93.30	3.02
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	105.00	3.40
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	95.50	2.05
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	97.70	2.10
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	102.00	2.19

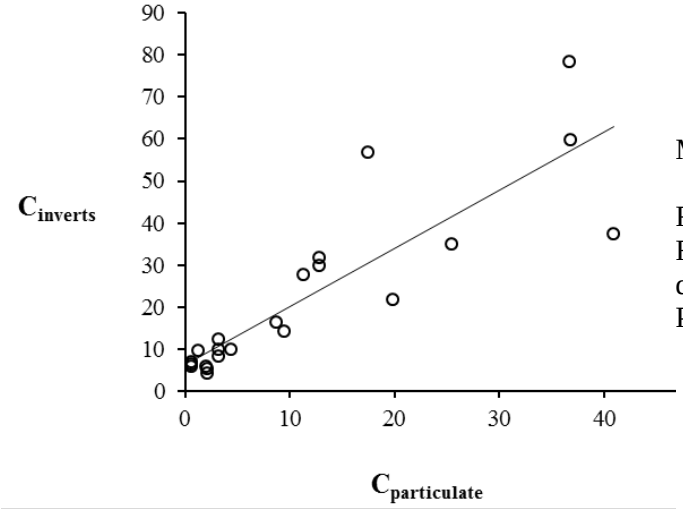


Median ratio: 2.81

R^2: 0.07
F: 0.46
df: 6
P: 0.65
Not used because P > 0.05 and negative slope.

Mayflies (Ephemeroptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Rinella et al. 1994	A	2.21		0.40	1.31	9.65	7.39
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.40	10.67
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	8.20	2.56
Casey 2005	Deerlick Creek		1.00	0.20	0.60	5.70	9.50
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	9.70	3.03
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.80	11.33
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	12.30	3.84
Conley et al. 2009	Plate 10A	4.40			4.40	9.70	2.20
Conley et al. 2009	Plate 20A	25.50			25.50	34.80	1.36
Conley et al. 2009	Plate 20B	17.50			17.50	56.70	3.24
Conley et al. 2009	Plate 20C	8.70			8.70	16.20	1.86
Conley et al. 2009	Plate 20D	11.30			11.30	27.50	2.43
Conley et al. 2009	Plate 5A	2.20			2.20	4.20	1.91
Conley et al. 2009	Plate 5B	2.00			2.00	5.70	2.85
Conley et al. 2011	2x-High	40.90			40.90	37.30	0.91
Conley et al. 2011	2x-Low	9.50			9.50	14.10	1.48
Conley et al. 2011	2x-Medium	19.90			19.90	21.60	1.09
Conley et al. 2013	Control	2.20			2.20	5.10	2.32
Conley et al. 2013	Selenate-high	36.80			36.80	59.80	1.63
Conley et al. 2013	Selenate-low	12.80			12.80	31.70	2.48
Conley et al. 2013	Selenite-high	36.70			36.70	78.40	2.14
Conley et al. 2013	Selenite-low	12.80			12.80	29.80	2.33

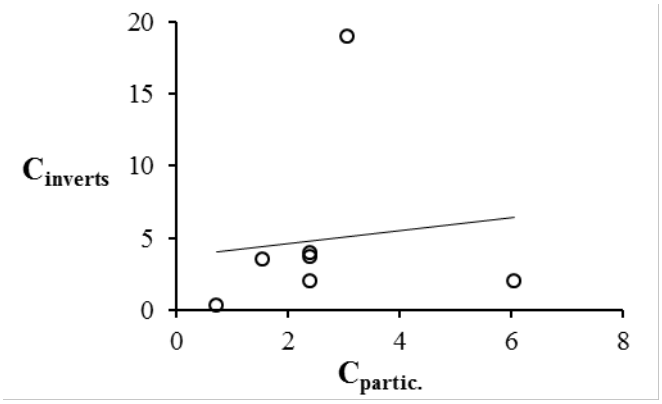


Median ratio: 2.38

R^2: 0.75
F: 59.19
df: 20
P: <0.001

Snails (Gastropoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Butler et al. 1995	WC	3.30		1.50	2.40	3.70	1.54
Butler et al. 1995	WC	3.30		1.50	2.40	3.90	1.63
Butler et al. 1995	WC	3.30		1.50	2.40	2.00	0.83
Butler et al. 1997	DCP1	1.00		2.10	1.55	3.50	2.26
Butler et al. 1997	MNP2	5.40		6.70	6.05	2.00	0.33
Butler et al. 1997	CHP	4.00		2.10	3.05	19.00	6.23
Butler et al. 1997	LCHP1	0.33		1.10	0.72	0.32	0.45



Median ratio: 1.54

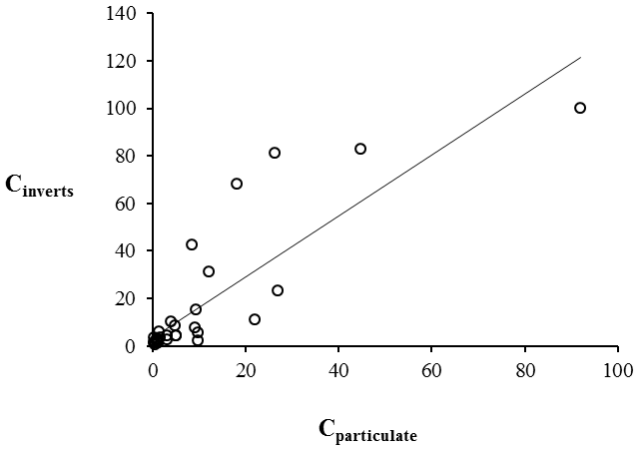
R^2: 0.01
F: 0.07
df: 5
P: 0.93
Not used because P > 0.05.

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	31.30	2.59
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	3.30	2.22
Birkner 1978	19	16.80		1.20	9.00	7.70	0.86
Birkner 1978	3	0.10		0.30	0.20	3.40	17.00
Birkner 1978	27	10.35		6.50	8.43	42.50	5.04
Birkner 1978	12	2.30		0.30	1.30	5.80	4.46
Birkner 1978	23	7.80		10.80	9.30	15.40	1.66
Bowie et al. 1996	Hyco Reservoir	27.0			27.0	23.0	0.85
Lambing et al. 1988	12	1.40		0.30	0.85	2.60	3.06
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	68.30	3.76
Saiki and Lowe 1987	Kesterson Pond 2	152.70	44.65	34.82	44.65	83.00	1.86
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	100.00	1.09
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.46	3.51
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	2.90	3.26
Saiki and Lowe 1987	Volta Wasteway	0.87	2.03	0.24	0.87	2.80	3.21
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.20	1.10
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.50	1.38

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	GT5	4.50	14.95		9.73	2.40	0.25
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.40	0.56
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.50	0.92
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.40	0.90
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.60	0.83
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.30	1.38
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.80	2.12
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.40	3.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.63	1.38
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.40	3.08



Median ratio: 1.89

R^2: 0.71
F: 76.3
df: 31
P: < 0.001

Special case of Odonates (Damselflies and Dragonflies) consuming invertebrates

n = Number of invertebrate food species co-occurring with an Odonate species.

C_{part} = Average selenium concentration in particulate material (mg/kg): $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$

C_{food} = Median selenium concentration in all invertebrate tissues that co-occur with an Odonate species (mg/kg)

C_{damsel} = Selenium concentration in damselfly tissue (mg/kg)

C_{dragon} = Selenium concentration in dragonfly tissue (mg/kg)

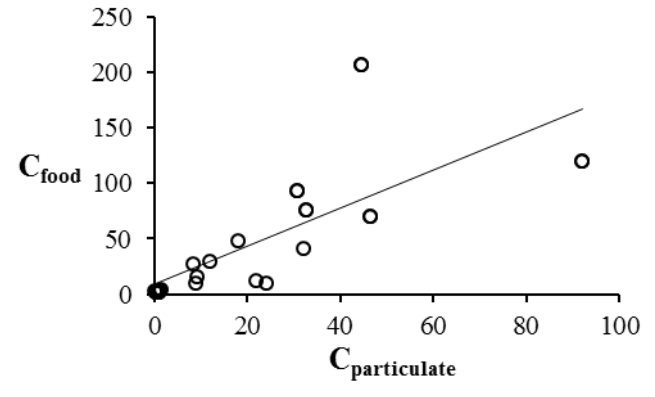
Ratio = $\frac{C_{food}}{C_{part}}$, $\frac{C_{damsel}}{C_{food}}$, or $\frac{C_{dragon}}{C_{food}}$

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Saiki and Lowe 1987	Kesterson Pond 11	dragonflies	4	18.15	47.5	2.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Wasteway	dragonflies	2	0.87	1.83	2.10
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Birkner 1978	29	damselflies	3	12.10	29.4	2.43
Birkner 1978	20	damselflies	2	22.00	11.2	0.51
Birkner 1978	7	damselflies	2	1.49	3.55	2.39
Birkner 1978	19	damselflies	2	9.00	9.8	1.09
Birkner 1978	30	damselflies	2	32.30	40.9	1.27
Birkner 1978	3	damselflies	3	0.20	2.5	12.50
Birkner 1978	22	damselflies	3	24.30	9.9	0.41
Birkner 1978	27	damselflies	1	8.43	26.7	3.17
Birkner 1978	23	damselflies	3	9.30	15.5	1.67
Grasso et al. 1995	17	damselflies	1	1.14	2.07	1.82



Median ratio: 2.21

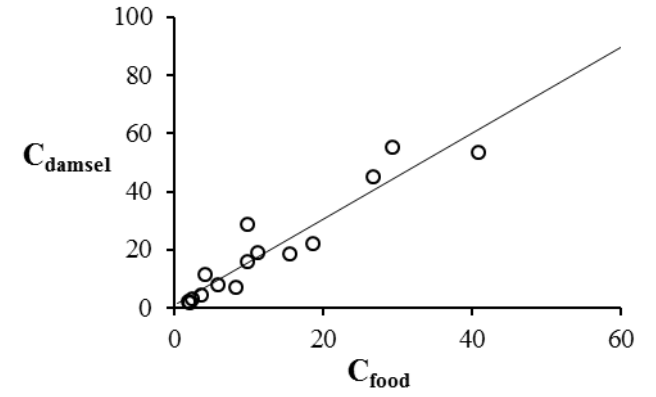
R^2: 0.54
F: 28.7
df: 24
P: < 0.001

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	29	29.4	55	1.87
Birkner 1978	4	1.95	1.8	0.92
Birkner 1978	25	18.7	21.9	1.17
Birkner 1978	20	11.2	18.7	1.67
Birkner 1978	7	3.55	4.4	1.24
Birkner 1978	19	9.8	28.4	2.90
Birkner 1978	6	4.2	11.1	2.64
Birkner 1978	30	40.9	53.3	1.30
Birkner 1978	3	2.5	3.1	1.24
Birkner 1978	22	9.9	15.8	1.60

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	27	26.7	45.1	1.69
Birkner 1978	23	15.5	18.4	1.19
Birkner 1978	11	5.9	7.7	1.31
Grasso et al. 1995	17	2.07	1.75	0.85
Grasso et al. 1995	9	8.2	6.98	0.85



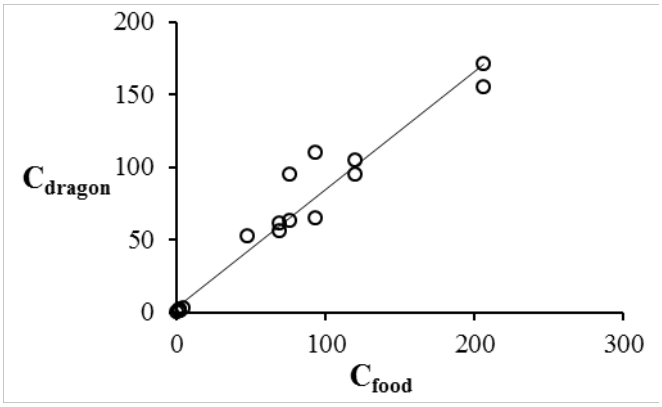
Median ratio: 1.30 x 2.21
(damselfly food to particulate) = 2.88

R^2: 0.89
F: 104.4
df: 13
P: <0.001

Dragonflies (Zygoptera)

Study	Site	C_{food}	C_{dragon}	Ratio
Mason et al. 2000	BK	1.845	1.665	0.90
Mason et al. 2000	HCRT	4.305	2.81	0.65
Saiki and Lowe 1987	Kesterson Pond 11	47.5	53	1.12
Saiki and Lowe 1987	Kesterson Pond 2	206.5	155	0.75
Saiki and Lowe 1987	Kesterson Pond 2	206.5	171	0.83
Saiki and Lowe 1987	Kesterson Pond 8	120	95.5	0.80
Saiki and Lowe 1987	Kesterson Pond 8	120	105	0.88
Saiki and Lowe 1987	Volta Pond 26	1.52	1.4	0.92
Saiki and Lowe 1987	Volta Pond 26	1.52	1.42	0.93
Saiki and Lowe 1987	Volta Pond 7	1.53	1.2	0.78
Saiki and Lowe 1987	Volta Pond 7	1.53	1.4	0.92
Saiki and Lowe 1987	Volta Wasteway	1.83	2.5	1.37
Schuler et al. 1990	Kesterson Pond 11	75.85	63.1	0.83
Schuler et al. 1990	Kesterson Pond 11	75.85	95.5	1.26
Schuler et al. 1990	Kesterson Pond 2	93.3	110	1.18
Schuler et al. 1990	Kesterson Pond 2	93.3	65	0.70
Schuler et al. 1990	Kesterson Pond 7	69.2	61.7	0.89
Schuler et al. 1990	Kesterson Pond 7	69.2	56.2	0.81
Sorenson & Schwarzbach 1991	5	0.42	0.49	1.17

Dragonflies (Zygoptera)



Median ratio: 0.89 x 2.21
(damselfly food to particulate) = 1.97

R^2: 0.95
F: 343.5
df: 17
P: <0.001

3.3.2 Vertebrates

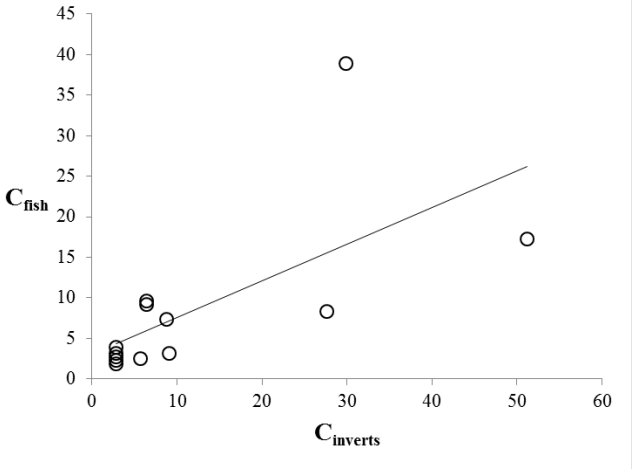
C_{invert} = Selenium concentration in invertebrate tissue (µg/g)
C_{fish} = Average selenium concentration in the whole-body of fish (µg/g)

Ratio = $\frac{C_{fish}}{C_{invert}}$

Black bullhead (*Ameiurus melas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	39.00	1.31
GEI 2013	SWA1	2.81	2.37	0.84
GEI 2013	SWA1	2.81	2.73	0.97
GEI 2013	SWA1	2.81	3.96	1.41
GEI 2013	SWA1	2.81	1.95	0.70
GEI 2013	SWA1	2.81	3.21	1.14
Mueller et al. 1991	R2	6.40	9.70	1.52
Mueller et al. 1991	R2	6.40	9.20	1.44
Mueller et al. 1991	R1	8.70	7.40	0.85
Lemly 1985	Badin Lake	5.70	2.58	0.45
Lemly 1985	Belews Lake	51.15	17.32	0.34
Lemly 1985	High Rock Lake	9.05	3.24	0.36
GEI 2014	SC-6	27.54	8.42	0.31

Black bullhead (*Ameiurus melas*)



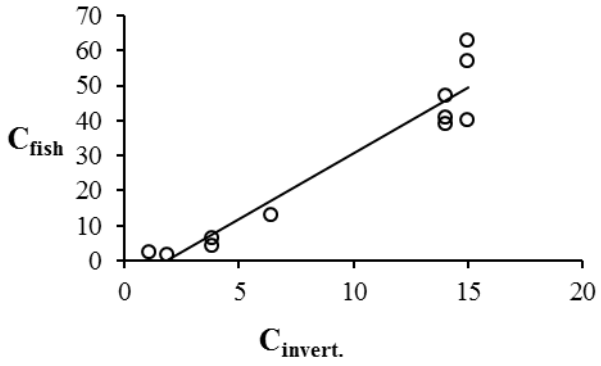
Median ratio: 0.85

R^2: 0.44
F: 8.52
df: 11
P: 0.006

Black crappie (*Pomoxis nigromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	Totten Reservoir	1.07	2.50	2.35
Butler et al. 1995	Summit Reservoir	1.85	1.70	0.92
Peterson et al. 1991	Ocean Lake, west side	3.83	4.20	1.10
Peterson et al. 1991	Ocean Lake, west side	3.83	6.32	1.65
Mueller et al. 1991	Lake Meredith near Ordway, CO	6.40	13.00	2.03
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	39.00	2.79
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	41.00	2.93
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	47.00	3.36
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	40.00	2.67
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	57.00	3.80
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	63.00	4.20

Black crappie (*Pomoxis nigromaculatus*)



Median ratio: 2.67

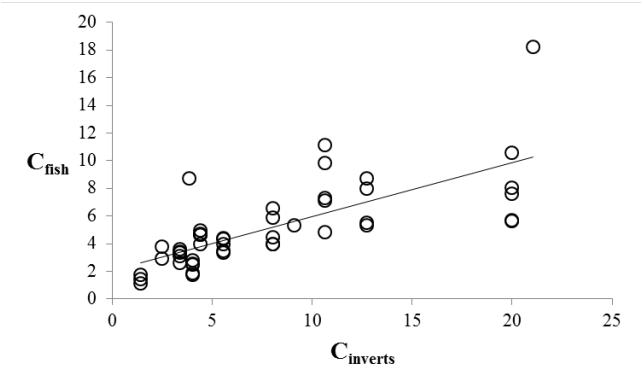
R^2: 0.92
F: 97.9
df: 9
P: < 0.001

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Cabin Creek, C-CC1	4.40	4.65	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	4.74	1.08
GEI 2014	Cabin Creek, C-CC1	4.40	4.95	1.12
GEI 2014	Cabin Creek, C-CC1	4.40	4.69	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	3.98	0.90
GEI 2014	Cabin Creek, C-CC2	5.56	3.46	0.62
GEI 2014	Cabin Creek, C-CC2	5.56	3.38	0.61
GEI 2014	Cabin Creek, C-CC2	5.56	3.95	0.71
GEI 2014	Cabin Creek, C-CC2	5.56	4.36	0.78
GEI 2014	Cabin Creek, C-CC2	5.56	4.39	0.79
GEI 2014	Coal Fork, C-CF1	3.39	3.58	1.06
GEI 2014	Coal Fork, C-CF1	3.39	3.09	0.91
GEI 2014	Coal Fork, C-CF1	3.39	3.37	0.99
GEI 2014	Coal Fork, C-CF1	3.39	2.64	0.78
GEI 2014	Coal Fork, C-CF1	3.39	3.42	1.01
GEI 2014	Hazy Creek, C-HC1	8.03	3.99	0.50
GEI 2014	Hazy Creek, C-HC1	8.03	5.88	0.73
GEI 2014	Hazy Creek, C-HC1	8.03	4.46	0.56
GEI 2014	Hazy Creek, C-HC1	8.03	6.55	0.82
GEI 2014	Hazy Creek, C-HC1	8.03	3.98	0.50
GEI 2014	Laurel Fork, C-LF1	12.73	5.36	0.42
GEI 2014	Laurel Fork, C-LF1	12.73	7.99	0.63
GEI 2014	Laurel Fork, C-LF1	12.73	8.72	0.68
GEI 2014	Laurel Fork, C-LF1	12.73	5.49	0.43
GEI 2014	Tenmile Fork, C-TF1	20.00	7.62	0.38
GEI 2014	Tenmile Fork, C-TF1	20.00	10.56	0.53

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Tenmile Fork, C-TF1	20.00	8.02	0.40
GEI 2014	Tenmile Fork, C-TF1	20.00	5.63	0.28
GEI 2014	Tenmile Fork, C-TF1	20.00	5.68	0.28
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.81	0.70
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.86	0.46
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.78	0.44
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.47	0.61
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.55	0.63
GEI 2014	Lukey Fork, H-LF1	9.09	5.32	0.59
GEI 2014	Mud River, H-MR3	3.86	8.72	2.26
GEI 2014	Mud River, H-MR6	2.49	3.80	1.53
GEI 2014	Mud River, H-MR6	2.49	2.93	1.18
GEI 2014	Sugartree Branch, H-SB1	10.62	9.82	0.92
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	11.14	1.05
GEI 2014	Sugartree Branch, H-SB1	10.62	4.85	0.46
GEI 2014	Sugartree Branch, H-SB1	10.62	7.16	0.67
GEI 2014	Stanley Fork, H-SF1	21.05	18.21	0.87



Median ratio: 0.71

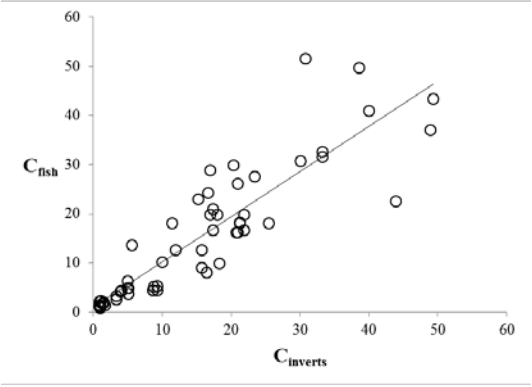
R^2: 0.52
F: 48.97
df: 45
P: < 0.001

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.30	2.16
Hermanutz et al. 1996	MSO II	16.63	24.29	1.46
Hermanutz et al. 1996	MSO III	5.55	13.77	2.48
Hermanutz et al. 1996	MSO I	21.19	18.28	0.86
Hermanutz et al. 1996	MSO I	21.19	18.13	0.86
Hermanutz et al. 1996	MSO II	17.30	20.99	1.21
Hermanutz et al. 1996	MSO II	5.05	4.88	0.97
Hermanutz et al. 1996	MSO I	0.87	1.55	1.78
Hermanutz et al. 1996	MSO II	1.70	1.55	0.91
Hermanutz et al. 1996	MSO III	1.20	1.83	1.52
Hermanutz et al. 1996	MSO III	10.00	10.32	1.03
Hermanutz et al. 1996	MSO III	3.95	4.21	1.06
Hermanutz et al. 1996	MSO II	17.30	16.76	0.97
Hermanutz et al. 1996	MSO II	5.05	3.86	0.76
Mueller et al. 1991	R1	8.70	5.20	0.60
Saiki et al. 1993	ET6	0.85	2.20	2.60
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.40	1.31
Saiki et al. 1993	GT5	4.90	5.00	1.02
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.30	1.06
Saiki et al. 1993	SJR2	3.30	3.30	1.00
Saiki et al. 1993	SJR2	3.30	2.70	0.82
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR3	1.50	1.90	1.27
Saiki et al. 1993	SJR1	0.95	0.87	0.92
Saiki et al. 1993	SJR1	0.95	1.40	1.48
Saiki et al. 1993	ET7	0.86	1.20	1.40
Saiki et al. 1993	ET7	0.86	1.20	1.40
Crutchfield 2000	transect 3	21.80	19.91	0.91
Crutchfield 2000	transect 3	21.80	16.72	0.77
Crutchfield 2000	transect 3	17.90	19.91	1.11
Crutchfield 2000	transect 3	20.70	16.26	0.79
Crutchfield 2000	transect 3	20.35	29.87	1.47
Crutchfield 2000	transect 3	23.40	27.59	1.18
Crutchfield 2000	transect 3	15.20	23.10	1.52
Crutchfield 2000	transect 3	16.95	28.96	1.71
Crutchfield 2000	transect 3	16.95	19.91	1.17
Crutchfield 2000	transect 3	11.95	12.69	1.06
Crutchfield 2000	transect 3	11.40	18.09	1.59
Crutchfield 2000	transect 3	9.25	4.56	0.49
Crutchfield 2000	transect 3	9.25	5.40	0.58

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 4	30.70	51.60	1.68
Crutchfield 2000	transect 4	30.00	30.78	1.03
Crutchfield 2000	transect 4	33.20	31.69	0.95
Crutchfield 2000	transect 4	48.90	37.09	0.76
Crutchfield 2000	transect 4	38.55	49.78	1.29
Crutchfield 2000	transect 4	49.30	43.40	0.88
Crutchfield 2000	transect 4	43.90	22.65	0.52
Crutchfield 2000	transect 4	33.25	32.60	0.98
Crutchfield 2000	transect 4	25.40	18.09	0.71
Crutchfield 2000	transect 4	20.90	16.26	0.78
Crutchfield 2000	transect 4	20.90	26.22	1.25
Crutchfield 2000	transect 4	15.70	12.69	0.81
Crutchfield 2000	transect 4	15.70	9.04	0.58
Crutchfield 2000	transect 4	16.45	8.13	0.49
Crutchfield 2000	transect 4	18.25	9.96	0.55
Bowie et al. 1996	Hyco Reservoir	40.00	41.00	1.03



Median ratio: 1.03

R^2: 0.80
F: 226.0
df: 58
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

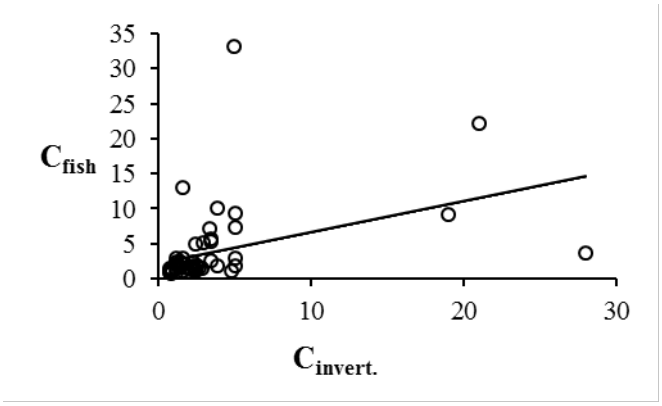
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	AK	0.78	0.94	1.21
Butler et al. 1995	HD1	0.83	0.83	1.01
Butler et al. 1995	HD1	0.83	0.86	1.04
Butler et al. 1995	HD1	0.83	1.20	1.45
Butler et al. 1995	HD1	0.83	1.40	1.70
Butler et al. 1995	DD	0.86	0.64	0.74
Butler et al. 1995	DD	0.86	0.88	1.02
Butler et al. 1995	DD	0.86	1.30	1.51

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	D1	1.20	2.80	2.33
Butler et al. 1993	B1	1.25	1.90	1.52
Butler et al. 1993	B1	1.25	2.20	1.76
Butler et al. 1995	ME2	1.25	0.83	0.66
Butler et al. 1995	ME2	1.25	1.30	1.04
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	SD	1.40	1.50	1.07
Butler et al. 1995	SD	1.40	1.80	1.29
Butler et al. 1993	D2	1.45	1.60	1.10
Butler et al. 1993	D2	1.45	2.30	1.59
Butler et al. 1993	P1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	RB3	1.60	13.00	8.13
Butler et al. 1995	YJ2	1.65	0.96	0.58
Butler et al. 1995	YJ2	1.65	2.80	1.70
Butler et al. 1994	NFK3	2.00	1.40	0.70
Butler et al. 1997	MN2	2.20	1.20	0.55
Butler et al. 1997	MUD	2.30	1.80	0.78
Butler et al. 1997	MUD	2.30	2.30	1.00
Butler et al. 1997	CHK	2.40	1.20	0.50
Butler et al. 1997	CHK	2.40	1.60	0.67
Butler et al. 1993	U1	2.45	4.80	1.96
Butler et al. 1995	SJ1	2.50	0.94	0.38
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.80	0.71
Butler et al. 1997	MN3	2.70	1.50	0.56
Butler et al. 1997	MN1	2.90	1.40	0.48
Butler et al. 1993	SP1	2.95	5.10	1.73
Butler et al. 1993	SP2	3.40	7.10	2.09
Butler et al. 1997	MUD2	3.45	2.50	0.72
Butler et al. 1997	MUD2	3.45	5.20	1.51
Butler et al. 1997	MUD2	3.45	5.60	1.62
Butler et al. 1993	F2	3.90	10.00	2.56
Butler et al. 1991	4	3.90	1.80	0.46
Butler et al. 1993	F2	4.80	0.94	0.20
Butler et al. 1994	BSW1	5.00	33.00	6.60
Butler et al. 1997	WBR	5.05	1.80	0.36
Butler et al. 1997	WBR	5.05	2.80	0.55
Butler et al. 1995	NW	5.10	7.20	1.41
Butler et al. 1995	NW	5.10	9.30	1.82

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	LZA1	19.00	9.00	0.47
Butler et al. 1994	RB1	21.00	22.00	1.05
Butler et al. 1994	GUN2	28.00	3.60	0.13



Median ratio: 1.04

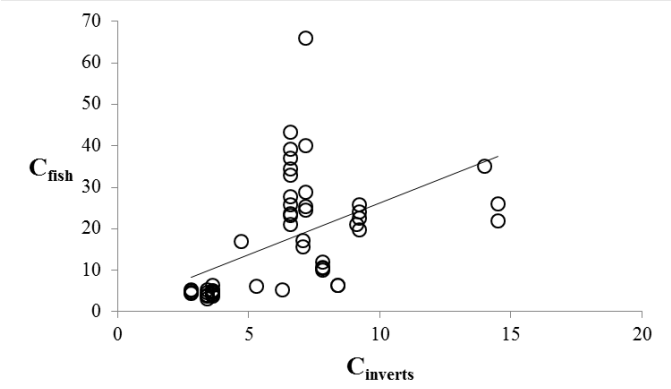
R^2: 0.16
F: 9.6
df: 51
P: < 0.001

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWA1	2.81	4.40	1.57
GEI 2013	SWA1	2.81	4.59	1.64
GEI 2013	SWA1	2.81	4.66	1.66
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.21	1.86
GEI 2013	SWA1	3.64	3.69	1.02
GEI 2013	SWA1	3.64	4.16	1.14
GEI 2013	SWA1	3.64	4.21	1.16
GEI 2013	SWA1	3.64	4.62	1.27
GEI 2013	SWA1	3.64	4.78	1.31
GEI 2013	SWA1	3.64	4.98	1.37
GEI 2013	SWA1	3.64	5.06	1.39
GEI 2013	SWA1	3.64	6.28	1.73
Lambing et al. 1994	S38	4.70	17.00	3.62
Lambing et al. 1994	S37	5.30	6.10	1.15
Lambing et al. 1994	S36	6.30	5.30	0.84
GEI 2014	Dry Creek, DC-2	3	3.14	0.92
GEI 2014	Dry Creek, DC-2	3	4.03	1.18
GEI 2014	Dry Creek, DC-2	3	3.76	1.10
GEI 2014	Dry Creek, DC-2	3	5.31	1.55
GEI 2014	Dry Creek, DC-2	3	4.59	1.34
GEI 2014	Dry Creek, DC-3	7	28.89	4.02
GEI 2014	Dry Creek, DC-3	7	66.07	9.20

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7	24.43	3.40
GEI 2014	Dry Creek, DC-3	7	25.36	3.53
GEI 2014	Dry Creek, DC-3	7	40.17	5.59
GEI 2014	Dry Creek, DC-3	9	25.80	2.80
GEI 2014	Dry Creek, DC-3	9	24.14	2.62
GEI 2014	Dry Creek, DC-3	9	22.46	2.43
GEI 2014	Dry Creek, DC-3	9	19.86	2.15
GEI 2013	SW2-1	6.60	21.14	3.21
GEI 2013	SW2-1	6.60	23.21	3.52
GEI 2013	SW2-1	6.60	23.64	3.58
GEI 2013	SW2-1	6.60	25.89	3.93
GEI 2013	SW2-1	6.60	27.71	4.20
GEI 2013	SW2-1	6.60	32.97	5.00
GEI 2013	SW2-1	6.60	34.54	5.24
GEI 2013	SW2-1	6.60	37.05	5.62
GEI 2013	SW2-1	6.60	39.26	5.95
GEI 2013	SW2-1	6.60	43.38	6.58
GEI 2013	SWB	7.06	15.74	2.23
GEI 2013	SWB	7.06	17.15	2.43
GEI 2013	SW1	7.82	9.96	1.27
GEI 2013	SW1	7.82	10.38	1.33
GEI 2013	SW1	7.82	10.58	1.35
GEI 2013	SW1	7.82	11.98	1.53
GEI 2013	SW11	8.41	6.36	0.76
GEI 2013	SW11	8.41	6.45	0.77
GEI 2013	SW2-1	9.14	21.09	2.31
Lambing et al. 1994	S34	14.00	35.00	2.50
Lambing et al. 1994	S11	14.50	22.00	1.52
Lambing et al. 1994	S11	14.50	26.00	1.79

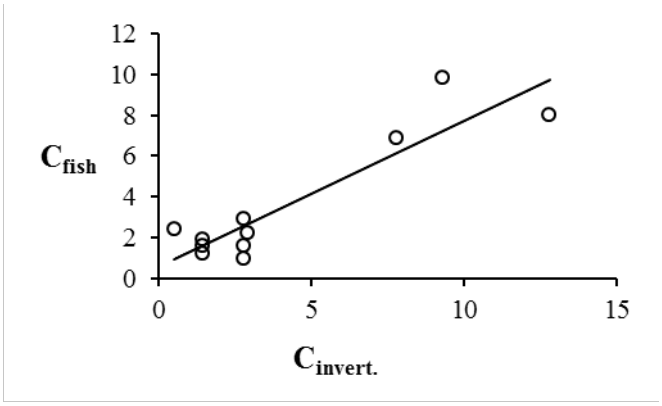


Median ratio: 1.79

R^2: 0.27
F: 18.48
df: 50
P: < 0.001

Brook trout (*Salvelinus fontinalis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	2.40	4.80
Mason et al. 2000	BK	1.43	1.21	0.84
Mason et al. 2000	BK	1.43	1.57	1.10
Mason et al. 2000	BK	1.43	1.90	1.33
Mason et al. 2000	HCRT	2.81	0.99	0.35
Mason et al. 2000	HCRT	2.81	1.59	0.57
Mason et al. 2000	HCRT	2.81	2.95	1.05
Butler et al. 1997	MN1	2.90	2.20	0.76
Hamilton and Buhl 2005	LGC	7.80	6.90	0.88
Hamilton and Buhl 2005	UGC	9.30	9.80	1.05
Hamilton and Buhl 2004	DVC	12.80	8.00	0.63
Hamilton and Buhl 2004	USC	0.50	2.40	4.80

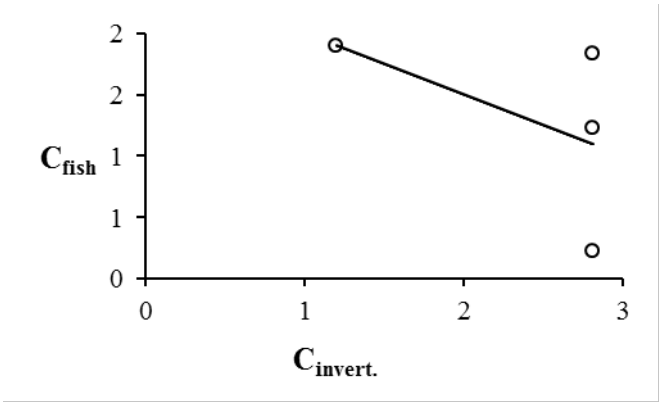


Median ratio: 0.88

R^2: 0.83
F: 43.6
df: 9
P: < 0.001

Brown bullhead (*Ameiurus nebulosus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Rinella and Schuler 1992		1.20	1.90	1.58
Mason et al. 2000	HCRT	2.81	0.22	0.08
Mason et al. 2000	HCRT	2.81	1.23	0.44
Mason et al. 2000	HCRT	2.81	1.83	0.65



Median ratio: 0.55

R^2: 0.27
F: 0.73
df: 2
P: 0.58
Not used because P > 0.05 and negative slope

Brown trout (*Salmo trutta*)

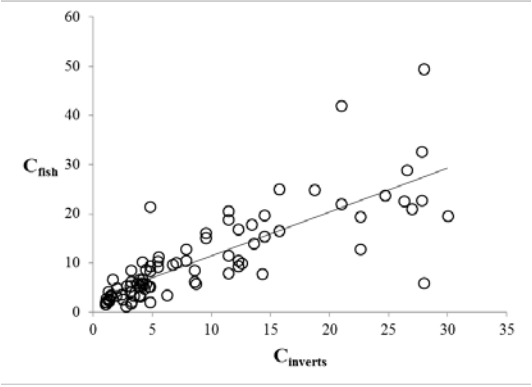
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.00	0.42
Butler et al. 1991	12	2.80	5.40	1.93
Butler et al. 1991	4	3.90	3.30	0.85
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1991	3	6.20	3.50	0.56
Butler et al. 1993	SP2	3.40	3.40	1.00
Butler et al. 1993	SP2	2.75	1.20	0.44
Butler et al. 1993	B2	1.35	2.40	1.78
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B1	1.25	4.20	3.36
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.20	2.21
Butler et al. 1993	P1	1.95	3.30	1.69
Butler et al. 1993	LP2	1.00	1.70	1.70
Butler et al. 1993	LP2	1.00	2.10	2.10
Butler et al. 1993	LP2	1.00	1.60	1.60
Butler et al. 1993	LP3	1.12	2.10	1.88
Butler et al. 1993	LP3	1.12	2.80	2.51
Butler et al. 1993	LP4	3.20	1.80	0.56

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1993	R2	3.90	6.70	1.72
Butler et al. 1993	R2	3.70	5.90	1.59
Butler et al. 1993	ST2	4.10	6.00	1.46
Butler et al. 1994	GUN2	28.00	49.45	1.77
Butler et al. 1994	GUN2	28.00	5.90	0.21
Butler et al. 1994	HCC1	21.00	21.98	1.05
Butler et al. 1994	HCC1	21.00	42.00	2.00
Butler et al. 1994	NFK3	2.00	5.00	2.50
Butler et al. 1994	SMF	4.80	21.44	4.47
Butler et al. 1994	SMF	4.80	5.26	1.10
Butler et al. 1994	SMF	4.80	8.40	1.75
Butler et al. 1994	SMF	4.80	9.40	1.96
Formation 2012	CC-1A	12.24	10.51	0.86
Formation 2012	CC-1A	12.24	9.33	0.76
Formation 2012	CC-1A	12.57	9.95	0.79
Formation 2012	CC-1A	12.24	16.85	1.38
Formation 2012	CC-1A	13.55	14.03	1.04
Formation 2012	CC-3A	5.45	10.44	1.92
Formation 2012	CC-3A	5.45	9.20	1.69
Formation 2012	CC-3A	5.48	11.25	2.05
Formation 2012	CC-3A	14.50	15.38	1.06
Formation 2012	CC-3A	14.50	19.68	1.36
Formation 2012	CC-150	4.46	5.83	1.31
Formation 2012	CC-150	4.46	8.67	1.94
Formation 2012	CC-150	4.70	5.20	1.11
Formation 2012	CC-150	7.03	10.14	1.44
Formation 2012	CC-150	14.32	7.83	0.55
Formation 2012	CC-350	3.16	6.28	1.99
Formation 2012	CC-350	3.16	8.53	2.70
Formation 2012	CC-350	4.20	5.78	1.38
Formation 2012	CC-350	11.45	11.50	1.00
Formation 2012	CC-350	11.45	7.95	0.69
Formation 2012	CC-75	3.11	4.05	1.30
Formation 2012	CC-75	3.11	5.35	1.72
Formation 2012	CC-75	3.97	3.18	0.80
Formation 2012	CC-75	4.16	10.32	2.48
Formation 2012	CC-75	4.16	6.60	1.59
Formation 2012	DC-600	8.53	8.54	1.00
Formation 2012	DC-600	8.53	6.20	0.73
Formation 2012	DC-600	8.65	5.85	0.68
Formation 2012	DC-600	7.83	12.83	1.64

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	7.83	10.54	1.35
Formation 2012	HS	15.70	16.52	1.05
Formation 2012	HS	15.70	25.00	1.59
Formation 2012	HS	18.70	24.90	1.33
Formation 2012	HS	27.80	32.63	1.17
Formation 2012	HS	27.80	22.80	0.82
Formation 2012	HS-3	11.40	20.60	1.81
Formation 2012	HS-3	11.40	18.83	1.65
Formation 2012	HS-3	13.41	17.89	1.33
Formation 2012	HS-3	24.70	23.68	0.96
Formation 2012	HS-3	26.55	28.97	1.09
Formation 2012	LSV-2C	22.62	19.45	0.86
Formation 2012	LSV-2C	22.62	12.78	0.56
Formation 2012	LSV-2C	26.31	22.67	0.86
Formation 2012	LSV-2C	30.00	19.53	0.65
Formation 2012	LSV-2C	26.95	20.96	0.78
Formation 2012	LSV-4	9.54	16.20	1.70
Formation 2012	LSV-4	9.54	15.18	1.59
Formation 2012	SFTC-1	2.42	3.68	1.52
Formation 2012	SFTC-1	3.21	2.25	0.70
Formation 2012	SFTC-1	1.63	6.70	4.11
Formation 2012	SFTC-1	2.49	2.64	1.06
Hamilton and Buhl 2005	CC	6.70	9.70	1.45
McDonald and Strosher 1998	ER 747	4.29	4.80	1.12

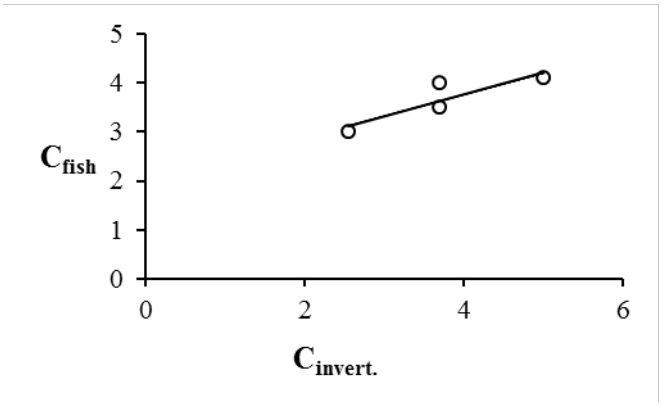


Median ratio: 1.38

R^2: 0.64
F: 151.8
df: 85
P: < 0.001

Bullhead (*Ameiurus sp.*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME3	2.55	3.00	1.18
Butler et al. 1993	R2	3.70	3.50	0.95
Butler et al. 1993	R2	3.70	4.00	1.08
Butler et al. 1994	BSW1	5.00	4.10	0.82



Median ratio: 1.01

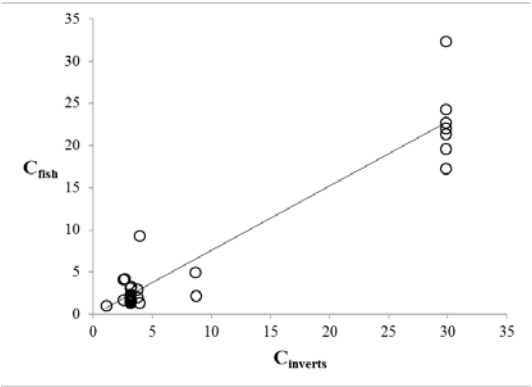
R^2: 0.77
F: 6.58
df: 2
P: 0.13
Not used because P > 0.05

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	21.36	0.72
Butler et al. 1991	7	29.80	22.05	0.74
Butler et al. 1991	7	29.80	17.27	0.58
Butler et al. 1991	7	29.80	19.62	0.66
Butler et al. 1991	7	29.80	22.76	0.76
Butler et al. 1991	7	29.80	24.33	0.82
Butler et al. 1991	7	29.80	32.40	1.09
Butler et al. 1993	LP4	3.20	1.65	0.52
Butler et al. 1993	LP4	3.20	3.30	1.03
Butler et al. 1993	R2	3.90	9.30	2.39
Butler et al. 1993	R2	3.90	1.33	0.34
Butler et al. 1993	R2	3.70	2.04	0.55
Butler et al. 1993	R2	3.70	3.00	0.81
Butler et al. 1995	SJ1	2.50	1.73	0.69
Butler et al. 1995	SJ1	2.50	4.10	1.64
Butler et al. 1995	TT	1.07	1.00	0.94
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	5.00	0.58
Mueller et al. 1991	R1	8.70	2.20	0.25
Roddy et al. 1991	18	3.10	1.40	0.45
Roddy et al. 1991	18	3.10	1.60	0.52
Roddy et al. 1991	18	3.10	1.70	0.55

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	1.90	0.61
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	1.50	0.48
Roddy et al. 1991	18	3.10	1.70	0.55
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	3.10	1.00



Median ratio: 0.74

R^2: 0.91
F: 332.8
df: 32
P: < 0.001

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	10.30	2.15
Butler et al. 1991	7	29.80	25.80	0.87
Butler et al. 1991	7	29.80	31.00	1.04
Butler et al. 1991	7	29.80	40.00	1.34
Butler et al. 1991	7	29.80	50.00	1.68
Butler et al. 1991	9	4.10	3.90	0.95
Butler et al. 1991	3	6.20	2.20	0.35
Butler et al. 1993	D2	1.45	3.70	2.55
Butler et al. 1993	F2	7.50	5.80	0.77
Butler et al. 1993	R2	4.30	5.00	1.16
Butler et al. 1993	R2	3.90	4.80	1.23
Butler et al. 1993	R2	3.70	3.30	0.89
Butler et al. 1994	GUN2	28.00	63.00	2.25
Butler et al. 1994	NFK2	3.10	4.90	1.58

Common carp (*Cyprinus carpio*)

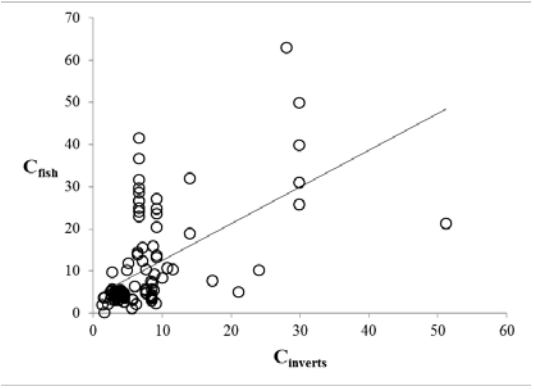
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	BSW1	5.00	12.00	2.40
Butler et al. 1994	RB1	21.00	5.10	0.24
Butler et al. 1995	ME4	1.55	3.90	2.52
Butler et al. 1995	ME4	1.55	3.70	2.39
Butler et al. 1995	ME4	1.55	3.80	2.45
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	ME3	2.55	5.20	2.04
Butler et al. 1995	SJ1	2.50	5.30	2.12
Butler et al. 1995	SJ1	2.50	3.40	1.36
Butler et al. 1995	MN1	2.70	5.80	2.15
Butler et al. 1995	MN1	2.70	9.80	3.63
Butler et al. 1995	MN1	2.70	5.40	2.00
Butler et al. 1997	MN5	8.60	16.00	1.86
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	3.30	1.10
GEI 2013	SWB	7.06	12.50	1.77
GEI 2013	SWB	7.06	15.61	2.21
GEI 2013	SW11	8.41	3.14	0.37
GEI 2013	SW11	8.41	3.52	0.42
GEI 2013	SW11	8.41	3.66	0.44
GEI 2013	SW11	8.41	3.85	0.46
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	3.60	0.43
GEI 2013	SW11	8.41	3.79	0.45
GEI 2013	SW11	8.41	3.95	0.47
GEI 2013	SW11	8.41	4.14	0.49
GEI 2013	SW11	8.41	4.34	0.52
GEI 2013	SW11	8.41	3.56	0.42
GEI 2013	SW2-1	6.60	26.73	4.05
GEI 2013	SW2-1	6.60	26.74	4.05
GEI 2013	SW2-1	6.60	28.74	4.36
GEI 2013	SW2-1	6.60	29.73	4.51
GEI 2013	SW2-1	6.60	41.57	6.30
GEI 2013	SW2-1	6.60	22.96	3.48
GEI 2013	SW2-1	6.60	24.27	3.68
GEI 2013	SW2-1	6.60	25.09	3.80
GEI 2013	SW2-1	6.60	31.74	4.81
GEI 2013	SW2-1	6.60	36.81	5.58
GEI 2013	SW2-1	9.14	13.29	1.45
GEI 2013	SW2-1	9.14	13.77	1.51
GEI 2013	SW2-1	9.14	20.49	2.24
GEI 2013	SW2-1	9.14	24.84	2.72

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW2-1	9.14	23.65	2.59
GEI 2013	SW2-1	9.14	27.27	2.99
GEI 2013	SW4-1	3.33	3.55	1.07
GEI 2013	SW4-1	3.33	4.68	1.41
GEI 2013	SW4-1	3.33	3.91	1.18
GEI 2013	SW4-1	3.33	4.36	1.31
GEI 2013	SW4-1	3.33	4.48	1.35
GEI 2013	SW4-1	3.33	4.60	1.38
GEI 2013	SW4-1	3.33	4.78	1.44
GEI 2013	SW9	4.45	2.73	0.61
GEI 2013	SW9	4.45	2.99	0.67
GEI 2013	SW9	4.45	3.64	0.82
GEI 2013	SW9	4.45	3.80	0.85
GEI 2013	SW9	4.45	3.90	0.88
GEI 2013	SW9	4.45	4.26	0.96
GEI 2013	SW9	4.45	4.53	1.02
GEI 2013	SW9	4.45	3.70	0.83
GEI 2013	SW9	4.45	3.77	0.85
GEI 2013	SW9	4.45	4.14	0.93
GEI 2013	SW9	4.45	4.41	0.99
GEI 2013	SW9	4.45	4.50	1.01
GEI 2013	SW9	4.45	4.69	1.05
GEI 2013	SW88	3.96	3.88	0.98
GEI 2013	SW88	3.96	5.33	1.35
GEI 2013	SW88	3.96	5.49	1.39
GEI 2013	SW88	3.96	5.66	1.43
Grasso et al. 1995	9	7.59	4.70	0.62
Grasso et al. 1995	9	7.59	4.93	0.65
Grasso et al. 1995	9	7.59	5.51	0.73
Lambing et al. 1994	S34	14.00	19.00	1.36
Lambing et al. 1994	S34	14.00	32.00	2.29
Low and Mullins 1990	5	5.60	1.20	0.21
Low and Mullins 1990	7	1.60	0.30	0.19
May et al. 2008	KR	17.20	7.78	0.45
May et al. 2008	NSCL	10.70	10.80	1.01
May et al. 2008	NSK	8.81	9.33	1.06
May et al. 2008	NSP	24.00	10.30	0.43
May et al. 2008	SSAL	11.50	10.50	0.91
May et al. 2008	SSAU	8.35	7.59	0.91
May et al. 2008	SSO	10.00	8.48	0.85
May et al. 2008	SSW	7.60	10.40	1.37
Mueller et al. 1991	R2	6.40	14.40	2.25

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.00	2.19
Mueller et al. 1991	R1	8.70	5.60	0.64
Mueller et al. 1991	A3	6.00	6.50	1.08
Mueller et al. 1991	A6	5.60	3.40	0.61
Mueller et al. 1991	A2	8.50	7.30	0.86
Peterson et al. 1991	7	3.83	4.24	1.11
Peterson et al. 1991	7	3.83	4.41	1.15
Peterson et al. 1991	7	3.83	4.73	1.23
Peterson et al. 1991	7	3.83	5.16	1.35
Peterson et al. 1991	7	3.83	5.21	1.36
Roddy et al. 1991	18	3.10	3.20	1.03
Roddy et al. 1991	18	3.10	3.90	1.26
Roddy et al. 1991	18	3.10	4.60	1.48
Roddy et al. 1991	18	3.10	4.70	1.52
Roddy et al. 1991	18	3.10	4.80	1.55
Roddy et al. 1991	18	3.10	5.30	1.71
Lemly 1985	Badin Lake	5.70	3.17	0.56
Lemly 1985	Belews Lake	51.15	21.29	0.42
Lemly 1985	High Rock Lake	9.05	2.45	0.27
Rinella and Schuler 1992	Harney Lake	2.05	2.20	1.07
Rinella and Schuler 1992	S. Malheur Lake	1.20	2.00	1.67



Median ratio: 1.20

R^2: 0.32
F: 54.27
df: 116
P: < 0.001

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	HCRT	2.81	0.49	0.18
Mason et al. 2000	HCRT	2.81	1.18	0.42
Mason et al. 2000	HCRT	2.81	1.97	0.70
GEI 2013	SW4-1	3.33	4.65	1.40
GEI 2013	SW4-1	3.33	4.96	1.49

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW4-1	3.33	5.52	1.66
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.31	1.90
GEI 2013	SW4-1	3.33	6.53	1.96
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	LG1	3.37	3.41	1.01
GEI 2013	LG1	3.37	3.58	1.06
GEI 2013	LG1	3.37	3.75	1.11
GEI 2013	LG1	3.37	3.78	1.12
GEI 2013	LG1	3.37	4.10	1.22
GEI 2013	LG1	3.39	3.23	0.95
GEI 2013	LG1	3.39	3.72	1.10
GEI 2013	LG1	3.39	3.74	1.10
GEI 2013	LG1	3.39	3.78	1.12
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.03	1.19
GEI 2013	LG1	3.39	4.12	1.22
GEI 2013	LG1	3.39	5.11	1.51
GEI 2013	LG1	3.39	5.21	1.54
GEI 2013	LG1	3.39	5.34	1.58
GEI 2013	LG1	3.56	3.28	0.92
GEI 2013	LG1	3.56	3.37	0.95
GEI 2013	LG1	3.56	3.82	1.07
GEI 2013	LG1	3.56	3.86	1.09
GEI 2013	LG1	3.56	4.02	1.13
GEI 2013	LG1	3.56	4.16	1.17
GEI 2013	LG1	3.56	4.49	1.26
GEI 2013	LG1	3.56	4.53	1.27
GEI 2013	LG1	3.56	4.63	1.30
GEI 2013	LG1	3.56	4.77	1.34
GEI 2013	CC1	3.76	5.43	1.44
GEI 2013	CC1	3.76	5.57	1.48
GEI 2013	CC1	3.76	6.51	1.73
GEI 2013	CC1	3.76	6.71	1.78
GEI 2013	CC1	3.76	7.12	1.89
GEI 2013	CC1	4.69	3.99	0.85
GEI 2013	CC1	4.69	4.06	0.87
GEI 2013	CC1	4.69	4.08	0.87
GEI 2013	CC1	4.69	4.25	0.91
GEI 2013	CC1	4.69	4.44	0.95
GEI 2013	CC1	4.69	4.48	0.96
GEI 2013	CC1	4.69	4.50	0.96

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	4.72	1.01
GEI 2013	CC1	4.69	5.24	1.12
GEI 2013	CC1	4.69	5.44	1.16
GEI 2013	CC1	5.86	4.98	0.85
GEI 2013	CC1	5.86	5.39	0.92
GEI 2013	CC1	5.86	5.77	0.99
GEI 2013	CC1	5.86	6.39	1.09
GEI 2013	CC1	5.86	6.43	1.10
GEI 2013	CC1	5.86	6.50	1.11
GEI 2013	CC1	5.86	6.57	1.12
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.47	1.28
GEI 2014	Bond Creek, BC-2	2.96	2.46	0.83
GEI 2014	Bond Creek, BC-2	2.96	3.22	1.09
GEI 2014	Bond Creek, BC-2	2.96	2.64	0.89
GEI 2014	Bond Creek, BC-2	2.96	2.96	1.00
GEI 2014	Bond Creek, BC-2	2.96	4.47	1.51
GEI 2014	Bond Creek, BC-3	3.02	3.09	1.02
GEI 2014	Bond Creek, BC-3	3.02	2.91	0.96
GEI 2014	Bond Creek, BC-3	3.02	3.45	1.14
GEI 2014	Bond Creek, BC-3	3.02	2.69	0.89
GEI 2014	Bond Creek, BC-3	3.02	3.30	1.09
GEI 2014	Bond Creek, BC-3	5.87	3.44	0.59
GEI 2014	Bond Creek, BC-3	5.87	2.62	0.45
GEI 2014	Bond Creek, BC-3	5.87	3.23	0.55
GEI 2014	Cow Camp Creek, CC-2	5.65	3.05	0.54
GEI 2014	Cow Camp Creek, CC-2	5.65	3.69	0.65
GEI 2014	Cow Camp Creek, CC-2	5.65	3.84	0.68
GEI 2014	Cow Camp Creek, CC-2	5.65	4.44	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	3.98	0.70
GEI 2014	Hazy Creek, C-HC1	8.03	6.84	0.85
GEI 2014	Hazy Creek, C-HC1	8.03	3.78	0.47
GEI 2014	Hazy Creek, C-HC1	8.03	5.81	0.72
GEI 2014	Hazy Creek, C-HC1	8.03	4.29	0.53
GEI 2014	Hazy Creek, C-HC1	8.03	3.59	0.45
GEI 2014	Laurel Fork, C-LF1	12.73	6.52	0.51
GEI 2014	Laurel Fork, C-LF1	12.73	6.81	0.54
GEI 2014	Laurel Fork, C-LF1	12.73	5.11	0.40
GEI 2014	Laurel Fork, C-LF1	12.73	5.16	0.41
GEI 2014	Laurel Fork, C-LF1	12.73	5.46	0.43
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.81	0.63

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.89	0.81
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.58	0.60
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.81	0.80
GEI 2014	Little Marsh Fork, C-LMF1	6.02	5.82	0.97
GEI 2014	Dry Creek, DC-1	3.37	4.62	1.37
GEI 2014	Dry Creek, DC-1	3.37	5.15	1.53
GEI 2014	Dry Creek, DC-1	3.37	6.46	1.92
GEI 2014	Dry Creek, DC-1	3.37	5.73	1.70
GEI 2014	Dry Creek, DC-1	3.37	4.59	1.36
GEI 2014	Dry Creek, DC-1	2.29	4.94	2.16
GEI 2014	Dry Creek, DC-1	2.29	4.04	1.76
GEI 2014	Dry Creek, DC-1	2.29	3.62	1.58
GEI 2014	Dry Creek, DC-1	2.29	3.84	1.68
GEI 2014	Dry Creek, DC-1	2.15	4.02	1.87
GEI 2014	Dry Creek, DC-1	2.15	3.60	1.67
GEI 2014	Dry Creek, DC-1	2.15	3.28	1.52
GEI 2014	Dry Creek, DC-1	2.15	3.03	1.41
GEI 2014	Dry Creek, DC-1	2.15	4.01	1.86
GEI 2014	Dry Creek, DC-1	1.97	3.51	1.78
GEI 2014	Dry Creek, DC-2	3.42	5.32	1.56
GEI 2014	Dry Creek, DC-2	3.42	4.62	1.35
GEI 2014	Dry Creek, DC-2	3.42	4.43	1.30
GEI 2014	Dry Creek, DC-2	3.42	4.56	1.34
GEI 2014	Dry Creek, DC-2	3.42	6.38	1.87
GEI 2014	Dry Creek, DC-2	3.42	2.96	0.87
GEI 2014	Dry Creek, DC-2	3.42	3.58	1.05
GEI 2014	Dry Creek, DC-2	3.42	3.22	0.94
GEI 2014	Dry Creek, DC-2	3.42	4.07	1.19
GEI 2014	Dry Creek, DC-2	3.42	3.28	0.96
GEI 2014	Dry Creek, DC-2	3.16	6.52	2.07
GEI 2014	Dry Creek, DC-2	3.16	4.92	1.56
GEI 2014	Dry Creek, DC-2	3.16	3.10	0.98
GEI 2014	Dry Creek, DC-2	3.16	3.14	1.00
GEI 2014	Dry Creek, DC-2	3.16	4.36	1.38
GEI 2014	Dry Creek, DC-2	3.16	3.12	0.99
GEI 2014	Dry Creek, DC-2	3.16	5.40	1.71
GEI 2014	Dry Creek, DC-2	2.93	2.85	0.97
GEI 2014	Dry Creek, DC-2	2.93	4.94	1.69
GEI 2014	Dry Creek, DC-2	2.93	5.17	1.76
GEI 2014	Dry Creek, DC-2	2.93	3.47	1.18
GEI 2014	Dry Creek, DC-2	2.93	2.49	0.85
GEI 2014	Dry Creek, DC-3	7.18	23.79	3.31

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7.18	16.06	2.24
GEI 2014	Dry Creek, DC-3	7.18	24.43	3.40
GEI 2014	Dry Creek, DC-3	7.18	22.20	3.09
GEI 2014	Dry Creek, DC-3	7.18	26.28	3.66
GEI 2014	Dry Creek, DC-3	9.23	21.48	2.33
GEI 2014	Dry Creek, DC-3	9.23	21.24	2.30
GEI 2014	Dry Creek, DC-3	9.23	21.46	2.33
GEI 2014	Dry Creek, DC-3	9.23	22.48	2.44
GEI 2014	Dry Creek, DC-3	9.23	18.80	2.04
GEI 2014	Dry Creek, DC-3	9.23	16.87	1.83
GEI 2014	Dry Creek, DC-4	19.42	15.86	0.82
GEI 2014	Dry Creek, DC-4	19.42	12.76	0.66
GEI 2014	Dry Creek, DC-4	19.42	28.50	1.47
GEI 2014	Dry Creek, DC-4	19.42	18.14	0.93
GEI 2014	Dry Creek, DC-4	19.42	17.55	0.90
GEI 2014	Dry Creek, DC-4	18.10	34.60	1.91
GEI 2014	Dry Creek, DC-4	18.10	23.70	1.31
GEI 2014	Foidel Creek, FOC-1	3.06	8.00	2.61
GEI 2014	Foidel Creek, FOC-1	3.06	9.68	3.16
GEI 2014	Foidel Creek, FOC-1	3.06	8.86	2.89
GEI 2014	Foidel Creek, FOC-1	3.06	2.51	0.82
GEI 2014	Foidel Creek, FOC-1	3.06	2.86	0.93
GEI 2014	Foidel Creek, FOC-1	3.06	4.24	1.39
GEI 2014	Foidel Creek, FOC-1	3.06	3.27	1.07
GEI 2014	Foidel Creek, FOC-1	3.06	5.03	1.64
GEI 2014	Foidel Creek, FOC-2	2.18	2.07	0.95
GEI 2014	Foidel Creek, FOC-2	2.18	3.06	1.41
GEI 2014	Foidel Creek, FOC-2	2.18	3.82	1.76
GEI 2014	Foidel Creek, FOC-2	2.18	2.26	1.04
GEI 2014	Foidel Creek, FOC-2	2.18	2.02	0.93
GEI 2014	Foidel Creek, FOC-2	2.18	2.28	1.05
GEI 2014	Foidel Creek, FOC-2	2.18	2.44	1.12
GEI 2014	Foidel Creek, FOC-2	2.18	2.62	1.21
GEI 2014	Grassy Creek, GC-2	4.20	5.28	1.26
GEI 2014	Grassy Creek, GC-2	4.20	6.13	1.46
GEI 2014	Grassy Creek, GC-2	4.20	6.29	1.50
GEI 2014	Grassy Creek, GC-2	4.20	4.80	1.15
GEI 2014	Grassy Creek, GC-2	4.20	4.59	1.09
GEI 2014	Grassy Creek, GC-2	4.58	3.27	0.71
GEI 2014	Grassy Creek, GC-2	4.58	5.50	1.20
GEI 2014	Grassy Creek, GC-2	4.58	3.64	0.80
GEI 2014	Grassy Creek, GC-2	4.58	4.29	0.94

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-2	4.58	3.01	0.66
GEI 2014	Grassy Creek, GC-2	3.96	7.48	1.89
GEI 2014	Grassy Creek, GC-2	3.96	6.12	1.55
GEI 2014	Grassy Creek, GC-2	3.96	8.61	2.18
GEI 2014	Grassy Creek, GC-2	3.96	7.09	1.79
GEI 2014	Grassy Creek, GC-2	3.96	5.06	1.28
GEI 2014	Grassy Creek, GC-2	3.96	4.95	1.25
GEI 2014	Grassy Creek, GC-2	3.96	3.85	0.97
GEI 2014	Grassy Creek, GC-2	3.96	5.32	1.34
GEI 2014	Grassy Creek, GC-2	3.96	4.04	1.02
GEI 2014	Grassy Creek, GC-2	3.96	4.25	1.08
GEI 2014	Grassy Creek, GC-2	3.97	3.48	0.88
GEI 2014	Grassy Creek, GC-2	3.97	3.86	0.97
GEI 2014	Grassy Creek, GC-2	3.97	4.08	1.03
GEI 2014	Grassy Creek, GC-2	3.97	4.58	1.15
GEI 2014	Grassy Creek, GC-2	3.97	3.53	0.89
GEI 2014	Grassy Creek, GC-3	4.54	6.07	1.34
GEI 2014	Grassy Creek, GC-3	4.54	7.25	1.60
GEI 2014	Grassy Creek, GC-3	4.54	5.25	1.16
GEI 2014	Grassy Creek, GC-3	4.54	5.65	1.25
GEI 2014	Grassy Creek, GC-3	4.54	10.75	2.37
GEI 2014	Grassy Creek, GC-3	4.55	3.30	0.73
GEI 2014	Grassy Creek, GC-3	4.55	3.98	0.88
GEI 2014	Grassy Creek, GC-3	4.55	3.46	0.76
GEI 2014	Grassy Creek, GC-3	4.55	4.14	0.91
GEI 2014	Grassy Creek, GC-3	4.55	3.81	0.84
GEI 2014	Grassy Creek, GC-3	4.34	11.05	2.55
GEI 2014	Grassy Creek, GC-3	4.34	7.22	1.66
GEI 2014	Grassy Creek, GC-3	4.34	9.61	2.22
GEI 2014	Grassy Creek, GC-3	4.34	6.03	1.39
GEI 2014	Grassy Creek, GC-3	4.34	3.95	0.91
GEI 2014	Grassy Creek, GC-3	4.34	4.82	1.11
GEI 2014	Grassy Creek, GC-3	4.34	5.18	1.19
GEI 2014	Grassy Creek, GC-3	4.34	4.46	1.03
GEI 2014	Grassy Creek, GC-3	4.34	4.21	0.97
GEI 2014	Grassy Creek, GC-3	4.35	2.97	0.68
GEI 2014	Grassy Creek, GC-3	4.35	4.16	0.96
GEI 2014	Grassy Creek, GC-3	4.35	4.29	0.99
GEI 2014	Grassy Creek, GC-3	4.35	3.69	0.85
GEI 2014	Grassy Creek, GC-3	4.35	4.73	1.09
GEI 2014	Grassy Creek, GC-4	5.10	4.30	0.84
GEI 2014	Grassy Creek, GC-4	5.10	5.17	1.01

Creek chub (*Semotilus atromaculatus*)

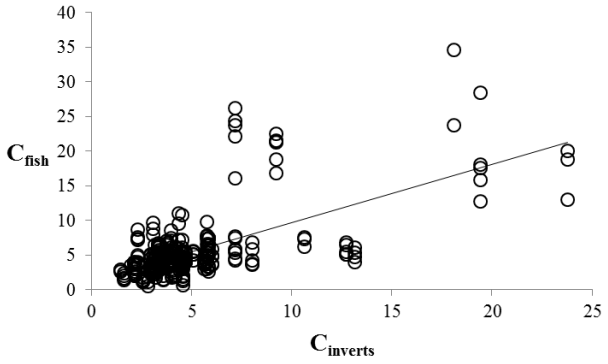
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-4	5.10	5.46	1.07
GEI 2014	Grassy Creek, GC-4	5.10	5.63	1.10
GEI 2014	Grassy Creek, GC-4	5.10	5.15	1.01
GEI 2014	Grassy Creek, GC-4	5.76	7.72	1.34
GEI 2014	Grassy Creek, GC-4	5.76	6.04	1.05
GEI 2014	Grassy Creek, GC-4	5.76	7.88	1.37
GEI 2014	Grassy Creek, GC-4	5.76	9.77	1.70
GEI 2014	Grassy Creek, GC-4	5.76	7.35	1.28
GEI 2014	Grassy Creek, GC-4	5.76	4.17	0.72
GEI 2014	Grassy Creek, GC-4	5.76	4.86	0.84
GEI 2014	Grassy Creek, GC-4	5.76	5.02	0.87
GEI 2014	Grassy Creek, GC-4	5.76	4.79	0.83
GEI 2014	Grassy Creek, GC-4	5.76	6.56	1.14
GEI 2014	Grassy Creek, GC-4	5.76	5.31	0.92
GEI 2014	Grassy Creek, GC-4 US	13.16	4.05	0.31
GEI 2014	Grassy Creek, GC-4 US	13.16	4.80	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	5.41	0.41
GEI 2014	Grassy Creek, GC-4 US	13.16	6.05	0.46
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.96	0.69
GEI 2014	Big Horse Creek, H-BHC3	5.78	2.97	0.51
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.84	0.66
GEI 2014	Sally Fork, H-BLB2	1.64	2.42	1.48
GEI 2014	Sally Fork, H-BLB2	1.64	1.65	1.01
GEI 2014	Sally Fork, H-BLB2	1.64	1.68	1.03
GEI 2014	Sally Fork, H-BLB2	1.64	2.02	1.23
GEI 2014	Sally Fork, H-BLB2	1.64	1.46	0.89
GEI 2014	Hubberson Gulch, HG-2	3.44	4.41	1.28
GEI 2014	Hubberson Gulch, HG-2	3.44	3.56	1.04
GEI 2014	Hubberson Gulch, HG-2	3.44	4.48	1.30
GEI 2014	Hubberson Gulch, HG-2	1.46	2.95	2.03
GEI 2014	Hubberson Gulch, HG-2	1.46	2.66	1.83
GEI 2014	Hubberson Gulch, HG-2	1.46	2.87	1.97
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	3.04	0.75
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.81	0.45
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.35	0.58
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.91	0.47
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.83	0.70
GEI 2014	Laurel Creek, H-LC1	4.57	1.29	0.28

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Laurel Creek, H-LC1	4.57	2.04	0.45
GEI 2014	Laurel Creek, H-LC1	4.57	1.49	0.33
GEI 2014	Laurel Creek, H-LC1	4.57	1.85	0.41
GEI 2014	Laurel Creek, H-LC1	4.57	0.67	0.15
GEI 2014	Lick Creek, H-LKC1	2.59	1.83	0.71
GEI 2014	Lick Creek, H-LKC1	2.59	1.40	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.41	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.19	0.46
GEI 2014	Lick Creek, H-LKC1	2.59	1.22	0.47
GEI 2014	Mud River, H-MR3	3.86	4.75	1.23
GEI 2014	Mud River, H-MR3	3.86	4.60	1.19
GEI 2014	Mud River, H-MR3	3.86	5.06	1.31
GEI 2014	Mud River, H-MR3	3.86	3.32	0.86
GEI 2014	Mud River, H-MR3	3.86	4.19	1.08
GEI 2014	Mud River, H-MR5	3.58	1.51	0.42
GEI 2014	Mud River, H-MR5	3.58	1.43	0.40
GEI 2014	Mud River, H-MR5	3.58	1.98	0.55
GEI 2014	Mud River, H-MR5	3.58	3.80	1.06
GEI 2014	Mud River, H-MR5	3.58	3.44	0.96
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	7.56	0.71
GEI 2014	Sugartree Branch, H-SB1	10.62	6.20	0.58
GEI 2014	Middle Creek, MC-1	3.21	2.75	0.86
GEI 2014	Middle Creek, MC-1	3.21	4.74	1.48
GEI 2014	Middle Creek, MC-1	3.21	4.01	1.25
GEI 2014	Middle Creek, MC-1	3.21	3.94	1.23
GEI 2014	Middle Creek, MC-1	3.21	3.63	1.13
GEI 2014	Middle Creek, MC-1	3.21	1.83	0.57
GEI 2014	Middle Creek, MC-1	3.21	1.85	0.57
GEI 2014	Middle Creek, MC-1	3.21	2.50	0.78
GEI 2014	Middle Creek, MC-1	3.21	2.33	0.73
GEI 2014	Middle Creek, MC-1	3.21	2.56	0.80
GEI 2014	Middle Creek, MC-2	4.19	1.93	0.46
GEI 2014	Middle Creek, MC-2	4.19	1.89	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.51	0.60
GEI 2014	Middle Creek, MC-2	4.19	1.87	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.13	0.51
GEI 2014	Sage Creek, SC-3	2.29	7.33	3.20
GEI 2014	Sage Creek, SC-3	2.29	7.23	3.16
GEI 2014	Sage Creek, SC-3	2.29	7.60	3.32
GEI 2014	Sage Creek, SC-3	2.29	4.77	2.08
GEI 2014	Sage Creek, SC-3	2.29	8.70	3.80

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Sage Creek, SC-4	23.79	18.77	0.79
GEI 2014	Sage Creek, SC-4	23.79	20.08	0.84
GEI 2014	Sage Creek, SC-4	23.79	13.05	0.55
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.41	1.04
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.65	1.07
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.47	0.62
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.83	0.81
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.37	0.75
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.69	0.80
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.31	0.60



Median ratio: 1.06

R^2: 0.41
F: 214.7
df: 303
P: < 0.001

Cutthroat trout (*Oncorhynchus clarkii*)

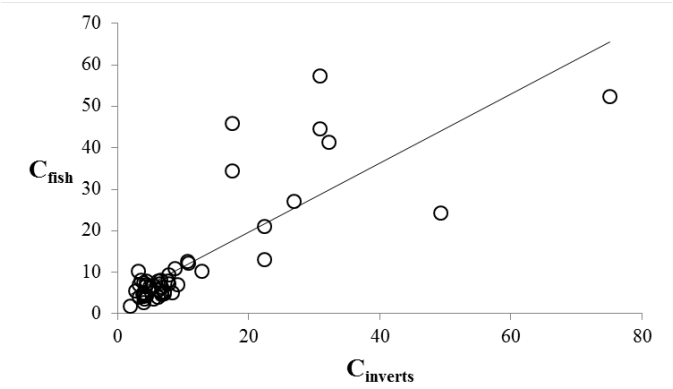
Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ShpC	1.90	1.80	0.95
McDonald and Strosher 1998	ER 745	2.74	5.40	1.97
Hamilton and Buhl 2005	SC	4.10	3.50	0.85
McDonald and Strosher 1998	ER 747	4.29	6.57	1.53
Hamilton and Buhl 2005	UAC	5.00	6.60	1.32
Hamilton and Buhl 2004	ACM	6.70	6.30	0.94
Hamilton and Buhl 2005	DC	8.70	11.00	1.26
McDonald and Strosher 1998	ER 746	10.70	12.71	1.19
Hamilton and Buhl 2005	BGS	10.80	12.20	1.13
Hamilton and Buhl 2004	DVC	12.80	10.20	0.80
Hamilton and Buhl 2004	UEMC	26.90	27.00	1.00
Hamilton and Buhl 2004	LEMC	75.20	52.30	0.70
Minnow 2007	BA6	3.27	6.98	2.13
Minnow 2007	AL4	3.92	4.44	1.13
Minnow 2007	MI5	4.00	5.12	1.28

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	EL12	4.01	7.42	1.85
Minnow 2007	EL14	4.41	4.52	1.02
Minnow 2007	FO9	4.44	7.80	1.76
Minnow 2007	MI3	6.21	5.65	0.91
Minnow 2007	MI2	6.69	5.16	0.77
Minnow 2007	EL1	7.08	4.82	0.68
Minnow 2007	LI8	7.81	9.36	1.20
Minnow 2007	FO10	17.51	45.94	2.62
Minnow 2007	HA7	22.41	21.10	0.94
Minnow 2007	CL11	30.87	57.27	1.86
Orr et al. 2012	Alexander Creek	3.92	2.71	0.69
Orr et al. 2012	Elk River 1	6.23	5.61	0.90
Orr et al. 2012	Elk River 1	6.23	7.94	1.27
Orr et al. 2012	Elk River 1	7.08	5.35	0.76
Orr et al. 2012	Elk River 12	3.81	4.58	1.20
Orr et al. 2012	Elk River 12	4.01	4.89	1.22
Orr et al. 2012	Fording River 22	3.10	10.28	3.32
Orr et al. 2012	Fording River 23	7.72	7.92	1.03
Orr et al. 2012	Fording River 9	4.44	6.92	1.56
Orr et al. 2012	Line Creek 8	6.61	7.99	1.21
Orr et al. 2012	Line Creek 8	7.81	7.13	0.91
Orr et al. 2012	Michel Creek 2	8.38	5.13	0.61
Orr et al. 2012	Michel Creek 2	6.69	4.63	0.69
Orr et al. 2012	Michel Creek 3	5.42	3.51	0.65
Orr et al. 2012	Michel Creek 3	6.21	4.02	0.65
Orr et al. 2012	Michel Creek 5	4.00	4.12	1.03
Orr et al. 2012	Fording River MP1	5.49	6.84	1.25
Orr et al. 2012	Barnes Lake Wetland 6	3.27	3.92	1.20
Orr et al. 2012	Clode Pond 11	32.22	41.27	1.28
Orr et al. 2012	Clode Pond 11	30.87	44.70	1.45
Orr et al. 2012	Elk Lakes 14	6.40	7.14	1.12
Orr et al. 2012	Elk Lakes 14	4.41	4.35	0.99
Orr et al. 2012	Fording River Oxbow 10	49.26	24.34	0.49
Orr et al. 2012	Fording River Oxbow 10	17.51	34.41	1.97
Orr et al. 2012	Henretta Lake 27	9.16	6.90	0.75
Orr et al. 2012	O'Rourke Lake 1	3.63	8.05	2.22
Orr et al. 2012	Harmer Pond 7	22.41	13.08	0.58

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.12

R^2: 0.66
F: 95.81
df: 50
P: < 0.001

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	4	1.80	2.10	1.17
Birkner 1978	22	11.30	11.00	0.97
Birkner 1978	27	34.60	79.00	2.28
Birkner 1978	23	15.50	34.50	2.23
Birkner 1978	1	1.75	2.10	1.20
Butler et al. 1991	10	4.80	8.10	1.69
Butler et al. 1991	3	6.20	9.50	1.53
Butler et al. 1993	SP2	3.40	6.00	1.76
Butler et al. 1993	SP2	3.15	8.20	2.60
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.80	3.17
Butler et al. 1993	U1	2.45	6.40	2.61
Butler et al. 1993	R2	3.90	6.60	1.69
Butler et al. 1993	R2	3.70	6.60	1.78
Butler et al. 1993	ST2	4.50	12.80	2.84
Butler et al. 1993	ST2	4.10	7.60	1.85
Butler et al. 1993	ST2	4.10	16.00	3.90
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	SB2	3.75	5.70	1.52
Butler et al. 1993	SB2	3.75	8.60	2.29
Butler et al. 1993	SB2	3.65	9.90	2.71
Butler et al. 1993	WSB2	4.75	17.10	3.60
Butler et al. 1993	WSB2	3.60	4.20	1.17
Butler et al. 1993	WSB2	3.60	10.00	2.78

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	WSB2	3.00	8.10	2.70
Butler et al. 1994	CRC	7.50	20.40	2.72
Butler et al. 1994	CF1	3.60	7.90	2.19
Butler et al. 1994	GUN2	28.00	7.50	0.27
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	TGC	4.90	11.00	2.24
Butler et al. 1994	AD	2.70	9.60	3.56
Butler et al. 1994	LSW1	3.90	73.00	18.72
Butler et al. 1994	OMD	73.00	13.00	0.18
Butler et al. 1994	PSW1	3.70	22.00	5.95
Butler et al. 1994	MKP	32.00	51.00	1.59
Butler et al. 1995	AK	0.78	2.60	3.35
Butler et al. 1995	AK	0.78	2.90	3.74
Butler et al. 1995	AK	0.78	2.80	3.61
Butler et al. 1995	DD	0.86	3.40	3.95
Butler et al. 1995	DD	0.86	3.90	4.53
Butler et al. 1995	DD	0.86	3.60	4.19
Butler et al. 1995	HD1	0.83	3.90	4.73
Butler et al. 1995	HD1	0.83	2.50	3.03
Butler et al. 1995	HD1	0.83	2.60	3.15
Butler et al. 1995	HD2	0.98	1.50	1.53
Butler et al. 1995	HD2	0.98	1.60	1.63
Butler et al. 1995	ME1	3.40	5.60	1.65
Butler et al. 1995	ME2	1.25	4.80	3.84
Butler et al. 1995	ME4	1.55	1.40	0.90
Butler et al. 1995	ME4	1.55	5.90	3.81
Butler et al. 1995	ME3	2.55	4.30	1.69
Butler et al. 1995	ME3	2.55	5.30	2.08
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	SD	1.40	4.90	3.50
Butler et al. 1995	SD	1.40	3.00	2.14
Butler et al. 1995	SD	1.40	4.00	2.86
Butler et al. 1995	WC	6.75	18.40	2.73
Butler et al. 1995	WC	6.75	22.90	3.39
Butler et al. 1995	WC	6.75	26.40	3.91
Butler et al. 1995	YJ2	1.65	11.00	6.67
Butler et al. 1995	YJ2	1.65	4.00	2.42
Butler et al. 1997	MNP2	4.40	11.00	2.50
Butler et al. 1997	MUD2	3.45	7.70	2.23
Butler et al. 1997	MUD2	3.45	12.00	3.48
Butler et al. 1997	MUD2	3.45	6.50	1.88
Butler et al. 1997	WCP	9.70	10.00	1.03

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	WCP	9.70	15.00	1.55
Butler et al. 1997	TR25	1.80	4.00	2.22
Butler et al. 1997	TR25	1.80	5.20	2.89
Butler et al. 1997	TR25	1.80	6.00	3.33
Butler et al. 1997	TRH	1.60	4.20	2.63
Butler et al. 1997	TRH	1.60	4.30	2.69
Butler et al. 1997	TRH	1.60	2.20	1.38
Butler et al. 1997	TRH	1.60	3.00	1.88
Butler et al. 1997	MN5	8.60	7.30	0.85
Butler et al. 1997	MNP1	0.70	1.70	2.43
Butler et al. 1997	MNP1	0.70	1.80	2.57
GEI 2013	SWA1	3.64	4.07	1.12
GEI 2013	SWA1	3.64	4.68	1.29
GEI 2013	SWA1	3.64	4.76	1.31
GEI 2013	SWA1	3.64	5.45	1.50
GEI 2013	SWA1	3.64	5.71	1.57
GEI 2013	SWA1	3.64	3.62	1.00
GEI 2013	SWA1	3.64	3.72	1.02
GEI 2013	SWA1	3.64	4.43	1.22
GEI 2013	SWA1	3.64	4.52	1.24
GEI 2013	SWA1	3.64	4.66	1.28
GEI 2013	SWA1	2.81	4.48	1.60
GEI 2013	SWA1	2.81	4.53	1.61
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.24	1.87
GEI 2013	SWA1	2.81	5.76	2.05
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	3.98	1.42
GEI 2013	SWA1	2.81	4.04	1.44
GEI 2013	SWA1	2.81	4.33	1.54
GEI 2013	SWA1	2.81	4.81	1.71
GEI 2013	SWB	7.06	7.38	1.05
GEI 2013	SWB	7.06	8.49	1.20
GEI 2013	SWB	7.06	8.72	1.24
GEI 2013	SWB	7.06	9.80	1.39
GEI 2013	SWB	7.06	8.61	1.22
GEI 2013	SWB	7.06	9.02	1.28
GEI 2013	SWB	7.06	9.11	1.29
GEI 2013	SWB	7.06	9.30	1.32
GEI 2013	SWB	7.06	9.53	1.35
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	11.22	1.51

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWB	7.44	12.25	1.65
GEI 2013	SWB	7.44	12.43	1.67
GEI 2013	SWB	7.44	12.46	1.68
GEI 2013	SWB	7.44	9.36	1.26
GEI 2013	SWB	7.44	9.46	1.27
GEI 2013	SWB	7.44	9.78	1.32
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.66	1.43
GEI 2013	SW11	8.41	5.70	0.68
GEI 2013	SW11	8.41	7.05	0.84
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW11	8.41	4.68	0.56
GEI 2013	SW11	8.41	5.29	0.63
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW2-1	6.60	12.83	1.95
GEI 2013	SW2-1	6.60	14.80	2.24
GEI 2013	SW2-1	6.60	20.13	3.05
GEI 2013	SW2-1	6.60	26.75	4.06
GEI 2013	SW2-1	6.60	30.48	4.62
GEI 2013	SW2-1	6.60	12.51	1.90
GEI 2013	SW2-1	6.60	16.70	2.53
GEI 2013	SW2-1	6.60	17.21	2.61
GEI 2013	SW2-1	6.60	18.27	2.77
GEI 2013	SW2-1	6.60	20.66	3.13
GEI 2013	SW2-1	9.14	13.31	1.46
GEI 2013	SW2-1	9.14	15.63	1.71
GEI 2013	SW2-1	9.14	15.77	1.73
GEI 2013	SW2-1	9.14	16.79	1.84
GEI 2013	SW2-1	9.14	17.00	1.86
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	19.39	2.12
GEI 2013	SW2-1	9.14	22.50	2.46
GEI 2013	SW1	7.82	9.11	1.16
GEI 2013	SW1	7.82	9.15	1.17
GEI 2013	SW1	7.82	11.15	1.43
GEI 2013	SW1	7.82	11.23	1.44
GEI 2013	SW1	7.82	13.76	1.76
GEI 2013	SW1	7.82	9.82	1.26
GEI 2013	SW1	7.82	8.45	1.08
GEI 2013	SW1	7.82	8.88	1.14
GEI 2013	SW1	7.82	9.41	1.20

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	11.07	1.42
GEI 2013	SW1	6.54	7.01	1.07
GEI 2013	SW1	6.54	7.86	1.20
GEI 2013	SW1	6.54	7.98	1.22
GEI 2013	SW1	6.54	8.23	1.26
GEI 2013	SW1	6.54	8.50	1.30
GEI 2013	SW1	6.54	9.48	1.45
GEI 2013	SW1	6.54	9.95	1.52
GEI 2013	SW1	6.54	10.09	1.54
GEI 2013	SW1	6.54	10.19	1.56
GEI 2013	SW4-1	3.33	5.88	1.77
GEI 2013	SW4-1	3.33	5.89	1.77
GEI 2013	SW4-1	3.33	6.07	1.83
GEI 2013	SW4-1	3.33	6.61	1.99
GEI 2013	SW4-1	3.33	6.87	2.07
GEI 2013	SW4-1	3.33	4.85	1.46
GEI 2013	SW4-1	3.33	5.25	1.58
GEI 2013	SW4-1	3.33	5.39	1.62
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	SW9	4.45	5.57	1.25
GEI 2013	SW9	4.45	5.93	1.33
GEI 2013	SW9	4.45	6.14	1.38
GEI 2013	SW9	4.45	6.20	1.39
GEI 2013	SW9	4.45	6.56	1.47
GEI 2013	SW9	4.45	7.57	1.70
GEI 2013	SW88	3.96	4.73	1.20
GEI 2013	SW88	3.96	4.96	1.25
GEI 2013	SW88	3.96	5.55	1.40
GEI 2013	SW88	3.96	5.56	1.41
GEI 2013	SW88	3.96	6.32	1.60
GEI 2013	SW88	3.96	5.13	1.30
GEI 2013	SW88	3.96	5.86	1.48
GEI 2013	SW88	3.96	6.07	1.53
GEI 2013	CC1	3.76	3.79	1.01
GEI 2013	CC1	3.76	5.23	1.39
GEI 2013	CC1	3.76	7.36	1.96
GEI 2013	CC1	3.76	8.69	2.31
GEI 2013	CC1	3.76	9.07	2.41
GEI 2013	CC1	4.69	5.92	1.26
GEI 2013	CC1	4.69	7.68	1.64
GEI 2013	CC1	4.69	7.59	1.62

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	6.49	1.39
GEI 2013	CC1	4.69	7.14	1.52
GEI 2013	CC1	5.86	6.68	1.14
GEI 2013	CC1	5.86	7.73	1.32
GEI 2013	CC1	5.86	7.88	1.35
GEI 2013	CC1	5.86	8.45	1.44
GEI 2013	CC1	5.86	11.69	2.00
GEI 2013	CC1	5.86	9.21	1.57
GEI 2013	CC1	5.86	9.70	1.66
GEI 2013	LG1	3.56	4.81	1.35
GEI 2013	LG1	3.56	4.86	1.37
GEI 2013	LG1	3.56	5.05	1.42
GEI 2013	LG1	3.56	5.47	1.54
GEI 2013	LG1	3.56	5.56	1.56
GEI 2013	LG1	3.56	3.72	1.05
GEI 2013	LG1	3.56	4.09	1.15
GEI 2013	LG1	3.56	3.26	0.92
GEI 2013	LG1	3.56	3.35	0.94
GEI 2013	LG1	3.56	4.20	1.18
GEI 2013	LG1	3.39	3.60	1.06
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.27	1.26
GEI 2013	LG1	3.39	4.45	1.31
GEI 2013	LG1	3.39	5.18	1.53
GEI 2013	LG1	3.39	5.51	1.63
Grasso et al. 1995	17	1.91	6.59	3.45
Grasso et al. 1995	17	1.91	6.60	3.46
Grasso et al. 1995	17	1.91	7.30	3.82
Grasso et al. 1995	10	1.85	2.74	1.48
Grasso et al. 1995	10	1.85	2.79	1.51
Grasso et al. 1995	10	1.85	2.90	1.57
Lambing et al. 1994	S46	6.20	5.10	0.82
Lambing et al. 1994	S48	3.05	2.50	0.82
Lambing et al. 1994	S11	14.50	11.00	0.76
Lambing et al. 1994	S11	14.50	33.00	2.28
Lambing et al. 1994	S34	14.00	25.00	1.79
Lambing et al. 1994	S39	5.85	7.90	1.35
Lambing et al. 1994	S39	5.85	21.00	3.59
Lemly 1985	Badin Lake	5.70	1.50	0.26
Lemly 1985	Belews Lake	51.15	13.60	0.27
Lemly 1985	High Rock Lake	9.05	1.89	0.21
GEI 2014	DC-4	19.42	27.69	1.43

Fathead minnow (*Pimephales promelas*)

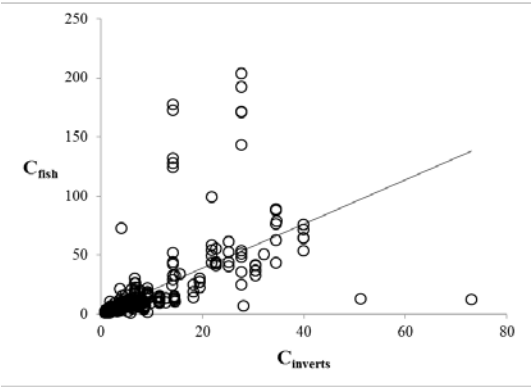
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	DC-4	19.42	27.88	1.44
GEI 2014	DC-4	19.42	23.05	1.19
GEI 2014	DC-4	19.42	30.61	1.58
GEI 2014	DC-4	18.10	19.32	1.07
GEI 2014	DC-4	18.10	14.48	0.80
GEI 2014	DC-4	18.10	25.42	1.40
GEI 2014	FOC-2	2.18	4.13	1.90
GEI 2014	FOC-2	2.18	4.10	1.89
GEI 2014	FOC-2	2.18	5.50	2.53
GEI 2014	FOC-2	2.18	4.85	2.23
GEI 2014	FOC-2	2.18	4.65	2.14
GEI 2014	SC-1	5.85	7.95	1.36
GEI 2014	SC-1	5.85	7.62	1.30
GEI 2014	SC-1	5.85	7.88	1.35
GEI 2014	SC-1	5.85	8.46	1.45
GEI 2014	SC-1	5.85	7.29	1.25
GEI 2014	SC-1	5.85	8.94	1.53
GEI 2014	SC-1	5.85	8.28	1.42
GEI 2014	SC-1	5.85	8.62	1.47
GEI 2014	SC-1	5.85	6.17	1.05
GEI 2014	SC-1	5.85	6.09	1.04
GEI 2014	SC-1	5.85	9.23	1.58
GEI 2014	SC-1	5.85	10.00	1.71
GEI 2014	SC-1	5.85	8.12	1.39
GEI 2014	SC-1	5.85	6.71	1.15
GEI 2014	SC-1	5.85	8.34	1.43
GEI 2014	SC-1	4.94	10.22	2.07
GEI 2014	SC-1	4.94	10.86	2.20
GEI 2014	SC-1	4.94	9.82	1.99
GEI 2014	SC-1	4.94	9.45	1.91
GEI 2014	SC-1	4.94	10.30	2.09
GEI 2014	SC-2	14.33	15.86	1.11
GEI 2014	SC-2	14.33	15.08	1.05
GEI 2014	SC-2	14.33	13.33	0.93
GEI 2014	SC-2	14.33	12.04	0.84
GEI 2014	SC-2	14.33	13.82	0.96
GEI 2014	SC-2	11.44	9.64	0.84
GEI 2014	SC-2	11.44	14.94	1.31
GEI 2014	SC-2	11.44	10.91	0.95
GEI 2014	SC-2	11.44	16.06	1.40
GEI 2014	SC-2	11.44	14.60	1.28
GEI 2014	SC-2	12.75	13.81	1.08

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-2	12.75	14.10	1.11
GEI 2014	SC-2	12.75	10.68	0.84
GEI 2014	SC-3	11.41	11.65	1.02
GEI 2014	SC-3	11.41	10.95	0.96
GEI 2014	SC-3	11.41	10.84	0.95
GEI 2014	SC-3	11.41	13.48	1.18
GEI 2014	SC-3	8.58	7.70	0.90
GEI 2014	SC-3	8.58	6.46	0.75
GEI 2014	SC-3	8.58	6.97	0.81
GEI 2014	SC-3	8.58	10.64	1.24
GEI 2014	SC-3	8.58	7.85	0.91
GEI 2014	SC-3	5.75	13.75	2.39
GEI 2014	SC-3	5.75	11.19	1.95
GEI 2014	SC-3	5.75	12.68	2.20
GEI 2014	SC-4	7.39	6.33	0.86
GEI 2014	SC-4	7.39	14.39	1.95
GEI 2014	SC-4	5.18	2.72	0.53
GEI 2014	SC-4	5.18	11.95	2.31
GEI 2014	SC-4	5.18	7.98	1.54
GEI 2014	SC-4	5.18	6.75	1.30
GEI 2014	SC-6	39.87	72.33	1.81
GEI 2014	SC-6	39.87	76.00	1.91
GEI 2014	SC-6	39.87	64.73	1.62
GEI 2014	SC-6	39.87	54.09	1.36
GEI 2014	SC-6	39.87	64.64	1.62
GEI 2014	SC-6	34.35	76.89	2.24
GEI 2014	SC-6	34.35	89.67	2.61
GEI 2014	SC-6	34.35	63.32	1.84
GEI 2014	SC-6	34.35	88.44	2.57
GEI 2014	SC-6	34.35	44.15	1.29
GEI 2014	SC-6	27.54	51.65	1.88
GEI 2014	SC-6	27.54	35.92	1.30
GEI 2014	SC-6	27.54	25.55	0.93
GEI 2014	SC-6	27.54	54.25	1.97
GEI 2014	SC-6	27.54	48.94	1.78
GEI 2014	SC-6	27.54	204.26	7.42
GEI 2014	SC-6	27.54	143.62	5.22
GEI 2014	SC-6	27.54	192.93	7.01
GEI 2014	SC-6	27.54	171.89	6.24
GEI 2014	SC-6	27.54	171.36	6.22
GEI 2014	SC-8	22.62	43.12	1.91
GEI 2014	SC-8	22.62	43.36	1.92

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-8	22.62	55.81	2.47
GEI 2014	SC-8	22.62	44.60	1.97
GEI 2014	SC-8	22.62	41.67	1.84
GEI 2014	SC-9	30.36	37.18	1.22
GEI 2014	SC-9	30.36	41.32	1.36
GEI 2014	SC-9	30.36	37.20	1.23
GEI 2014	SC-9	30.36	33.25	1.10
GEI 2014	SC-9	30.36	41.94	1.38
GEI 2014	SC-8	21.77	99.40	4.57
GEI 2014	SC-8	21.77	54.47	2.50
GEI 2014	SC-8	21.77	59.07	2.71
GEI 2014	SC-8	21.77	43.70	2.01
GEI 2014	SC-8	21.77	50.18	2.31
GEI 2014	SC-9	25.06	40.82	1.63
GEI 2014	SC-9	25.06	61.80	2.47
GEI 2014	SC-9	25.06	44.74	1.79
GEI 2014	SC-9	25.06	52.97	2.11
GEI 2014	SC-8	14.15	52.46	3.71
GEI 2014	SC-8	14.15	29.43	2.08
GEI 2014	SC-8	14.15	44.58	3.15
GEI 2014	SC-8	14.15	33.44	2.36
GEI 2014	SC-8	14.15	42.86	3.03
GEI 2014	SC-8	14.15	128.33	9.07
GEI 2014	SC-8	14.15	173.33	12.25
GEI 2014	SC-8	14.15	132.34	9.35
GEI 2014	SC-8	14.15	124.90	8.83
GEI 2014	SC-8	14.15	177.97	12.58



Median ratio: 1.57

R^2: 0.35
F: 185.7
df: 344
P: < 0.001

Flannelmouth sucker (*Catostomus latipinnis*)

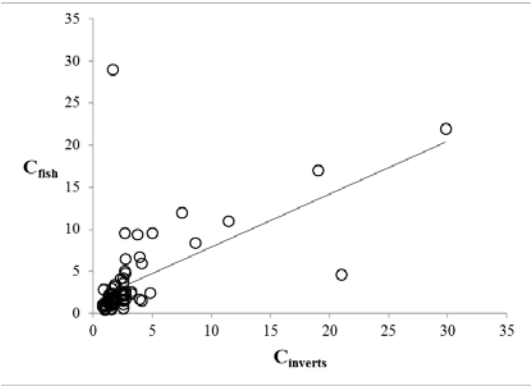
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.50	0.52
Butler et al. 1991	7	29.80	22.00	0.74
Butler et al. 1991	4	3.90	1.70	0.44
Butler et al. 1991	9	4.10	1.50	0.37
Butler et al. 1991	9	4.10	6.00	1.46
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	P1	1.50	2.40	1.60
Butler et al. 1993	LP3	1.12	0.92	0.83
Butler et al. 1993	LP3	1.12	1.40	1.26
Butler et al. 1993	LP4	3.20	2.40	0.75
Butler et al. 1993	LP4	3.20	2.60	0.81
Butler et al. 1994	CRC	7.50	12.00	1.60
Butler et al. 1994	LZA1	19.00	17.00	0.89
Butler et al. 1994	BSW1	5.00	9.60	1.92
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	COL1	1.50	0.50	0.33
Butler et al. 1994	COL1	1.50	0.60	0.40
Butler et al. 1994	COL1	1.50	0.63	0.42
Butler et al. 1994	COL1	1.50	0.92	0.61
Butler et al. 1994	COL1	1.50	1.00	0.67
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	COL1	1.50	1.70	1.13
Butler et al. 1994	COL1	1.50	1.80	1.20
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	RB3	1.60	29.00	18.13
Butler et al. 1994	RB1	21.00	4.60	0.22
Butler et al. 1994	LSW1	3.90	6.70	1.72
Butler et al. 1994	PSW1	3.70	9.40	2.54
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	AK	0.78	0.90	1.16
Butler et al. 1995	AK	0.78	0.82	1.06
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	HD1	0.83	2.90	3.52
Butler et al. 1995	HD2	0.98	0.49	0.50
Butler et al. 1995	HD2	0.98	0.54	0.55
Butler et al. 1995	HD2	0.98	0.62	0.63
Butler et al. 1995	HD2	0.98	0.96	0.98
Butler et al. 1995	ME2	1.25	1.60	1.28
Butler et al. 1995	ME2	1.25	1.40	1.12
Butler et al. 1995	ME2	1.25	2.00	1.60
Butler et al. 1995	ME2	1.25	2.20	1.76
Butler et al. 1995	ME4	1.55	1.50	0.97

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME4	1.55	1.30	0.84
Butler et al. 1995	ME4	1.55	1.90	1.23
Butler et al. 1995	ME4	1.55	2.40	1.55
Butler et al. 1995	ME4	1.55	3.00	1.94
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	2.10	0.82
Butler et al. 1995	ME3	2.55	2.40	0.94
Butler et al. 1995	ME3	2.55	3.60	1.41
Butler et al. 1995	SJ1	2.50	1.71	0.68
Butler et al. 1995	SJ1	2.50	1.50	0.60
Butler et al. 1995	SJ1	2.50	2.20	0.88
Butler et al. 1995	SJ1	2.50	0.61	0.24
Butler et al. 1995	SJ1	2.50	1.10	0.44
Butler et al. 1995	SJ1	2.50	4.20	1.68
Butler et al. 1995	YJ2	1.65	1.60	0.97
Butler et al. 1995	YJ2	1.65	2.40	1.45
Butler et al. 1995	MN1	2.70	6.50	2.41
Butler et al. 1995	MN1	2.70	1.70	0.63
Butler et al. 1995	MN1	2.70	4.80	1.78
Butler et al. 1995	MP	1.60	1.20	0.75
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1997	MN3	2.70	2.30	0.85
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MUD	2.30	4.10	1.78
Butler et al. 1997	MUD	2.30	2.70	1.17
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	MN4	2.65	5.10	1.92
Butler et al. 1997	MN4	2.65	9.60	3.62
Butler et al. 1997	MN5	8.60	8.40	0.98
Butler et al. 1997	MNQ	1.80	2.10	1.17
Butler et al. 1997	MNQ	1.80	3.20	1.78
Butler et al. 1997	MNQ	1.80	3.50	1.94

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio

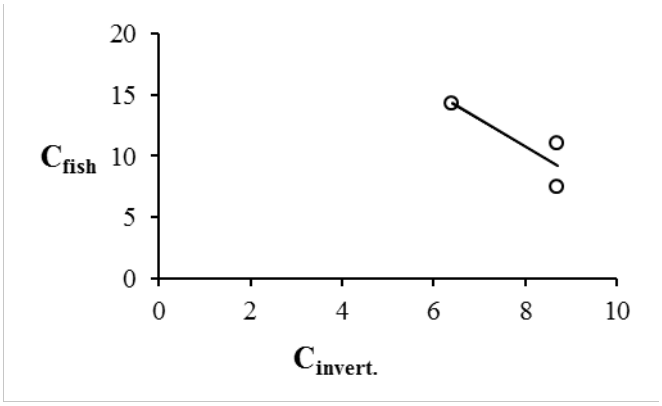


Median ratio: 0.98

R^2: 0.36
F: 41.6
df: 73
P: < 0.001

Gizzard shad (*Dorosoma cepedianum*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.30	2.23
Mueller et al. 1991	R1	8.70	7.50	0.86
Mueller et al. 1991	R1	8.70	11.00	1.26



Median ratio: 1.26

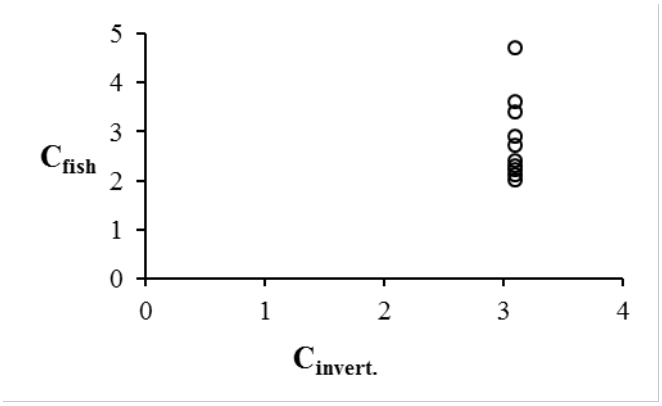
R^2: 0.74
F: 2.78
df: 1
P: 0.39
Not used because P > 0.05 and negative slope.

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	2.70	0.87

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	4.70	1.52



Median ratio: 0.82

R^2: 0.0
F: 0.0
df: 8
P: 1.0

Not used because no slope and P>0.05.

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	7.90	1.65
Butler et al. 1991	7	29.80	15.20	0.51
Butler et al. 1991	7	29.80	25.10	0.84
Butler et al. 1991	3	6.20	6.40	1.03
Butler et al. 1994	LZA1	19.00	37.00	1.95
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	ME3	2.55	5.00	1.96
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	CH1	7.50	9.50	1.27
Butler et al. 1997	MUD2	3.45	7.60	2.20
Butler et al. 1997	MUD2	3.45	7.00	2.03
Butler et al. 1997	TR25	1.80	4.40	2.44
Butler et al. 1997	TRH	1.60	3.30	2.06
GEI 2013	SWA1	2.81	2.96	1.06
GEI 2013	SWA1	2.81	3.21	1.14
GEI 2013	SWA1	2.81	3.24	1.16
GEI 2013	SWA1	2.81	3.69	1.32
GEI 2013	SWA1	2.81	3.88	1.38
GEI 2013	SWB	7.44	11.94	1.61
GEI 2013	SW11	8.41	4.54	0.54

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW11	8.41	4.84	0.58
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	7.00	0.83
GEI 2013	SW11	8.41	7.13	0.85
GEI 2013	SW9	4.45	4.38	0.98
GEI 2013	SW9	4.45	5.06	1.14
GEI 2013	SW9	4.45	5.53	1.24
GEI 2013	SW9	4.45	5.80	1.30
GEI 2013	SW9	4.45	7.29	1.64
GEI 2013	SW88	3.96	7.14	1.81
GEI 2013	SW88	3.96	7.41	1.87
GEI 2013	LG1	3.39	4.11	1.21
GEI 2013	LG1	3.39	4.33	1.28
GEI 2013	LG1	3.39	5.71	1.68
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	4.00	1.29
Roddy et al. 1991	18	3.10	5.20	1.68
Roddy et al. 1991	18	3.10	5.70	1.84
Lemly 1985	Badin Lake	5.70	2.18	0.38
Lemly 1985	Belews Lake	51.15	13.99	0.27
Lemly 1985	High Rock Lake	9.05	2.10	0.23
GEI 2014	C-BCR2	6.81	9.47	1.39
GEI 2014	C-BCR2	6.81	9.29	1.37
GEI 2014	C-BCR2	6.81	8.04	1.18
GEI 2014	C-CC1	4.40	7.23	1.64
GEI 2014	C-CC1	4.40	11.76	2.67
GEI 2014	C-CC2	5.56	4.59	0.83
GEI 2014	C-CC2	5.56	3.04	0.55
GEI 2014	C-CC2	5.56	5.34	0.96
GEI 2014	C-CF1	3.39	3.62	1.07
GEI 2014	C-CF1	3.39	2.95	0.87
GEI 2014	C-CF1	3.39	3.23	0.95
GEI 2014	C-CF1	3.39	5.55	1.64
GEI 2014	C-CLF1	9.30	3.99	0.43
GEI 2014	C-CLF1	9.30	5.23	0.56
GEI 2014	C-CLF1	9.30	4.75	0.51
GEI 2014	C-CLF1	9.30	4.87	0.52
GEI 2014	C-CLF1	9.30	3.58	0.39
GEI 2014	C-CLF2	6.85	5.76	0.84
GEI 2014	C-CLF2	6.85	5.89	0.86
GEI 2014	C-CLF2	6.85	4.78	0.70

Green sunfish (*Lepomis cyanellus*)

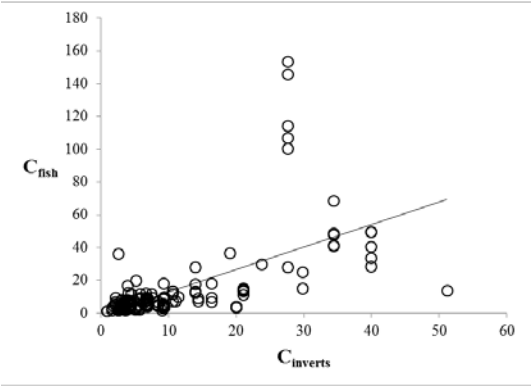
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	C-CLF2	6.85	5.11	0.75
GEI 2014	C-CLF2	6.85	4.10	0.60
GEI 2014	C-LFWOC1	9.32	10.10	1.08
GEI 2014	C-TF1	20.00	4.15	0.21
GEI 2014	C-TF1	20.00	3.47	0.17
GEI 2014	C-TF1	20.00	4.14	0.21
GEI 2014	C-TF1	20.00	4.11	0.21
GEI 2014	C-TF1	20.00	3.41	0.17
GEI 2014	C-WOC1	6.65	12.05	1.81
GEI 2014	C-WOC1	6.65	9.60	1.44
GEI 2014	C-WOC1	6.65	8.66	1.30
GEI 2014	C-WOC1	6.65	5.81	0.87
GEI 2014	C-WOC1	6.65	7.54	1.13
GEI 2014	H-BB1	16.29	9.55	0.59
GEI 2014	H-BB1	16.29	18.27	1.12
GEI 2014	H-BB1	16.29	7.08	0.43
GEI 2014	H-BHC1	5.08	3.69	0.73
GEI 2014	H-BHC1	5.08	2.48	0.49
GEI 2014	H-BHC1	5.08	3.29	0.65
GEI 2014	H-BHC1	5.08	3.49	0.69
GEI 2014	H-BHC1	5.08	3.70	0.73
GEI 2014	H-JSB1	4.03	4.83	1.20
GEI 2014	H-JSB1	4.03	2.57	0.64
GEI 2014	H-JSB1	4.03	3.73	0.93
GEI 2014	H-LF1	9.09	3.12	0.34
GEI 2014	H-LF1	9.09	5.96	0.66
GEI 2014	H-LF1	9.09	4.30	0.47
GEI 2014	H-LF1	9.09	4.02	0.44
GEI 2014	H-LF1	9.09	5.29	0.58
GEI 2014	H-MR2	2.14	9.57	4.47
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	5.80	2.71
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	6.88	3.22
GEI 2014	H-MR3	3.86	8.09	2.10
GEI 2014	H-MR3	3.86	16.98	4.40
GEI 2014	H-MR3	3.86	6.80	1.76
GEI 2014	H-MR3	3.86	8.52	2.21
GEI 2014	H-MR3	3.86	6.62	1.72
GEI 2014	H-MR4	9.26	9.01	0.97
GEI 2014	H-MR4	9.26	8.78	0.95
GEI 2014	H-MR4	9.26	18.33	1.98

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	H-MR4	9.26	9.84	1.06
GEI 2014	H-MR4	9.26	5.94	0.64
GEI 2014	H-MR5	3.58	5.50	1.54
GEI 2014	H-MR5	3.58	3.52	0.98
GEI 2014	H-MR5	3.58	2.41	0.67
GEI 2014	H-MR5	3.58	3.09	0.86
GEI 2014	H-MR5	3.58	1.94	0.54
GEI 2014	H-MR6	2.49	36.20	14.54
GEI 2014	H-MR6	2.49	2.58	1.04
GEI 2014	H-MR6	2.49	1.94	0.78
GEI 2014	H-MR6	2.49	1.74	0.70
GEI 2014	H-MR6	2.49	2.69	1.08
GEI 2014	H-SB1	10.62	11.90	1.12
GEI 2014	H-SB1	10.62	13.39	1.26
GEI 2014	H-SB1	10.62	7.64	0.72
GEI 2014	H-SB1	10.62	13.45	1.27
GEI 2014	H-SF1	21.05	13.59	0.65
GEI 2014	H-SF1	21.05	14.22	0.68
GEI 2014	H-SF1	21.05	15.27	0.73
GEI 2014	H-SF1	21.05	14.58	0.69
GEI 2014	H-SF1	21.05	11.25	0.53
GEI 2014	H-SF2	13.95	17.74	1.27
GEI 2014	H-SF2	13.95	12.86	0.92
GEI 2014	H-SF2	13.95	12.76	0.91
GEI 2014	H-SF2	13.95	13.41	0.96
GEI 2014	H-SF2	13.95	28.23	2.02
GEI 2014	H-UB1	3.02	4.43	1.47
GEI 2014	H-UB1	3.02	4.91	1.63
GEI 2014	H-UB1	3.02	3.73	1.23
GEI 2014	H-UB1	3.02	8.00	2.65
GEI 2014	H-UB1	3.02	8.36	2.77
GEI 2014	SC-1-25	4.00	12.78	3.19
GEI 2014	SC-2	14.33	9.31	0.65
GEI 2014	SC-2	14.33	7.59	0.53
GEI 2014	SC-2	11.44	10.23	0.89
GEI 2014	SC-2-27	23.76	30.00	1.26
GEI 2014	SC-3	5.75	8.66	1.51
GEI 2014	SC-3	5.75	11.72	2.04
GEI 2014	SC-3	5.75	9.59	1.67
GEI 2014	SC-4	5.18	19.86	3.83
GEI 2014	SC-6	39.87	49.55	1.24
GEI 2014	SC-6	39.87	49.56	1.24

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-6	39.87	33.71	0.85
GEI 2014	SC-6	39.87	28.73	0.72
GEI 2014	SC-6	39.87	40.64	1.02
GEI 2014	SC-6	34.35	41.51	1.21
GEI 2014	SC-6	34.35	48.02	1.40
GEI 2014	SC-6	34.35	68.66	2.00
GEI 2014	SC-6	34.35	41.27	1.20
GEI 2014	SC-6	34.35	48.76	1.42
GEI 2014	SC-6	27.54	28.17	1.02
GEI 2014	SC-6	27.54	106.88	3.88
GEI 2014	SC-6	27.54	114.55	4.16
GEI 2014	SC-6	27.54	100.41	3.65
GEI 2014	SC-6	27.54	153.64	5.58
GEI 2014	SC-6	27.54	145.62	5.29
GEI 2014	S-SC1	11.01	7.47	0.68



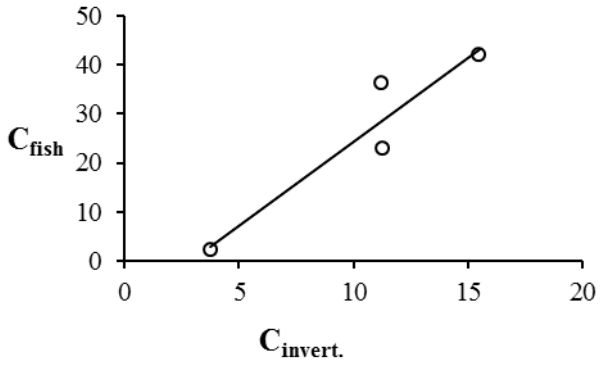
Median ratio: 1.12

R^2: 0.37
F: 93.17
df: 160
P: < 0.001

Iowa darter (*Etheostoma exile*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	7	3.75	2.10	0.56
Birkner 1978	20	11.20	36.30	3.24
Birkner 1978	22	11.30	23.00	2.04
Birkner 1978	23	15.50	41.90	2.70

Iowa darter (*Etheostoma exile*)



Median ratio: 2.37

R^2: 0.90
F: 17.3
df: 2
P: 0.055
Not used because P > 0.05

Largemouth bass (*Micropterus salmoides*)

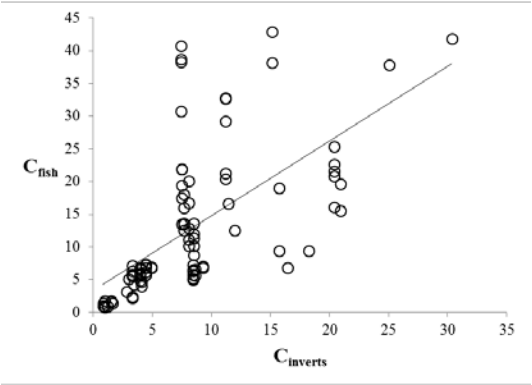
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	MP	1.60	1.40	0.88
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	5.10	1.70
GEI 2013	SWA1	2.81	3.17	1.13
GEI 2013	SW11	8.41	5.02	0.60
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	5.19	0.62
GEI 2013	SW11	8.41	6.26	0.74
GEI 2013	SW11	8.41	6.48	0.77
GEI 2013	SW11	8.41	7.22	0.86
GEI 2013	SW4-1	3.33	5.53	1.66
GEI 2013	SW4-1	3.33	5.65	1.70
GEI 2013	SW4-1	3.33	5.72	1.72
GEI 2013	SW4-1	3.33	5.80	1.74
GEI 2013	SW4-1	3.33	6.34	1.91
GEI 2013	SW4-1	3.33	7.14	2.15
GEI 2013	SW9	4.45	5.78	1.30
GEI 2013	SW9	4.45	5.79	1.30
GEI 2013	SW9	4.45	6.19	1.39
GEI 2013	SW9	4.45	6.87	1.54
GEI 2013	SW9	4.45	7.27	1.63
GEI 2013	SW9	4.45	7.36	1.65
GEI 2013	SW88	3.96	4.87	1.23
GEI 2013	SW88	3.96	5.73	1.45
GEI 2013	SW88	3.96	5.77	1.46
GEI 2013	SW88	3.96	5.93	1.50
GEI 2013	SW88	3.96	6.62	1.67
GEI 2013	SW88	3.96	6.84	1.73

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	LG1	3.39	4.29	1.27
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.80	1.39
Saiki et al. 1993	GT5	4.90	6.90	1.41
Saiki et al. 1993	GT4	4.05	4.70	1.16
Saiki et al. 1993	GT4	4.05	4.00	0.99
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR2	3.30	2.40	0.73
Saiki et al. 1993	SJR3	1.50	1.80	1.20
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR1	0.95	0.80	0.85
Saiki et al. 1993	SJR1	0.95	1.80	1.90
Saiki et al. 1993	ET7	0.86	0.86	1.00
Saiki et al. 1993	ET7	0.86	1.00	1.16
Rinella and Schuler 1992	S. Malheur Lake	1.20	0.92	0.77
Crutchfield 2000	transect 3	11.95	12.52	1.05
Crutchfield 2000	transect 3	11.40	16.67	1.46
Crutchfield 2000	transect 3	9.25	6.83	0.74
Crutchfield 2000	transect 3	9.25	6.99	0.76
Crutchfield 2000	transect 3	8.60	6.59	0.77
Crutchfield 2000	transect 3	8.60	5.69	0.66
Crutchfield 2000	transect 4	20.90	15.53	0.74
Crutchfield 2000	transect 4	20.90	19.68	0.94
Crutchfield 2000	transect 4	15.70	18.95	1.21
Crutchfield 2000	transect 4	15.70	9.43	0.60
Crutchfield 2000	transect 4	16.45	6.83	0.42
Crutchfield 2000	transect 4	18.25	9.43	0.52
GEI 2014	ARB	11.21	20.41	1.82
GEI 2014	ARB	11.21	32.75	2.92
GEI 2014	ARB	11.21	32.73	2.92
GEI 2014	ARB	11.21	29.23	2.61
GEI 2014	ARB	11.21	21.26	1.90
GEI 2014	ARE	20.40	25.35	1.24
GEI 2014	ARE	20.40	20.80	1.02
GEI 2014	ARE	20.40	22.67	1.11
GEI 2014	ARE	20.40	21.57	1.06
GEI 2014	ARE	20.40	16.05	0.79
GEI 2014	ARM	8.51	13.62	1.60
GEI 2014	ARM	8.51	10.13	1.19
GEI 2014	ARM	8.51	12.00	1.41
GEI 2014	ARM	8.51	11.40	1.34

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARM	8.51	8.71	1.02
GEI 2014	ARM	7.68	12.52	1.63
GEI 2014	ARM	7.68	13.59	1.77
GEI 2014	ARM	7.68	17.99	2.34
GEI 2014	ARM	7.68	13.49	1.76
GEI 2014	ARM	7.68	15.98	2.08
GEI 2014	ARN	8.06	11.16	1.39
GEI 2014	ARN	8.06	12.89	1.60
GEI 2014	ARN	8.06	16.74	2.08
GEI 2014	ARN	8.06	10.12	1.26
GEI 2014	ARN	8.06	20.08	2.49
GEI 2014	ARN	7.49	17.47	2.33
GEI 2014	ARN	7.49	13.48	1.80
GEI 2014	ARN	7.49	19.44	2.60
GEI 2014	ARN	7.49	21.87	2.92
GEI 2014	ARN	7.49	21.91	2.92
GEI 2014	ARN	7.44	30.73	4.13
GEI 2014	ARN	7.44	40.71	5.47
GEI 2014	ARN	7.44	38.75	5.21
GEI 2014	ARN	7.44	38.24	5.14
GEI 2014	SC-5	15.13	38.13	2.52
GEI 2014	SC-5	15.13	42.86	2.83
GEI 2014	SC-9	30.36	41.87	1.38
GEI 2014	SC-9	25.06	37.84	1.51

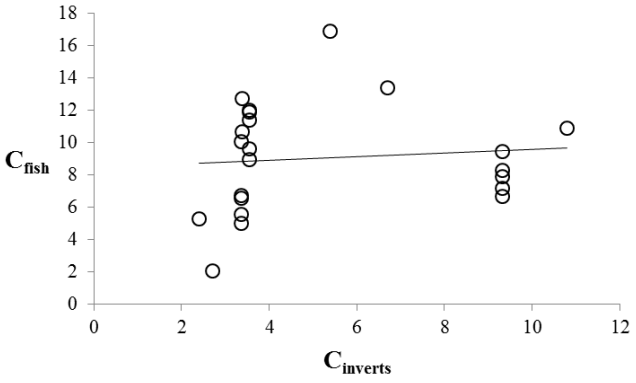


Median ratio: 1.39

R^2: 0.40
F: 61.45
df: 91
P: < 0.001

Longnose dace (*Rhinichthys cataractae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Lambing et al. 1994	S33	2.40	5.30	2.21
Mueller et al. 1991	A1	2.70	2.10	0.78
GEI 2013	LG1	3.37	5.05	1.50
GEI 2013	LG1	3.37	5.57	1.65
GEI 2013	LG1	3.37	6.57	1.95
GEI 2013	LG1	3.37	6.75	2.00
GEI 2013	LG1	3.37	10.08	2.99
GEI 2013	LG1	3.39	10.69	3.15
GEI 2013	LG1	3.39	12.77	3.77
GEI 2013	LG1	3.56	8.95	2.52
GEI 2013	LG1	3.56	9.63	2.71
GEI 2013	LG1	3.56	11.41	3.21
GEI 2013	LG1	3.56	11.94	3.36
GEI 2013	LG1	3.56	12.04	3.39
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	8.32	0.89
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	9.47	1.02
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.94	0.85
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	6.67	0.72
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.19	0.77
Mueller et al. 1991	T1	5.40	16.90	3.13
Hamilton and Buhl 2005	CC	6.70	13.40	2.00
Hamilton and Buhl 2005	BGS	10.80	10.90	1.01

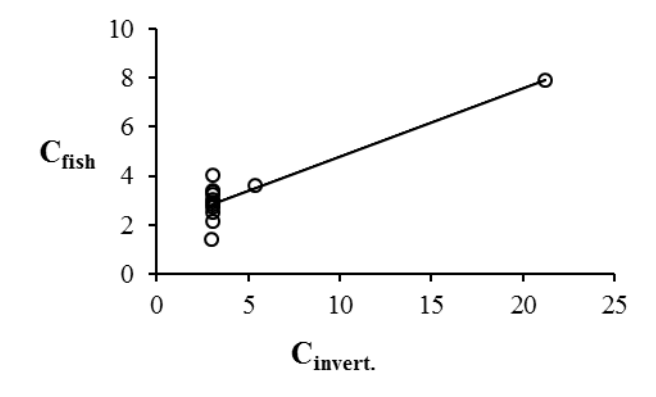


Median ratio: 2.00

R^2: 0.01
F: 0.19
df: 20
P: 0.83
Not used because P > 0.05

Longnose sucker (*Catostomus catostomus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	FL17	3.03	1.40	0.46
Butler et al. 1994	NFK2	3.10	2.10	0.68
Butler et al. 1994	NFK2	3.10	2.50	0.81
Butler et al. 1994	NFK2	3.10	2.70	0.87
Butler et al. 1994	NFK2	3.10	2.80	0.90
Butler et al. 1994	NFK2	3.10	2.90	0.94
Butler et al. 1994	NFK2	3.10	3.00	0.97
Butler et al. 1994	NFK2	3.10	3.20	1.03
Butler et al. 1994	NFK2	3.10	3.30	1.06
Butler et al. 1994	NFK2	3.10	3.40	1.10
Butler et al. 1994	NFK2	3.10	4.00	1.29
Mueller et al. 1991	T1	5.40	3.60	0.67
Minnow 2007	FL17	21.22	7.90	0.37



Median ratio: 0.90

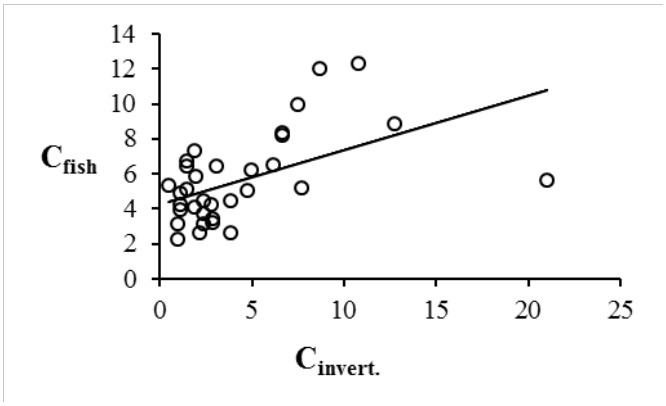
R^2: 0.83
F: 54.66
df: 11
P: <0.001

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	5.30	10.60
Butler et al. 1993	LP2	1.00	2.20	2.20
Butler et al. 1993	LP2	1.00	3.10	3.10
Butler et al. 1993	LP3	1.12	3.90	3.50
Butler et al. 1993	LP3	1.12	4.20	3.77
Butler et al. 1993	LP3	1.12	4.90	4.39
Butler et al. 1993	P1	1.50	5.10	3.40
Butler et al. 1993	P1	1.50	6.40	4.27
Butler et al. 1993	P1	1.50	6.70	4.47
Hamilton and Buhl 2004	ShpC	1.90	4.10	2.16
Butler et al. 1993	P1	1.95	7.30	3.74
Butler et al. 1994	NFK3	2.00	5.80	2.90

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	MN2	2.20	2.60	1.18
Butler et al. 1997	CHK	2.40	3.10	1.29
Butler et al. 1997	CHK	2.40	4.40	1.83
Lambing et al. 1994	S33	2.40	3.70	1.54
Butler et al. 1991	12	2.80	4.20	1.50
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	MN1	2.90	3.40	1.17
Butler et al. 1994	NFK2	3.10	6.40	2.06
Butler et al. 1991	4	3.90	2.60	0.67
Butler et al. 1991	4	3.90	4.40	1.13
Butler et al. 1991	10	4.80	5.00	1.04
Hamilton and Buhl 2005	UAC	5.00	6.20	1.24
Butler et al. 1991	3	6.20	6.50	1.05
Hamilton and Buhl 2004	ACM	6.70	8.30	1.24
Hamilton and Buhl 2005	CC	6.70	8.20	1.22
Butler et al. 1993	F2	7.50	9.90	1.32
Hamilton and Buhl 2004	LBR	7.70	5.20	0.68
Hamilton and Buhl 2005	DC	8.70	12.00	1.38
Hamilton and Buhl 2005	BGS	10.80	12.30	1.14
Hamilton and Buhl 2004	DVC	12.80	8.80	0.69
Butler et al. 1994	HCC1	21.00	5.60	0.27



Median ratio: 1.38

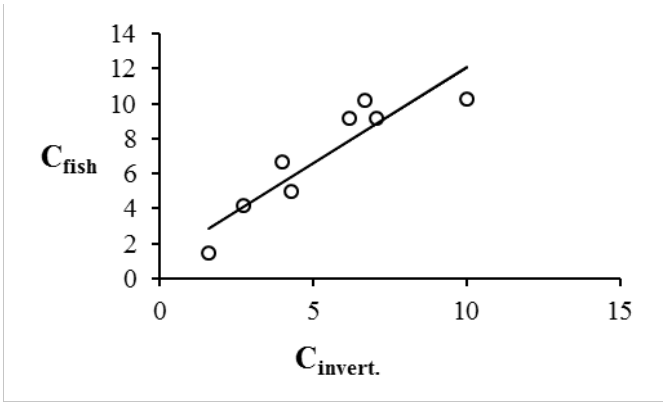
R^2: 0.27
F: 11.62
df: 31
P: < 0.001

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Low and Mullins 1990	7	1.60	1.40	0.88
McDonald and Strosher 1998	ER 745	2.74	4.17	1.52
Minnow 2007	EL12	4.01	6.60	1.65
McDonald and Strosher 1998	ER 747	4.29	4.93	1.15

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	MI3	6.21	9.12	1.47
Minnow 2007	MI2	6.69	10.16	1.52
Minnow 2007	EL1	7.08	9.12	1.29
Minnow 2007	FO23	10.00	10.20	1.02



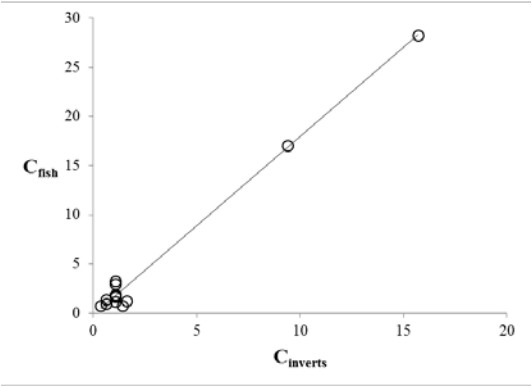
Median ratio: 1.38

R^2: 0.83
F: 30.27
df: 6
P: <0.001

Northern pike (*Esox lucius*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.96	2.78
Butler et al. 1995	TT	1.07	3.24	3.04
Butler et al. 1995	TT	1.07	1.65	1.55
Butler et al. 1995	TT	1.07	1.18	1.11
Butler et al. 1995	TT	1.07	1.90	1.78
Butler et al. 1995	TT	1.07	1.80	1.69
Butler et al. 1995	PU	0.61	0.93	1.52
Butler et al. 1995	PU	0.61	1.40	2.30
Muscatello et al. 2008	David Lake	1.39	0.78	0.56
Muscatello et al. 2008	Delta Lake	9.38	17.02	1.81
Muscatello et al. 2008	Unknown Lake	15.71	28.28	1.80
Muscatello and Janz 2009	Indigo Lake	0.36	0.75	2.08
Muscatello and Janz 2009	Vulture Lake	1.62	1.26	0.78

Northern pike (*Esox lucius*)

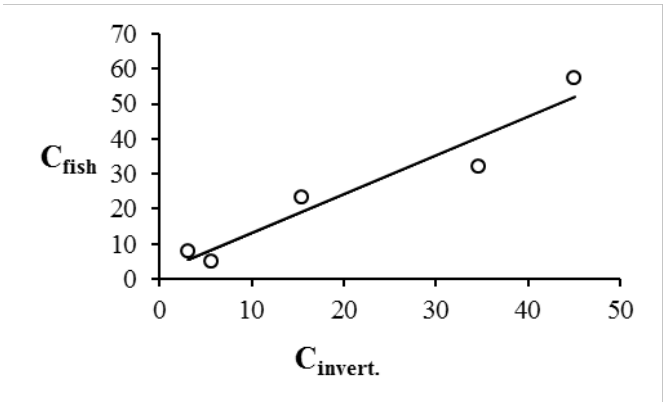


Median ratio: 1.78

R^2: 0.99
F: 982.9
df: 11
P: <0.001

Northern plains killfish (*Fundulus kansae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	3	3.10	7.70	2.48
Birkner 1978	11	5.65	5.00	0.88
Birkner 1978	23	15.50	23.10	1.49
Birkner 1978	27	34.60	31.90	0.92
Birkner 1978	30	45.05	57.40	1.27



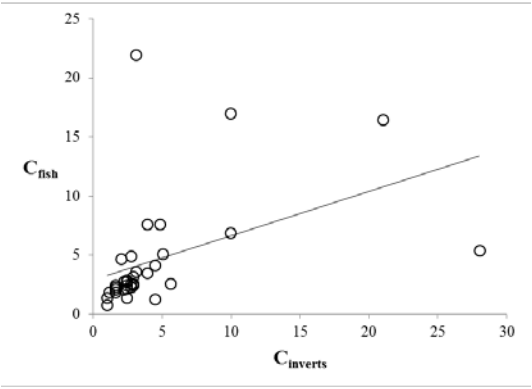
Median ratio: 1.27

R^2: 0.93
F: 37.8
df: 3
P: 0.008

Rainbow trout (*Oncorhynchus mykiss*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1993	F2	4.80	7.60	1.58
Butler et al. 1993	F2	3.90	7.60	1.95
Butler et al. 1993	LP2	1.00	0.78	0.78
Butler et al. 1993	LP2	1.00	1.40	1.40
Butler et al. 1993	LP3	1.12	1.90	1.70
Butler et al. 1994	GUN2	28.00	5.40	0.19
Butler et al. 1994	HCC1	21.00	16.48	0.78
Butler et al. 1994	NFK3	2.00	4.70	2.35
Butler et al. 1994	NFK2	3.10	21.98	7.09
Butler et al. 1994	NFK2	3.10	3.60	1.16
Butler et al. 1995	MP	1.60	1.88	1.18
Butler et al. 1995	MP	1.60	2.30	1.44
Butler et al. 1995	MP	1.60	2.50	1.56
Butler et al. 1995	MP	1.60	2.10	1.31
Butler et al. 1997	CHK	2.40	1.41	0.59
Butler et al. 1997	CHK	2.40	2.20	0.92
Butler et al. 1997	CHK	2.40	2.50	1.04
Butler et al. 1997	CHK	2.40	2.80	1.17
Butler et al. 1997	CHK	2.40	2.90	1.21
Butler et al. 1997	MN3	2.70	2.28	0.84
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MN3	2.70	4.90	1.81
Butler et al. 1997	MN2	2.20	2.10	0.95
Butler et al. 1997	MN2	2.20	2.80	1.27
Butler et al. 1997	MN1	2.90	2.50	0.86
Butler et al. 1997	MN1	2.90	2.60	0.90
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	WBR	5.05	5.10	1.01
Low and Mullins 1990	5	5.60	2.60	0.46
Casey 2005	Deerlick Creek	4.45	1.29	0.29
Casey 2005	Deerlick Creek	4.45	4.15	0.93
Casey 2005	Luscar Creek	9.95	6.88	0.69
Casey 2005	Luscar Creek	9.95	17.04	1.71

Rainbow trout (*Oncorhynchus mykiss*)



Median ratio: 1.07

R^2: 0.19
F: 7.52
df: 32
P: 0.002

Red shiner (*Cyprinella lutrensis*)

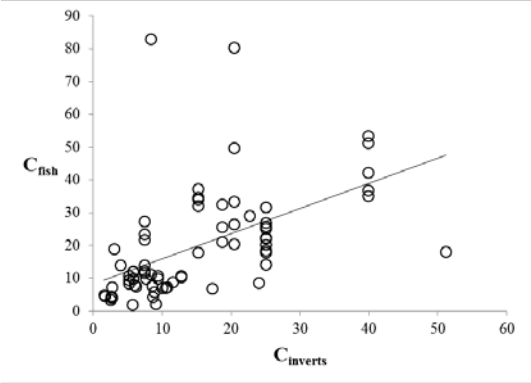
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	3	6.20	7.70	1.24
Butler et al. 1994	IW	8.35	83.00	9.94
Butler et al. 1994	AD	2.70	7.30	2.70
Butler et al. 1994	LW	3.00	19.00	6.33
Butler et al. 1994	LSW1	3.90	14.00	3.59
Butler et al. 1995	ME4	1.55	5.10	3.29
Butler et al. 1995	ME3	2.55	4.60	1.80
Butler et al. 1995	ME3	2.55	4.20	1.65
Butler et al. 1995	SJ1	2.50	3.50	1.40
Butler et al. 1995	YJ2	1.65	4.50	2.73
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	4.40	0.51
May et al. 2008	KR	17.20	7.03	0.41
May et al. 2008	NSCL	10.70	7.36	0.69
May et al. 2008	NSCU	10.50	7.24	0.69
May et al. 2008	NSK	8.81	5.81	0.66
May et al. 2008	NSP	24.00	8.62	0.36
May et al. 2008	SSAL	11.50	9.00	0.78
May et al. 2008	SSAU	8.35	11.20	1.34
May et al. 2008	SSO	10.00	7.16	0.72
May et al. 2008	SSW	7.60	10.00	1.32
Mueller et al. 1991	A3	6.00	8.10	1.35
Mueller et al. 1991	A2	8.50	7.90	0.93
Lemly 1985	Badin Lake	5.70	2.10	0.37
Lemly 1985	Belews Lake	51.15	18.25	0.36
Lemly 1985	High Rock Lake	9.05	2.18	0.24
GEI 2014	ARE	20.40	49.84	2.44

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARE	20.40	80.50	3.95
GEI 2014	ARE	20.40	20.57	1.01
GEI 2014	ARE	20.40	33.37	1.64
GEI 2014	ARE	20.40	26.50	1.30
GEI 2014	ARN	7.44	27.50	3.70
GEI 2014	ARN	7.44	23.58	3.17
GEI 2014	ARN	7.44	21.74	2.92
GEI 2014	FC-4	18.65	21.20	1.14
GEI 2014	FC-4	18.65	32.68	1.75
GEI 2014	FC-4	18.65	25.73	1.38
GEI 2014	GC-1	9.33	10.78	1.16
GEI 2014	GC-1	9.33	9.97	1.07
GEI 2014	SC-2	12.75	10.44	0.82
GEI 2014	SC-2	12.75	10.87	0.85
GEI 2014	SC-3	5.75	12.05	2.10
GEI 2014	SC-3	5.75	12.17	2.12
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-4	7.39	12.26	1.66
GEI 2014	SC-4	7.39	11.68	1.58
GEI 2014	SC-4	7.39	14.15	1.92
GEI 2014	SC-4	5.18	9.58	1.85
GEI 2014	SC-4	5.18	8.43	1.63
GEI 2014	SC-4	5.18	10.83	2.09
GEI 2014	SC-5	15.13	17.96	1.19
GEI 2014	SC-5	15.13	34.71	2.29
GEI 2014	SC-5	15.13	34.05	2.25
GEI 2014	SC-5	15.13	37.28	2.46
GEI 2014	SC-5	15.13	32.18	2.13
GEI 2014	SC-6	39.87	53.60	1.34
GEI 2014	SC-6	39.87	37.00	0.93
GEI 2014	SC-6	39.87	35.11	0.88
GEI 2014	SC-6	39.87	51.39	1.29
GEI 2014	SC-6	39.87	42.31	1.06
GEI 2014	SC-8	22.62	29.20	1.29
GEI 2014	SC-9	25.06	22.55	0.90
GEI 2014	SC-9	25.06	18.02	0.72
GEI 2014	SC-9	25.06	25.94	1.04
GEI 2014	SC-9	25.06	18.68	0.75
GEI 2014	SC-9	25.06	14.28	0.57
GEI 2014	SC-9	25.06	31.67	1.26
GEI 2014	SC-9	25.06	20.43	0.82

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-9	25.06	22.27	0.89
GEI 2014	SC-9	25.06	27.05	1.08
GEI 2014	SC-9	25.06	25.28	1.01



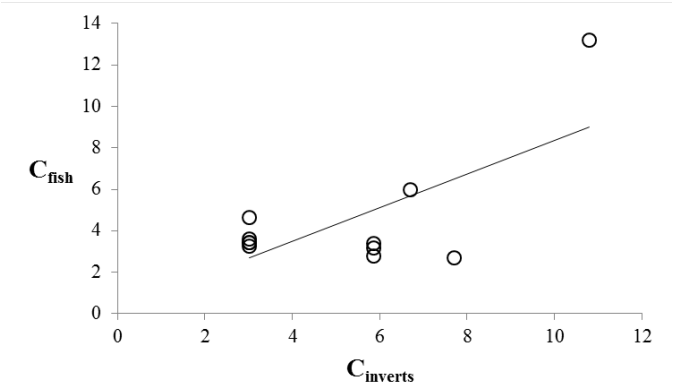
Median ratio: 1.31

R^2: 0.28
F: 26.57
df: 70
P: <0.001

Redside shiner (*Richardsonius balteatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ACM	6.70	6.00	0.90
Hamilton and Buhl 2004	LBR	7.70	2.70	0.35
Hamilton and Buhl 2005	BGS	10.80	13.20	1.22
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	4.64	1.54
GEI 2014	Bond Creek, BC-3	3.02	3.26	1.08
GEI 2014	Bond Creek, BC-3	5.87	3.18	0.54
GEI 2014	Bond Creek, BC-3	5.87	3.37	0.57
GEI 2014	Bond Creek, BC-3	5.87	2.79	0.47
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19

Redside shiner (*Richardsonius balteatus*)



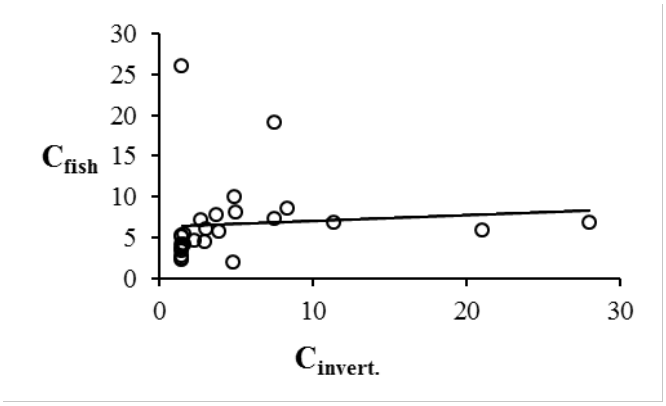
Median ratio: 1.08

R^2: 0.47
F: 7.84
df: 9
P: 0.011

Roundtail chub (*Gila robusta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	COL1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	2.50	1.67
Butler et al. 1994	COL1	1.50	2.70	1.80
Butler et al. 1994	COL1	1.50	3.30	2.20
Butler et al. 1994	COL1	1.50	3.70	2.47
Butler et al. 1994	COL1	1.50	4.10	2.73
Butler et al. 1994	COL1	1.50	5.10	3.40
Butler et al. 1994	COL1	1.50	5.30	3.53
Butler et al. 1994	COL1	1.50	26.00	17.33
Butler et al. 1994	RB3	1.60	5.40	3.38
Butler et al. 1995	MP	1.60	4.20	2.63
Butler et al. 1997	MUD	2.30	4.60	2.00
Butler et al. 1994	AD	2.70	7.10	2.63
Butler et al. 1994	LW	3.00	4.50	1.50
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	PSW1	3.70	7.70	2.08
Butler et al. 1994	LSW1	3.90	5.80	1.49
Butler et al. 1991	10	4.80	1.90	0.40
Butler et al. 1994	TGC	4.90	10.00	2.04
Butler et al. 1994	BSW1	5.00	8.10	1.62
Butler et al. 1993	F2	7.50	7.30	0.97
Butler et al. 1994	CRC	7.50	19.00	2.53
Butler et al. 1994	IW	8.35	8.50	1.02
Butler et al. 1997	NW2	11.40	6.90	0.61
Butler et al. 1994	RB1	21.00	5.90	0.28
Butler et al. 1994	GUN2	28.00	6.80	0.24

Roundtail chub (*Gila robusta*)



Median ratio: 1.98

R^2: 0.01
F: 0.18
df: 24
P: 0.834
Not used because P > 0.05

Sand shiner (*Notropis stramineus*)

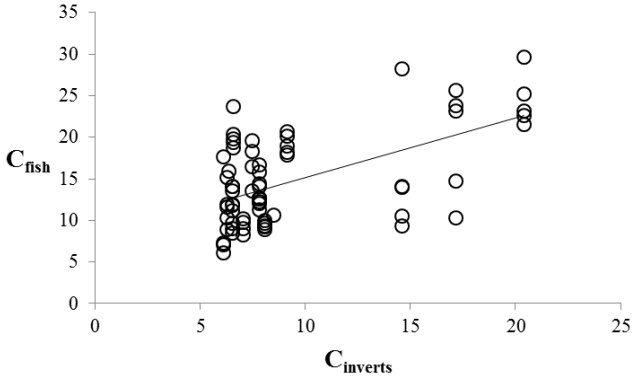
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	6.54	8.43	1.29
GEI 2013	SW1	6.54	9.02	1.38
GEI 2013	SW1	6.54	9.66	1.48
GEI 2013	SW1	6.54	11.21	1.71
GEI 2013	SW1	6.54	11.85	1.81
GEI 2013	SW1	6.54	11.94	1.83
GEI 2013	SW1	6.54	13.50	2.06
GEI 2013	SW1	6.54	14.05	2.15
GEI 2013	SW1	6.54	14.14	2.16
GEI 2013	SW2-1	6.60	18.70	2.84
GEI 2013	SW2-1	6.60	19.33	2.93
GEI 2013	SW2-1	6.60	19.77	3.00
GEI 2013	SW2-1	6.60	20.39	3.09
GEI 2013	SW2-1	6.60	23.70	3.59
GEI 2013	SWB	7.06	8.27	1.17
GEI 2013	SWB	7.06	9.01	1.28
GEI 2013	SWB	7.06	9.81	1.39
GEI 2013	SWB	7.06	10.22	1.45
GEI 2013	SW1	7.82	11.33	1.45
GEI 2013	SW1	7.82	12.05	1.54
GEI 2013	SW1	7.82	12.22	1.56
GEI 2013	SW1	7.82	12.55	1.60
GEI 2013	SW1	7.82	12.65	1.62
GEI 2013	SW1	7.82	12.68	1.62
GEI 2013	SW1	7.82	14.13	1.81
GEI 2013	SW1	7.82	14.43	1.85
GEI 2013	SW1	7.82	15.87	2.03

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	16.63	2.13
GEI 2013	SW2-1	9.14	17.84	1.95
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	18.98	2.08
GEI 2013	SW2-1	9.14	20.12	2.20
GEI 2013	SW2-1	9.14	20.73	2.27
GEI 2014	Arkansas River, ARE	20.40	21.50	1.05
GEI 2014	Arkansas River, ARE	20.40	23.20	1.14
GEI 2014	Arkansas River, ARE	20.40	22.64	1.11
GEI 2014	Arkansas River, ARE	20.40	25.24	1.24
GEI 2014	Arkansas River, ARE	20.40	29.70	1.46
GEI 2014	Arkansas River, ARM	8.51	10.67	1.25
GEI 2014	Arkansas River, ARN	8.06	9.69	1.20
GEI 2014	Arkansas River, ARN	8.06	9.27	1.15
GEI 2014	Arkansas River, ARN	8.06	9.96	1.24
GEI 2014	Arkansas River, ARN	8.06	9.29	1.15
GEI 2014	Arkansas River, ARN	8.06	8.86	1.10
GEI 2014	Arkansas River, ARN	7.49	13.60	1.82
GEI 2014	Arkansas River, ARN	7.49	18.34	2.45
GEI 2014	Arkansas River, ARN	7.49	16.46	2.20
GEI 2014	Arkansas River, ARN	7.49	19.64	2.62
GEI 2014	Fountain Creek, FC-4	14.59	13.95	0.96
GEI 2014	Fountain Creek, FC-4	14.59	9.34	0.64
GEI 2014	Fountain Creek, FC-4	14.59	14.06	0.96
GEI 2014	Fountain Creek, FC-4	14.59	28.26	1.94
GEI 2014	Fountain Creek, FC-4	14.59	10.53	0.72
GEI 2014	Fountain Creek, FC-4	17.15	10.28	0.60
GEI 2014	Fountain Creek, FC-4	17.15	23.76	1.39
GEI 2014	Fountain Creek, FC-4	17.15	14.77	0.86
GEI 2014	Fountain Creek, FC-4	17.15	23.13	1.35
GEI 2014	Fountain Creek, FC-4	17.15	25.62	1.49
GEI 2014	Fountain Creek, FCP	6.13	17.62	2.88
GEI 2014	Fountain Creek, FCP	6.13	7.32	1.19
GEI 2014	Fountain Creek, FCP	6.13	7.14	1.17
GEI 2014	Fountain Creek, FCP	6.13	6.05	0.99
GEI 2014	Fountain Creek, FCP	6.13	7.11	1.16
GEI 2014	Fountain Creek, FCP	6.35	15.93	2.51
GEI 2014	St. Charles River, SC-4	6.29	11.92	1.90
GEI 2014	St. Charles River, SC-4	6.29	15.14	2.41
GEI 2014	St. Charles River, SC-4	6.29	8.94	1.42
GEI 2014	St. Charles River, SC-4	6.29	10.33	1.64
GEI 2014	St. Charles River, SC-4	6.29	11.58	1.84

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.56

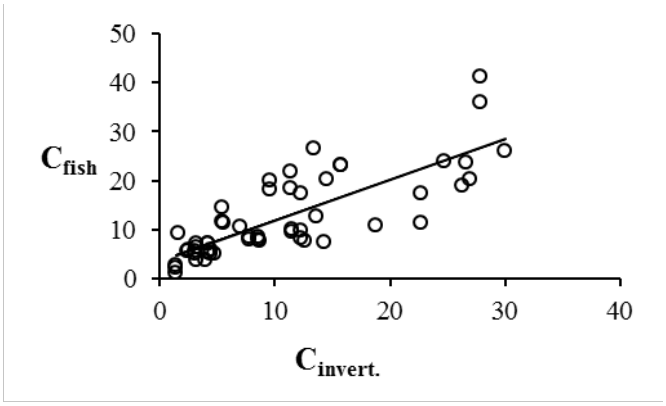
R^2: 0.32
F: 32.15
df: 67
P: <0.001

Sculpin (*Cottoidea*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	BK	1.43	1.16	0.81
Mason et al. 2000	BK	1.43	2.35	1.64
Mason et al. 2000	BK	1.43	2.64	1.84
Formation 2012	SFTC-1	1.63	9.31	5.71
Formation 2012	SFTC-1	2.42	5.68	2.35
Formation 2012	SFTC-1	2.49	5.87	2.36
Formation 2012	CC-75	3.11	5.03	1.62
Formation 2012	CC-75	3.11	5.58	1.79
Formation 2012	CC-350	3.16	6.47	2.05
Formation 2012	CC-350	3.16	7.12	2.26
Formation 2012	SFTC-1	3.21	3.75	1.17
Formation 2012	CC-75	3.97	3.77	0.95
Formation 2012	CC-75	4.16	7.08	1.70
Formation 2012	CC-75	4.16	7.19	1.73
Formation 2012	CC-350	4.20	5.28	1.26
Formation 2012	CC-150	4.46	5.04	1.13
Formation 2012	CC-150	4.46	6.01	1.35
Formation 2012	CC-150	4.70	5.14	1.09
Formation 2012	CC-3A	5.45	11.65	2.14
Formation 2012	CC-3A	5.45	14.45	2.65
Formation 2012	CC-3A	5.48	11.47	2.09
Formation 2012	CC-150	7.03	10.73	1.53
Formation 2012	DC-600	7.83	7.96	1.02
Formation 2012	DC-600	7.83	8.62	1.10

Sculpin (*Cottoidea*)

Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	8.53	7.87	0.92
Formation 2012	DC-600	8.53	8.50	1.00
Formation 2012	DC-600	8.65	7.63	0.88
Formation 2012	LSV-4	9.54	18.28	1.92
Formation 2012	LSV-4	9.54	20.01	2.10
Formation 2012	HS-3	11.40	18.57	1.63
Formation 2012	HS-3	11.40	21.85	1.92
Formation 2012	CC-350	11.45	9.53	0.83
Formation 2012	CC-350	11.45	10.03	0.88
Formation 2012	CC-1A	12.24	8.34	0.68
Formation 2012	CC-1A	12.24	9.94	0.81
Formation 2012	CC-1A	12.24	17.47	1.43
Formation 2012	CC-1A	12.57	7.78	0.62
Formation 2012	HS-3	13.41	26.63	1.99
Formation 2012	CC-1A	13.55	12.63	0.93
Formation 2012	CC-150	14.32	7.35	0.51
Formation 2012	CC-3A	14.50	20.20	1.39
Formation 2012	HS	15.70	23.23	1.48
Formation 2012	HS	15.70	23.25	1.48
Formation 2012	HS	18.70	10.95	0.59
Formation 2012	LSV-2C	22.62	11.38	0.50
Formation 2012	LSV-2C	22.62	17.47	0.77
Formation 2012	HS-3	24.70	23.93	0.97
Formation 2012	LSV-2C	26.31	18.85	0.72
Formation 2012	HS-3	26.55	23.68	0.89
Formation 2012	LSV-2C	26.95	20.32	0.75
Formation 2012	HS	27.80	35.93	1.29
Formation 2012	HS	27.80	41.30	1.49
Formation 2012	LSV-2C	30.00	25.95	0.87

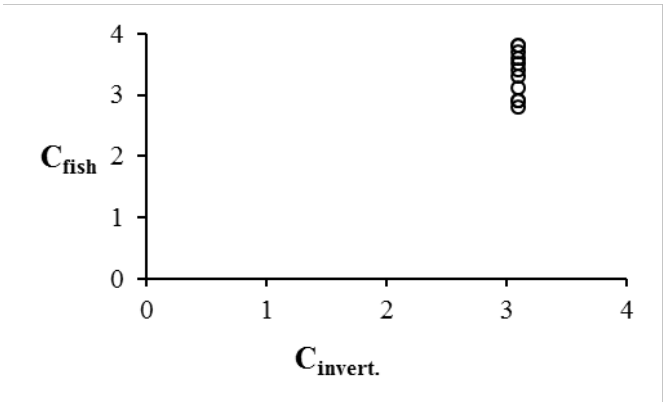


Median ratio: 1.29

R^2: 0.63
F: 87.0
df: 51
P: <0.001

Shorthead redhorse (*Moxostoma macrolepidotum*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.10	1.00
Roddy et al. 1991	18	3.10	3.30	1.06
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.50	1.13
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	3.70	1.19
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	3.80	1.23



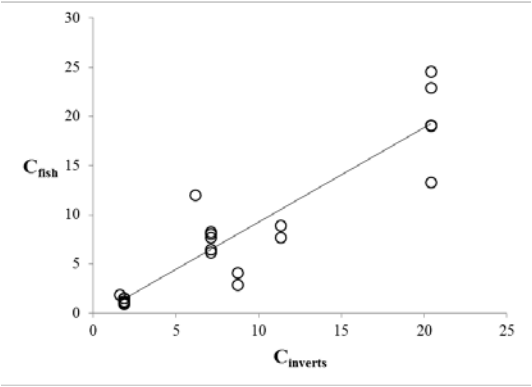
Median ratio: 1.10

R^2: 0.00
F: 0.00
df: 9
P: 1.0
Not used because P > 0.05

Smallmouth bass (*Micropterus dolomieu*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	SU	1.85	1.55	0.84
Butler et al. 1995	SU	1.85	1.22	0.66
Butler et al. 1995	SU	1.85	0.98	0.53
Butler et al. 1995	SU	1.85	1.14	0.62
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	MNP3	6.15	12.00	1.95
Mueller et al. 1991	R1	8.70	2.90	0.33
Mueller et al. 1991	R1	8.70	4.10	0.47
GEI 2014	ARE	20.40	24.61	1.21
GEI 2014	ARE	20.40	22.97	1.13
GEI 2014	ARE	20.40	13.28	0.65

Smallmouth bass (*Micropterus dolomieu*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	ARE	20.40	19.06	0.93
GEI 2014	ARE	20.40	19.11	0.94
GEI 2014	ARM	7.10	8.25	1.16
GEI 2014	ARM	7.10	8.04	1.13
GEI 2014	ARM	7.10	7.72	1.09
GEI 2014	ARM	7.10	6.21	0.87
GEI 2014	ARM	7.10	6.51	0.92
GEI 2014	C-SC1	11.30	8.94	0.79
GEI 2014	C-SC1	11.30	7.68	0.68



Median ratio: 0.86

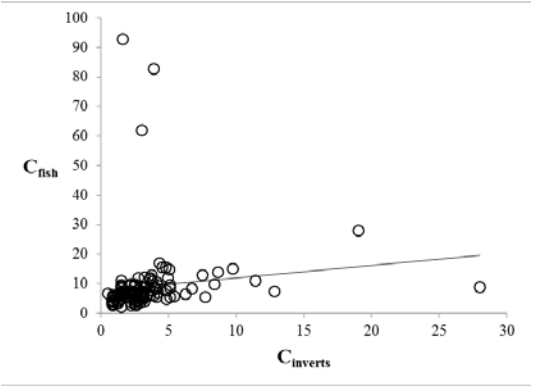
R^2: 0.84
F: 107.1
df: 20
P: <0.001

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1991	10	4.80	4.80	1.00
Butler et al. 1991	9	4.10	5.70	1.39
Butler et al. 1991	3	6.20	6.50	1.05
Butler et al. 1993	SP2	2.75	12.00	4.36
Butler et al. 1993	B2	1.35	5.80	4.30
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	D1	1.20	3.50	2.92
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.40	2.83
Butler et al. 1993	D2	1.45	4.90	3.38
Butler et al. 1993	D2	1.45	6.80	4.69
Butler et al. 1993	D2	1.45	6.50	4.48
Butler et al. 1993	F2	3.90	8.90	2.28
Butler et al. 1993	P1	1.95	5.50	2.82
Butler et al. 1993	SP1	2.95	7.30	2.47

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	SP1	2.95	8.90	3.02
Butler et al. 1993	SP1	2.95	7.00	2.37
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1993	U1	2.45	6.90	2.82
Butler et al. 1993	U1	2.45	7.30	2.98
Butler et al. 1993	U1	2.45	9.20	3.76
Butler et al. 1993	U1	2.45	9.40	3.84
Butler et al. 1993	U1	2.45	9.80	4.00
Butler et al. 1993	LP3	1.12	6.00	5.38
Butler et al. 1993	LP4	3.20	8.70	2.72
Butler et al. 1993	R2	4.30	17.10	3.98
Butler et al. 1993	R2	3.90	6.00	1.54
Butler et al. 1993	ST2	4.50	15.70	3.49
Butler et al. 1993	ST2	4.10	8.50	2.07
Butler et al. 1993	ST2	4.10	10.70	2.61
Butler et al. 1993	ST2	3.35	9.30	2.78
Butler et al. 1993	R1	4.00	8.50	2.13
Butler et al. 1993	ST1	2.25	6.80	3.02
Butler et al. 1993	SB2	3.60	12.10	3.36
Butler et al. 1993	SB2	3.75	7.80	2.08
Butler et al. 1993	SB2	3.75	10.80	2.88
Butler et al. 1993	SB1	2.15	10.00	4.65
Butler et al. 1993	SB1	2.15	9.50	4.42
Butler et al. 1993	SB1	2.15	7.80	3.63
Butler et al. 1993	WSB2	4.75	15.60	3.28
Butler et al. 1993	WSB2	3.60	11.70	3.25
Butler et al. 1993	WSB2	3.00	6.20	2.07
Butler et al. 1993	WSB2	3.00	7.60	2.53
Butler et al. 1994	CRC	7.50	13.00	1.73
Butler et al. 1994	CF1	3.60	6.10	1.69
Butler et al. 1994	GUN2	28.00	8.90	0.32
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	LZA1	19.00	28.00	1.47
Butler et al. 1994	NFK3	2.00	7.10	3.55
Butler et al. 1994	NFK2	3.10	6.90	2.23
Butler et al. 1994	NFK2	3.10	4.80	1.55
Butler et al. 1994	NFK2	3.10	5.40	1.74
Butler et al. 1994	NFK2	3.10	5.70	1.84
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	NFK2	3.10	6.20	2.00
Butler et al. 1994	NFK2	3.10	6.30	2.03
Butler et al. 1994	NFK2	3.10	6.40	2.06

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1994	NFK2	3.10	6.70	2.16
Butler et al. 1994	NFK2	3.10	7.40	2.39
Butler et al. 1994	NFK2	3.10	8.70	2.81
Butler et al. 1994	TGC	4.90	12.00	2.45
Butler et al. 1994	BSW1	5.00	15.00	3.00
Butler et al. 1994	COL1	1.50	2.30	1.53
Butler et al. 1994	COL1	1.50	5.00	3.33
Butler et al. 1994	COL1	1.50	7.30	4.87
Butler et al. 1994	COL1	1.50	7.40	4.93
Butler et al. 1994	COL1	1.50	8.40	5.60
Butler et al. 1994	COL1	1.50	8.60	5.73
Butler et al. 1994	COL1	1.50	9.30	6.20
Butler et al. 1994	COL1	1.50	9.60	6.40
Butler et al. 1994	COL1	1.50	11.00	7.33
Butler et al. 1994	RB3	1.60	93.00	58.13
Butler et al. 1994	SMF	4.80	7.80	1.63
Butler et al. 1994	LW	3.00	62.00	20.67
Butler et al. 1994	LSW1	3.90	83.00	21.28
Butler et al. 1994	PSW1	3.70	13.00	3.51
Butler et al. 1995	AK	0.78	4.30	5.55
Butler et al. 1995	AK	0.78	3.10	4.00
Butler et al. 1995	AK	0.78	4.00	5.16
Butler et al. 1995	DD	0.86	5.60	6.51
Butler et al. 1995	DD	0.86	4.40	5.12
Butler et al. 1995	DD	0.86	6.00	6.98
Butler et al. 1995	HD1	0.83	2.80	3.39
Butler et al. 1995	HD1	0.83	3.20	3.88
Butler et al. 1995	HD1	0.83	5.30	6.42
Butler et al. 1995	ME1	3.40	6.40	1.88
Butler et al. 1995	ME2	1.25	6.10	4.88
Butler et al. 1995	ME3	2.55	2.80	1.10
Butler et al. 1995	ME3	2.55	7.00	2.75
Butler et al. 1995	ME3	2.55	5.50	2.16
Butler et al. 1995	NW	5.10	8.70	1.71
Butler et al. 1995	SJ1	2.50	4.30	1.72
Butler et al. 1995	SJ1	2.50	5.10	2.04
Butler et al. 1995	SJ1	2.50	2.90	1.16
Butler et al. 1995	YJ2	1.65	6.50	3.94
Butler et al. 1995	YJ2	1.65	6.30	3.82
Butler et al. 1995	YJ2	1.65	7.10	4.30
Butler et al. 1995	MN1	2.70	5.50	2.04
Butler et al. 1997	CHK	2.40	5.20	2.17

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1997	CHK	2.40	3.80	1.58
Butler et al. 1997	MN3	2.70	6.00	2.22
Butler et al. 1997	MN3	2.70	4.30	1.59
Butler et al. 1997	MN2	2.20	2.70	1.23
Butler et al. 1997	MN2	2.20	3.60	1.64
Butler et al. 1997	MN1	2.90	3.70	1.28
Butler et al. 1997	MUD	2.30	7.20	3.13
Butler et al. 1997	MUD	2.30	6.10	2.65
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	WBR	5.05	9.70	1.92
Butler et al. 1997	WBR	5.05	5.50	1.09
Butler et al. 1997	MN4	2.65	7.90	2.98
Butler et al. 1997	MN5	8.60	14.00	1.63
Butler et al. 1997	MNQ	1.80	5.90	3.28
Hamilton and Buhl 2004	DVC	12.80	7.50	0.59
Hamilton and Buhl 2004	USC	0.50	6.90	13.80
Hamilton and Buhl 2004	ACM	6.70	8.50	1.27
Hamilton and Buhl 2004	LBR	7.70	5.60	0.73
Hamilton and Buhl 2005	LiB	5.40	5.80	1.07
Hamilton and Buhl 2005	SLC	9.70	15.20	1.57



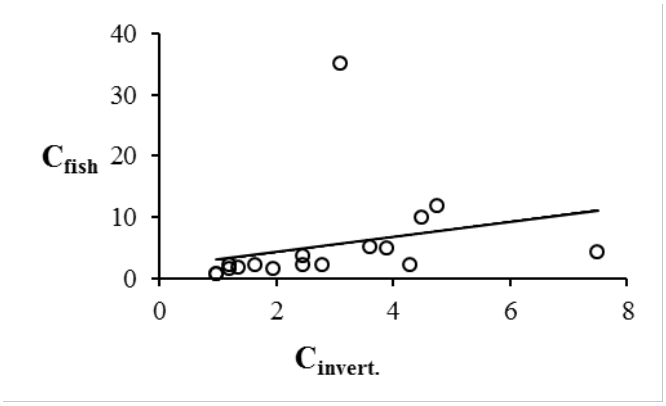
Median ratio: 2.76

R^2: 0.01
F: 1.76
df: 118
P: 0.177
Not used because P > 0.05

Sucker (Catostomidae)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	HD2	0.98	0.68	0.69
Butler et al. 1995	HD2	0.98	0.76	0.78
Butler et al. 1993	D1	1.20	2.30	1.92
Rinella and Schuler 1992	Malheur Lake	1.20	1.60	1.33

Sucker (Catostomidae)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	YJ2	1.65	2.20	1.33
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	U1	2.45	2.30	0.94
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1991	12	2.80	2.10	0.75
Butler et al. 1994	NFK2	3.10	35.00	11.29
Butler et al. 1993	SB2	3.60	5.10	1.42
Butler et al. 1993	R2	3.90	5.00	1.28
Butler et al. 1993	R2	4.30	2.20	0.51
Butler et al. 1993	ST2	4.50	10.00	2.22
Butler et al. 1993	WSB2	4.75	11.80	2.48
Butler et al. 1993	F2	7.50	4.20	0.56



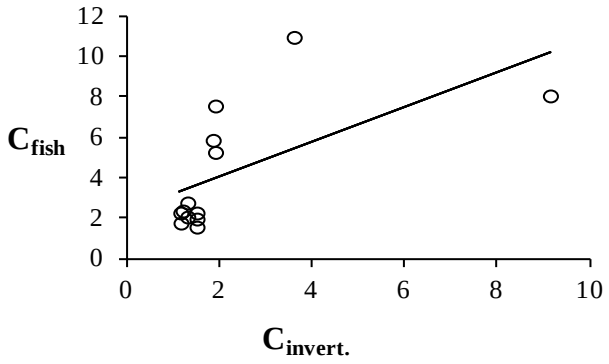
Median ratio: 1.33

R^2: 0.07
F: 1.10
df: 15
P: 0.360
Not used because P > 0.05

Sunfish (Centrarchidae)

Study	Site	C_{invert}	C_{fish}	Ratio
Welsh and Maughan 1994	outfall drain	1.15	2.30	2.00
Welsh and Maughan 1994	Pretty Water	1.16	1.80	1.56
Welsh and Maughan 1994	Hart Mine Marsh	1.20	2.40	2.00
Welsh and Maughan 1994	outfall drain	1.30	2.10	1.62
Welsh and Maughan 1994	outfall drain	1.30	2.80	2.15
Welsh and Maughan 1994	Pretty Water	1.50	1.60	1.07
Welsh and Maughan 1994	Old Channel	1.50	2.00	1.33
Welsh and Maughan 1994	Pretty Water	1.50	2.30	1.53
Welsh and Maughan 1994	Cibola Lake	1.85	5.90	3.19
Welsh and Maughan 1994	Cibola Lake	1.90	5.30	2.79
Welsh and Maughan 1994	Cibola Lake	1.90	7.60	4.00
Welsh and Maughan 1994	Oxbow Lake	3.60	11.00	3.06

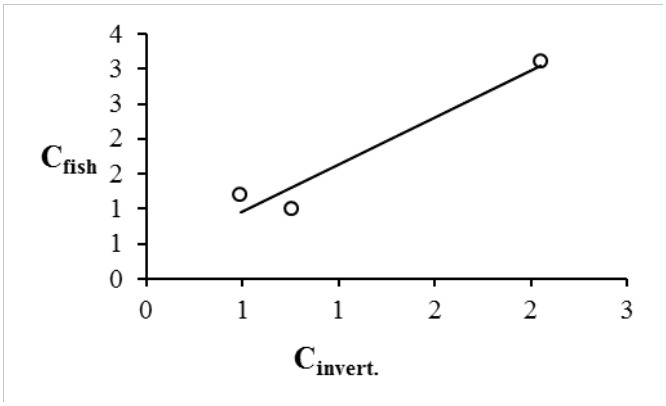
Sunfish (Centrarchidae)				
GEI 2013	SW2-1	9.14	8.10	0.89



Median ratio: 2.00

R^2: 0.38
F: 6.66
df: 11
P: 0.013

Tui chub (*Gila bicolor*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Sorenson & Schwarzbach 1991	5	0.49	1.20	2.45
Sorenson & Schwarzbach 1991	4	0.76	1.00	1.32
Rinella and Schuler 1992	Harney Lake	2.05	3.10	1.51

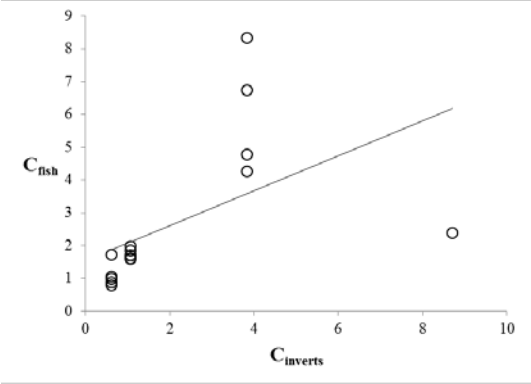


Median ratio: 1.51

R^2: 0.94
F: 15.9
df: 1
P: 0.175
Not used because P > 0.05

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.86	1.75
Butler et al. 1995	TT	1.07	1.62	1.52
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	2.00	1.88

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	PU	0.61	1.72	2.82
Butler et al. 1995	PU	0.61	1.05	1.73
Butler et al. 1995	PU	0.61	0.81	1.33
Butler et al. 1995	PU	0.61	1.00	1.64
Butler et al. 1995	PU	0.61	0.89	1.46
Mueller et al. 1991	R1	8.70	2.40	0.28
Peterson et al. 1991	7	3.83	4.27	1.11
Peterson et al. 1991	7	3.83	4.79	1.25
Peterson et al. 1991	7	3.83	6.76	1.77
Peterson et al. 1991	7	3.83	8.35	2.18



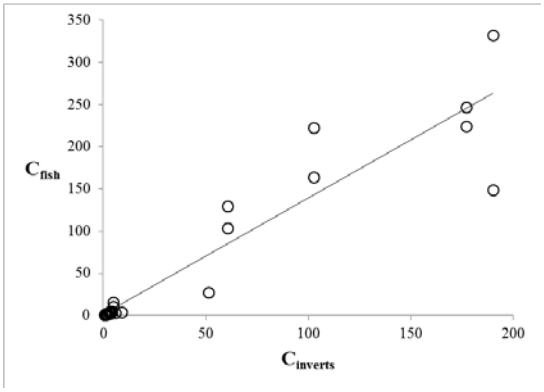
Median ratio: 1.60

R^2: 0.28
F: 5.46
df: 14
P: 0.018

Western mosquitofish (*Gambusia affinis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	SWA1	3.64	2.91	0.80
GEI 2013	SWA1	2.81	3.01	1.07
GEI 2013	SWA1	2.81	3.49	1.24
GEI 2013	SWA1	2.81	3.66	1.30
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.27	1.52
Lemly 1985	Badin Lake	5.70	3.35	0.59
Lemly 1985	Belews Lake	51.15	27.20	0.53
Lemly 1985	High Rock Lake	9.05	3.54	0.39
Saiki and Lowe 1987	Kesterson Pond 11	60.65	130.00	2.14
Saiki and Lowe 1987	Kesterson Pond 11	60.65	104.00	1.71
Saiki and Lowe 1987	Kesterson Pond 2	177.00	224.00	1.27
Saiki and Lowe 1987	Kesterson Pond 2	177.00	247.00	1.40
Saiki and Lowe 1987	Kesterson Pond 8	102.50	164.00	1.60

Western mosquitofish (*Gambusia affinis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Saiki and Lowe 1987	Kesterson Pond 8	102.50	223.00	2.18
Saiki and Lowe 1987	San Luis Drain	190.00	149.00	0.78
Saiki and Lowe 1987	San Luis Drain	190.00	332.00	1.75
Saiki and Lowe 1987	Volta Pond 26	1.42	1.28	0.90
Saiki and Lowe 1987	Volta Pond 26	1.42	1.24	0.87
Saiki and Lowe 1987	Volta Wasteway	2.23	1.35	0.61
Saiki and Lowe 1987	Volta Wasteway	2.23	1.36	0.61
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.30	1.54
Saiki et al. 1993	GT5	4.90	16.00	3.27
Saiki et al. 1993	GT5	4.90	11.00	2.24
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.90	1.21
Saiki et al. 1993	SJR2	3.30	4.50	1.36
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR1	0.95	0.95	1.01
Saiki et al. 1993	SJR1	0.95	1.30	1.38
Saiki et al. 1993	ET7	0.86	0.90	1.05
Saiki et al. 1993	ET7	0.86	1.00	1.16



Median ratio: 1.21

R^2: 0.89
F: 263.3
df: 33
P: <0.001

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	LP3	1.12	2.50	2.24
Butler et al. 1993	B1	1.25	2.60	2.08
Butler et al. 1993	D2	1.45	1.90	1.31
Butler et al. 1993	D2	1.45	2.50	1.72

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	P1	1.50	1.70	1.13
Butler et al. 1993	P1	1.50	1.80	1.20
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1995	SU	1.85	1.20	0.65
GEI 2014	Arkansas River, ARB	11.21	14.90	1.33
GEI 2014	Arkansas River, ARB	11.21	20.39	1.82
GEI 2014	Arkansas River, ARB	11.21	13.82	1.23
GEI 2014	Arkansas River, ARB	11.21	8.36	0.75
GEI 2014	Arkansas River, ARB	11.21	10.88	0.97
GEI 2014	Arkansas River, ARB	11.21	21.55	1.92
GEI 2014	Arkansas River, ARB	11.21	18.70	1.67
GEI 2014	Arkansas River, ARB	11.21	24.53	2.19
GEI 2014	Arkansas River, ARB	11.21	15.02	1.34
GEI 2014	Arkansas River, ARB	11.21	28.29	2.52
GEI 2014	Arkansas River, ARE	20.4	18.21	0.89
GEI 2014	Arkansas River, ARE	20.4	19.54	0.96
GEI 2014	Arkansas River, ARE	20.4	15.27	0.75
GEI 2014	Arkansas River, ARE	20.4	11.37	0.56
GEI 2014	Arkansas River, ARE	20.4	17.86	0.88
GEI 2014	Arkansas River, ARE	20.4	10.62	0.52
GEI 2014	Arkansas River, ARE	20.4	17.51	0.86
GEI 2014	Arkansas River, ARE	20.4	24.66	1.21
GEI 2014	Arkansas River, ARE	20.4	18.92	0.93
GEI 2014	Arkansas River, ARE	20.4	21.70	1.06
GEI 2014	Arkansas River, ARM	8.51	10.13	1.19
GEI 2014	Arkansas River, ARM	8.51	9.24	1.09
GEI 2014	Arkansas River, ARM	8.51	8.30	0.97
GEI 2014	Arkansas River, ARM	8.51	10.09	1.19
GEI 2014	Arkansas River, ARM	7.68	16.18	2.11
GEI 2014	Arkansas River, ARM	7.68	13.21	1.72
GEI 2014	Arkansas River, ARM	7.68	11.96	1.56
GEI 2014	Arkansas River, ARM	7.68	8.58	1.12
GEI 2014	Arkansas River, ARM	7.68	9.73	1.27
GEI 2014	Arkansas River, ARM	7.1	8.19	1.15
GEI 2014	Arkansas River, ARM	7.1	7.96	1.12
GEI 2014	Arkansas River, ARN	7.49	9.15	1.22
GEI 2014	Arkansas River, ARN	7.49	8.61	1.15
GEI 2014	Arkansas River, ARN	7.49	7.06	0.94
GEI 2014	Arkansas River, ARN	7.49	11.57	1.54
GEI 2014	Arkansas River, ARN	7.49	11.56	1.54
GEI 2014	Arkansas River, ARN	7.44	21.20	2.85
GEI 2014	Arkansas River, ARN	7.44	23.28	3.13

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Arkansas River, ARN	7.44	20.85	2.80
GEI 2014	Arkansas River, ARN	7.44	25.91	3.48
GEI 2014	Bond Creek, BC-2	2.96	3.06	1.03
GEI 2014	Bond Creek, BC-2	2.96	3.54	1.19
GEI 2014	Bond Creek, BC-2	2.96	3.04	1.03
GEI 2014	Bond Creek, BC-3	3.02	2.76	0.91
GEI 2014	Bond Creek, BC-3	3.02	2.85	0.94
GEI 2014	Bond Creek, BC-3	3.02	2.47	0.82
GEI 2014	Bond Creek, BC-3	3.02	2.03	0.67
GEI 2014	Bond Creek, BC-3	3.02	2.23	0.74
GEI 2014	Cow Camp Creek, CC-2	5.65	4.46	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	4.45	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	6.19	1.09
GEI 2014	Cow Camp Creek, CC-2	5.65	4.71	0.83
GEI 2014	Cow Camp Creek, CC-2	5.65	5.38	0.95
GEI 2014	Seng Creek, C-SC1	11.302	20.32	1.80
GEI 2014	Dry Creek, DC-4	19.42	26.07	1.34
GEI 2014	Dry Creek, DC-4	19.42	22.55	1.16
GEI 2014	Dry Creek, DC-4	19.42	14.29	0.74
GEI 2014	Dry Creek, DC-4	19.42	14.25	0.73
GEI 2014	Dry Creek, DC-4	19.42	14.67	0.76
GEI 2014	Dry Creek, DC-4	18.1	29.83	1.65
GEI 2014	Dry Creek, DC-4	18.1	30.65	1.69
GEI 2014	Dry Creek, DC-4	18.1	20.87	1.15
GEI 2014	Dry Creek, DC-4	18.1	12.06	0.67
GEI 2014	Fountain Creek, FC-4	18.65	24.54	1.32
GEI 2014	Fountain Creek, FCP	6.13	5.33	0.87
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.75	0.94
GEI 2014	Fountain Creek, FCP	6.13	4.37	0.71
GEI 2014	Fountain Creek, FCP	5.38	8.50	1.58
GEI 2014	Fountain Creek, FCP	5.38	5.94	1.10
GEI 2014	Fountain Creek, FCP	5.38	5.97	1.11
GEI 2014	Fountain Creek, FCP	5.38	5.76	1.07
GEI 2014	Fountain Creek, FCP	5.38	5.61	1.04
GEI 2014	Fountain Creek, FCP	6.35	15.82	2.49
GEI 2014	Fountain Creek, FCP	6.35	5.68	0.90
GEI 2014	Fountain Creek, FCP	6.35	10.17	1.60
GEI 2014	Fountain Creek, FCP	6.35	12.34	1.94
GEI 2014	Fountain Creek, FCP	6.35	10.64	1.68
GEI 2014	Foidel Creek, FOC-2	2.175	1.74	0.80

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Foidel Creek, FOC-2	2.175	1.25	0.57
GEI 2014	Foidel Creek, FOC-2	2.175	1.76	0.81
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	1.64	0.76
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	2.29	1.05
GEI 2014	Grassy Creek, GC-2	4.195	4.45	1.06
GEI 2014	Grassy Creek, GC-2	4.195	4.42	1.05
GEI 2014	Grassy Creek, GC-2	4.195	2.51	0.60
GEI 2014	Grassy Creek, GC-3	4.535	2.78	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.84	0.63
GEI 2014	Grassy Creek, GC-3	4.535	4.37	0.96
GEI 2014	Grassy Creek, GC-3	4.535	2.89	0.64
GEI 2014	Grassy Creek, GC-3	4.545	4.29	0.94
GEI 2014	Grassy Creek, GC-3	4.545	3.16	0.69
GEI 2014	Grassy Creek, GC-3	4.545	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.34	4.12	0.95
GEI 2014	Grassy Creek, GC-3	4.35	3.96	0.91
GEI 2014	Grassy Creek, GC-4	5.1	0.93	0.18
GEI 2014	Grassy Creek, GC-4	5.1	1.40	0.27
GEI 2014	Grassy Creek, GC-4	5.1	4.12	0.81
GEI 2014	Grassy Creek, GC-4	5.76	7.04	1.22
GEI 2014	Grassy Creek, GC-4	5.76	7.42	1.29
GEI 2014	Grassy Creek, GC-4	5.76	4.22	0.73
GEI 2014	Grassy Creek, GC-4	5.76	4.75	0.82
GEI 2014	Grassy Creek, GC-4 US	13.16	6.32	0.48
GEI 2014	Grassy Creek, GC-4 US	13.16	4.74	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.98	0.38
GEI 2014	Grassy Creek, GC-4 US	13.16	4.75	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.88	0.37
GEI 2014	Middle Creek, MC-1	3.21	2.03	0.63
GEI 2014	Middle Creek, MC-1	3.21	2.18	0.68
GEI 2014	Middle Creek, MC-1	3.21	1.79	0.56
GEI 2014	Middle Creek, MC-1	3.21	2.28	0.71
GEI 2014	Middle Creek, MC-1	3.21	2.54	0.79
GEI 2014	Middle Creek, MC-2	4.19	2.58	0.62
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	1.86	0.44
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.10	0.50
GEI 2014	St. Charles River, SC-3	5.75	7.74	1.35

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	St. Charles River, SC-3	5.75	12.34	2.15
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.76	3.09
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	38.23	2.53
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.59	3.08
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	60.66	4.01
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	43.70	2.89
GEI 2014	St. Charles River, SC-9	25.06	35.97	1.44
GEI 2014	St. Charles River, SC-9	25.06	38.53	1.54
GEI 2014	St. Charles River at I-25, SC-I	4.335	7.62	1.76
GEI 2014	St. Charles River at I-25, SC-I	4.335	8.20	1.89
GEI 2014	St. Charles River at I-25, SC-I	4.335	6.10	1.41
GEI 2014	St. Charles River at I-25, SC-I	4.335	22.44	5.18
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.29	0.99
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.79	1.11
GEI 2014	St. Charles River at I-25, SC-I	4.335	47.18	10.88
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.88	1.12
GEI 2014	Wildhorse Creek, WHC	56.14	5.43	0.10
GEI 2014	Wildhorse Creek, WHC	34.24	36.23	1.06
GEI 2014	Wildhorse Creek, WHC	34.24	10.22	0.30
GEI 2014	Wildhorse Creek, WHC	34.24	52.16	1.52
GEI 2014	Wildhorse Creek, WHC	34.24	40.81	1.19
GEI 2014	Wildhorse Creek, WHC	34.24	26.45	0.77
GEI 2014	Wildhorse Creek, WHC	62.34	61.90	0.99
GEI 2014	Wildhorse Creek, WHC	62.34	15.88	0.25
GEI 2014	Wildhorse Creek, WHC	62.34	27.40	0.44
GEI 2014	Wildhorse Creek, WHC	62.34	23.10	0.37
Muscatello and Janz 2009	Indigo Lake	0.36	0.99	2.75
Muscatello and Janz 2009	Vulture Lake	1.62	3.37	2.08
Grasso et al. 1995	17	1.91	2.84	1.49
Grasso et al. 1995	17	1.91	3.19	1.67
Grasso et al. 1995	17	1.91	3.44	1.80
Grasso et al. 1995	17	1.91	3.64	1.91

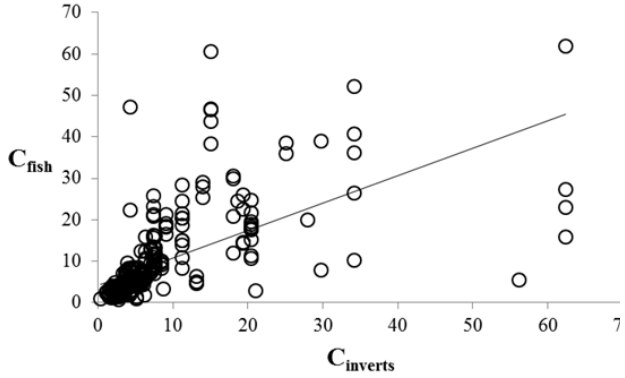
White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Grasso et al. 1995	17	1.91	4.00	2.09
Grasso et al. 1995	17	1.91	4.01	2.10
Butler et al. 1994	NFK3	2.00	3.90	1.95
Butler et al. 1993	ST1	2.25	4.90	2.18
Lambing et al. 1994	S33	2.40	3.50	1.46
Mueller et al. 1991	A1	2.70	4.20	1.56
GEI 2013	SWA1	2.81	2.83	1.01
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.18	1.49
Mason et al. 2000	HCRT	2.81	0.81	0.29
Mason et al. 2000	HCRT	2.81	1.43	0.51
Mason et al. 2000	HCRT	2.81	1.43	0.51
Butler et al. 1993	WSB2	3.00	3.90	1.30
Butler et al. 1993	SP2	3.15	3.50	1.11
Butler et al. 1993	LP4	3.20	2.80	0.88
GEI 2013	SW4-1	3.33	3.01	0.91
GEI 2013	SW4-1	3.33	3.45	1.04
GEI 2013	SW4-1	3.33	3.50	1.05
GEI 2013	SW4-1	3.33	3.62	1.09
GEI 2013	SW4-1	3.33	4.04	1.22
GEI 2013	SW4-1	3.33	4.08	1.23
GEI 2013	SW4-1	3.33	4.13	1.24
GEI 2013	SW4-1	3.33	4.17	1.25
GEI 2013	SW4-1	3.33	4.34	1.31
GEI 2013	SW4-1	3.33	4.78	1.44
Butler et al. 1993	ST2	3.35	7.00	2.09
GEI 2013	LG1	3.37	3.54	1.05
GEI 2013	LG1	3.37	3.55	1.05
GEI 2013	LG1	3.37	3.90	1.16
GEI 2013	LG1	3.37	3.95	1.17
GEI 2013	LG1	3.37	4.48	1.33
GEI 2013	LG1	3.39	3.00	0.88
GEI 2013	LG1	3.56	2.72	0.77
GEI 2013	LG1	3.56	2.80	0.79
GEI 2013	LG1	3.56	2.89	0.81
GEI 2013	LG1	3.56	2.99	0.84
GEI 2013	LG1	3.56	3.04	0.86
GEI 2013	LG1	3.56	3.08	0.87
GEI 2013	LG1	3.56	3.13	0.88
GEI 2013	LG1	3.56	3.18	0.89
GEI 2013	LG1	3.56	3.25	0.91
GEI 2013	LG1	3.56	3.27	0.92

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	WSB2	3.60	4.30	1.19
Butler et al. 1993	WSB2	3.60	6.30	1.75
GEI 2013	SWA1	3.64	2.83	0.78
GEI 2013	SWA1	3.64	3.39	0.93
GEI 2013	SWA1	3.64	3.47	0.95
GEI 2013	SWA1	3.64	3.55	0.98
GEI 2013	SWA1	3.64	3.63	1.00
GEI 2013	SWA1	3.64	3.75	1.03
Butler et al. 1993	SB2	3.65	4.30	1.18
Butler et al. 1993	R2	3.70	4.20	1.14
Butler et al. 1993	SB2	3.75	4.80	1.28
GEI 2013	CC1	3.76	5.99	1.59
GEI 2013	CC1	3.76	6.56	1.74
GEI 2013	CC1	3.76	7.21	1.92
GEI 2013	CC1	3.76	7.42	1.97
GEI 2013	CC1	3.76	7.62	2.03
Peterson et al. 1991	7	3.83	3.30	0.86
Peterson et al. 1991	7	3.83	4.64	1.21
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1991	4	3.90	5.30	1.36
GEI 2013	SW88	3.96	4.63	1.17
GEI 2013	SW88	3.96	4.75	1.20
Butler et al. 1993	R1	4.00	9.50	2.38
Butler et al. 1993	ST2	4.10	8.30	2.02
GEI 2013	SW9	4.45	4.07	0.91
GEI 2013	SW9	4.45	4.18	0.94
GEI 2013	SW9	4.45	4.19	0.94
GEI 2013	SW9	4.45	4.20	0.94
GEI 2013	SW9	4.45	4.40	0.99
GEI 2013	SW9	4.45	5.18	1.16
GEI 2013	CC1	4.69	4.51	0.96
GEI 2013	CC1	4.69	4.57	0.98
GEI 2013	CC1	4.69	4.94	1.05
GEI 2013	CC1	4.69	5.02	1.07
GEI 2013	CC1	4.69	5.81	1.24
GEI 2013	CC1	4.69	6.01	1.28
GEI 2013	CC1	4.69	6.43	1.37
GEI 2013	CC1	4.69	7.25	1.55
GEI 2013	CC1	4.69	8.00	1.71
GEI 2013	CC1	4.69	8.52	1.82
Butler et al. 1993	F2	4.80	5.20	1.08
GEI 2013	CC1	5.86	5.00	0.85

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	CC1	5.86	5.37	0.92
GEI 2013	CC1	5.86	5.59	0.95
GEI 2013	CC1	5.86	5.71	0.98
GEI 2013	CC1	5.86	5.90	1.01
GEI 2013	CC1	5.86	6.61	1.13
GEI 2013	CC1	5.86	6.79	1.16
GEI 2013	CC1	5.86	6.82	1.16
GEI 2013	CC1	5.86	7.29	1.25
GEI 2013	CC1	5.86	7.48	1.28
Butler et al. 1991	3	6.20	1.80	0.29
GEI 2013	SWB	7.06	7.18	1.02
GEI 2013	SWB	7.06	7.36	1.04
GEI 2013	SWB	7.06	7.98	1.13
GEI 2013	SWB	7.06	8.03	1.14
GEI 2013	SWB	7.06	9.65	1.37
GEI 2013	SWB	7.06	12.76	1.81
GEI 2013	SWB	7.06	12.85	1.82
GEI 2013	SWB	7.06	13.16	1.86
GEI 2013	SWB	7.44	8.21	1.10
GEI 2013	SWB	7.44	8.77	1.18
GEI 2013	SWB	7.44	8.85	1.19
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	13.59	1.83
GEI 2013	SWB	7.44	15.75	2.12
GEI 2013	SWB	7.44	16.40	2.21
Butler et al. 1994	IW	8.35	9.70	1.16
Mueller et al. 1991	R1	8.70	3.40	0.39
GEI 2013	SW2-1	9.14	16.54	1.81
GEI 2013	SW2-1	9.14	18.14	1.99
GEI 2013	SW2-1	9.14	18.54	2.03
GEI 2013	SW2-1	9.14	19.16	2.10
GEI 2013	SW2-1	9.14	21.29	2.33
Lambing et al. 1994	S34	14.00	25.30	1.81
Lambing et al. 1994	S34	14.00	28.00	2.00
Lambing et al. 1994	S34	14.00	29.00	2.07
Butler et al. 1994	HCC1	21.00	3.00	0.14
Butler et al. 1994	GUN2	28.00	20.00	0.71
Butler et al. 1991	7	29.80	7.90	0.27

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio



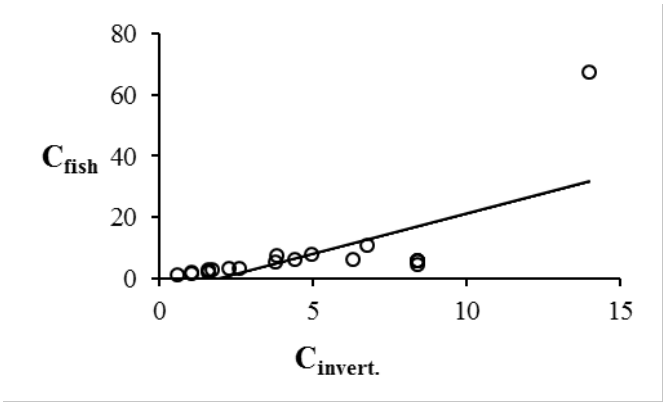
Median ratio: 1.11

R^2: 0.38
F: 174.4
df: 284
P: <0.001

Yellow perch (*Perca flavescens*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	PU	0.61	1.10	1.80
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	MP	1.60	2.00	1.25
Butler et al. 1995	MP	1.60	2.20	1.38
Butler et al. 1995	MP	1.60	2.70	1.69
Belize et al. 2006	Halfway	1.74	2.72	1.56
Belize et al. 2006	Geneva	2.29	3.30	1.44
Belize et al. 2006	Bethel	2.61	3.09	1.19
Belize et al. 2006	McFarlane	3.79	5.40	1.42
Peterson et al. 1991	7	3.83	7.33	1.91
Belize et al. 2006	Long	4.42	6.28	1.42
Belize et al. 2006	Ramsey	4.97	7.64	1.54
Belize et al. 2006	Windy	6.32	6.06	0.96
Belize et al. 2006	Nelson	6.79	10.68	1.57
GEI 2013	SW11	8.41	4.54	0.54
GEI 2013	SW11	8.41	5.49	0.65
GEI 2013	SW11	8.41	5.50	0.65
GEI 2013	SW11	8.41	5.58	0.66
GEI 2013	SW11	8.41	5.68	0.68
Lambing et al. 1994	S34	14.00	67.00	4.79

Yellow perch (*Perca flavescens*)



Median ratio: 1.42

R^2: 0.46
F: 16.24
df: 19
P: <0.001

Table B-7. Final vertebrate Trophic Transfer Factor (TTF) values, including estimated values using taxonomic classification.

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
alligator gar	*Atractosteus spatula*	Lepistosteiformes	Lepisosteidae	Atractosteus	1.21	All fish
black bullhead	*Ameiurus melas*	Siluriformes	Ictaluridae	Ameiurus	0.85	Exact match
black crappie	*Pomoxis nigromaculatus*	Perciformes	Centrarchidae	Pomoxis	2.67	Exact match
black redhorse	*Moxostoma duquesnei*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
blacknose dace	*Rhinichthys atratulus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Exact match
blue catfish	*Ictalurus furcatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Genus Ictalurus
bluegill	*Lepomis macrochirus*	Perciformes	Centrarchidae	Lepomis	1.03	Exact match
bluehead sucker	*Catostomus discobolus*	Cypriniformes	Catostomidae	Catostomus	1.04	Exact match
brassy minnow	*Hybognathus hankinsoni*	Cypriniformes	Cyprinidae	Hybognathus	1.20	Family Cyprinidae
brook stickleback	*Culaea inconstans*	Gasterosteiformes	Gasterosteidae	Culaea	1.79	Exact match
brook trout	*Salvelinus fontinalis*	Salmoniformes	Salmonidae	Salvelinus	0.88	Exact match
brown bullhead	*Ameiurus nebulosus*	Siluriformes	Ictaluridae	Ameiurus	0.85	Genus Ameiurus
brown trout	*Salmo trutta*	Salmoniformes	Salmonidae	Salmo	1.38	Exact match
bullhead		Siluriformes	Ictaluridae		0.77	Family Ictaluridae
burbot	*Lota lota*	lota	Gadiformes	Lotidae	1.21	All fish
chain pickerel	*Esox niger*	Esociformes	Esocidae	Esox	1.78	Genus Esox
channel catfish	*Ictalurus punctatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Exact match
common carp	*Cyprinus carpio*	Cypriniformes	Cyprinidae	Cyprinus	1.20	Exact match
common snook	*Centropomus undecimalis*	Perciformes	Centropomidae	Centropomus	1.41	Order Perciformes
crappie	*Pomoxis sp.*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
creek chub	*Semotilus atromaculatus*	Cypriniformes	Cyprinidae	Semotilus	1.06	Exact match
cutthroat trout	*Oncorhynchus clarkii*	Salmoniformes	Salmonidae	Oncorhynchus	1.12	Exact match
desert pupfish	*Cyprinodon macularius*	Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.24	Order Cyprinodontiformes
dolly varden	*Salvelinus malma*	Salmoniformes	Salmonidae	Salvelinus	0.88	Genus Salvelinus
fathead minnow	*Pimephales promelas*	Cypriniformes	Cyprinidae	Pimephales	1.57	Exact match
flannelmouth sucker	*Catostomus latipinnis*	Cypriniformes	Catostomidae	Catostomus	0.98	Exact match
flathead catfish	*Pylodictis olivaris*	Siluriformes	Ictaluridae	Pylodictus	0.77	Family Ictaluridae
flathead chub	*Platygobio gracilis*	Cypriniformes	Cyprinidae	Platygobio	1.20	Family Cyprinidae
freshwater drum	*Aplodinotus grunniens*	Perciformes	Sciaenidae	Aplodinotus	1.41	Order Perciformes
gizzard shad	*Dorosoma cepedianum*	Clupeiformes	Clupeidae	Dorosoma	1.21	All fish
goldeye	*Hiodon alosoides*	Hiodontiformes	Hiodontidae	Hiodon	1.21	All fish
green sunfish	*Lepomis cyanellus*	Perciformes	Centrarchidae	Lepomis	1.12	Exact match
iowa darter	*Etheostoma exile*	Perciformes	Percidae	Etheostoma	1.51	Family Percidae
kokanee salmon	*Oncorhynchus nerka*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
largemouth bass	*Micropterus salmoides*	Perciformes	Centrarchidae	Micropterus	1.39	Exact match
largescale sucker	*Catostomus macrocheilus*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
longnose dace	*Rhinichthys cataractae*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
longnose sucker	*Catostomus catostomus*	Cypriniformes	Catostomidae	Catostomus	0.90	Exact match
mottled sculpin	*Cottus bairdi*	Scorpaeniformes	Cottidae	Cottus	1.38	Exact match
mountain whitefish	*Prosopium williamsoni*	Salmoniformes	Salmonidae	Prosopium	1.38	Exact match
ninespine stickleback	Pungitius pungitius	pungitius	Gasterosteiformes	Gasterosteidae	1.79	Family Gasterosteidae
northern pike	*Esox lucius*	Esociformes	Esocidae	Esox	1.78	Exact match
northern pikeminnow	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
northern plains killifish	*Fundulus kansae*	Cyprinodontiformes	Fundulidae	Fundulus	1.27	Exact match
northern redbelly dace	*Chrosomus eos*	Cypriniformes	Cyprinidae	Chrosomus	1.20	Family Cyprinidae
northern squawfish	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
quillback	*Carpiodes cyprinus*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
rainbow trout	*Oncorhynchus mykiss*	Salmoniformes	Salmonidae	Oncorhynchus	1.07	Exact match
razorback sucker	*Xyrauchen texanus*	Cypriniformes	Catostomidae	Xyrauchen	1.01	Family Catostomidae
red shiner	*Cyprinella lutrensis*	Cypriniformes	Cyprinidae	Cyprinella	1.31	Family Cyprinidae
redbreast sunfish	*Lepomis auritus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redear sunfish	*Lepomis microlophus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redside shiner	*Richardsonius balteatus*	Cypriniformes	Cyprinidae	Richardsonius	1.08	Exact match
river carpsucker	*Carpiodes carpio*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
river redhorse	*Moxostoma carinatum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
rock bass	*Ambloplites rupestris*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
roundtail chub	*Gila robusta*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
sacramento perch	*Archoplites interruptus*	Perciformes	Centrarchidae	Archoplites	1.12	Family Centrarchidae
sacramento pikeminnow	*Ptychocheilus grandis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
sailfin molly	*Poecilia latipinna*	Cyprinodontiformes	Poeciliidae	Poecilia	1.21	Family Poeciliidae
sand shiner	*Notropis stramineus*	Cypriniformes	Cyprinidae	Notropis	1.56	Exact match
sauger	*Sander canadensis*	Perciformes	Percidae	Sander	1.60	Genus Sander
sculpin	*Cottus sp.*	Scorpaeniformes	Cottidae	Cottus	1.29	Exact match
shadow bass	*Ambloplites ariommus*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
shorthead redhorse	*Moxostoma macrolepidotum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
silver carp	*Hypophthalmichthys molitrix*	Cypriniformes	Cyprinidae	Hypophthalmichthys	1.20	Family Cyprinidae
smallmouth bass	*Micropterus dolomieu*	Perciformes	Centrarchidae	Micropterus	0.86	Exact match
smallmouth buffalo	*Ictiobus bubalus*	Cypriniformes	Catostomidae	Ictiobus	1.01	Family Catostomidae
speckled dace	*Rhinichthys osculus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys
spottail shiner	Notropis hudsonius	hudsonius	Cypriniformes	Cyprinidae	1.56	Genus Notropis
spotted bass	*Micropterus punctulatus*	Perciformes	Centrarchidae	Micropterus	1.12	Genus Micropterus
spotted gar	*Lepisosteus oculatus*	Lepistosteiformes	Lepisosteidae	Lepisosteus	1.21	All fish
stonecat	*Noturus flavus*	Siluriformes	Ictaluridae	Noturus	0.77	Family Ictaluridae
striped bass	*Morone saxatilis*	Perciformes	Moronidae	Morone	1.48	Exact match
striped mullet	*Mugil cephalus*	Mugiliformes	Mugilidae	Mugil	1.21	All fish

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
sucker		Cypriniformes	Catostomidae		1.01	Family Catostomidae
tilapia		Perciformes	Cichlidae		1.41	Order Perciformes
trout species	*Oncorhynchus sp.*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
tui chub	*Gila bicolor*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
utah sucker	*Catostomus ardens*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
walleye	*Sander vitreus*	Perciformes	Percidae	Sander	1.60	Exact match
western mosquitofish	*Gambusia affinis*	Cyprinodontiformes	Poeciliidae	Gambusia	1.21	Exact match
white bass	*Morone chrysops*	Perciformes	Moronidae	Morone	1.48	Genus Morone
white crappie	*Pomoxis annularis*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
white sturgeon	*Acipenser transmontanus*	Acipenseriformes	Acipenseridae	Acipenser	1.21	All fish
white sucker	*Catostomus commersonii*	Cypriniformes	Catostomidae	Catostomus	1.11	Exact match
wiper	*Morone chrysops x Moron saxatilis*	Perciformes	Moronidae	Morone	1.48	Genus Morone
yellow perch	*Perca flavescens*	Perciformes	Percidae	Perca	1.42	Exact match

Table B-8. Food web models used to calculate composite TTFs to translate the egg-ovary FCV to a water-column value at aquatic sites where sufficient data was available to calculate an enrichment factor (EF).

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			black bullhead	0.85	Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion		Median of all insects	in	2.14	0.45	Median of all crustaceans	crs	1.41	0.45	Median of all bivalves	bvs	4.29	0.10											2.03	0.85	1.72
Default			black crappie	2.67	Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			blacknose dace	0.71	Eats immature aquatic insects, amphipods, and various other aquatic invertebrates; also eats algae and diatoms, which may be of little nutritional value (Smith 1979, Becker 1983).		Median of all insects	in	2.14	0.50	Median of all invertebrates except bivalves	all	1.48	0.50														1.81	0.71	1.29
Default			blue catfish	0.68	Bottom feeder. Eats mostly crustaceans and aquatic insects when young. Later, fishes and large invertebrates become most important (Moyle 1976). Also scavenges.		Median of all insects	in	2.14	0.36	Median insects and benthic crustaceans	in,bc	1.74	0.20	Median of all bivalves	bvs	4.29	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.36	2.28	0.68	1.56
Default			bluegill	1.03	Feeds opportunistically on aquatic insect larvae, planktonic crustaceans, flying insects, snails, and other small invertebrates; small fishes, fish eggs, crayfish, and algae sometimes are eaten. Larvae and juveniles often eat cladocerans and copepod nauplii. Adults eats mainly aquatic insects, crayfishes, and small fishes, or, in some bodies of water, mostly zooplankton. Feeds at all levels of water column.		Median of all insects	in	2.14	0.68	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.04	1.95	1.03	2.00
Default			bluehead sucker	1.04	Herbivore, Invertivore		TL1	TL1	1.00	0.60	Median of all invertebrates except bivalves	all	1.48	0.40														1.19	1.04	1.24
Default			brassy minnow	1.20	Eats algae, phyto- and zooplankton, benthic invertebrates, surface drift, bottom ooze (Becker 1983).		TL1	TL1	1.00	0.50	Median of planktonic crustacean	pc	1.41	0.40	Median of all insects	in	2.14	0.10										1.28	1.20	1.54

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			brook stickleback	1.79	Eats various aquatic invertebrates (including eggs and larvae), eggs and larvae of fishes, and algae. In a Manitoba lake, was opportunistic but heavily dependent on arthropods (Moodie 1986).		Median of all invertebrates except bivalves	all	1.48	0.80	TL1	TL1	1.00	0.20															1.38	1.79	2.47
Default			brook trout	0.88	Feeds opportunistically on various invertebrate and vertebrate animals, including primarily terrestrial and aquatic insects and planktonic crustaceans.		Median of all insects	in	2.14	0.60	crayfish	cr	1.46	0.10	Median of all bivalves	bvs	4.29	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.22	0.88	1.96
Default			brown bullhead	0.85	Bottom feeder. Young eat chironomid larvae and small crustaceans. Adults eat larger insect larvae and fishes, also fish eggs, mollusks, carrion, and plant material (Becker 1983, Moyle 1976).		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.85	1.79
Default			brown trout	1.38	Eats aquatic and terrestrial insects and their larvae, crustaceans (especially crayfish), molluscs, fishes, and other animals. In streams, young feed mainly on aquatic and terrestrial drift invertebrates; in lakes, they feed on zooplankton and benthic invertebrates (Sublette et al. 1990). Large adults feed on fishes, crayfish, and other benthic invertebrates.		Median of planktonic crustaceans	pc	1.41	0.20	Median of all insects	in	2.14	0.12	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.02	1.38	2.78
Default			bullhead	0.77	Black (not exotic to CO and NM): Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion. Stomach often contain substantial amounts of plant material of unknown nutritional value (Moyle 1976). Juveniles planktivorous; at about 27 mm TL, feed largely on crustaceans and midge larvae		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.77	1.62
Default			burbot	1.21	Young eat mainly immature aquatic insects, crayfish, molluscs, and other deepwater invertebrates. Larger individuals feed mostly on fishes (Becker 1983, Scott and Crossman 1973).		Median of all insects	in	2.14	0.25	Median of all crustaceans	crs	1.41	0.25									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.03	1.21	2.45
Default			channel catfish	0.68	Bottom feeder. Young eat mainly small invertebrates; as they grow, fishes and crayfish become increasingly important, though individuals of all sizes eat abundant aquatic insects. Large fish are mainly piscivorous (Moyle 1976).		Median of all insects	in	2.14	0.48	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	1.97	0.68	1.35

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			common carp	1.20	Omnivorous; adults eat mainly invertebrates, detritus, fish eggs, and plant material (Jester 1974, Becker 1983, Sublette et al. 1990).		Median of all invertebrates except bivalves	all	1.48	0.65	TL1	TL1	1.00	0.35															1.31	1.20	1.58
Default			crappie	2.67	Black: Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals. White: eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			creek chub	1.06	Feeds opportunistically on various plants and animals, from surface drift to benthos; mostly invertivorous but large individuals often picivorous (Becker 1983). Chironomid larvae and other larval insects important in diet of young.		Median of all invertebrates except bivalves	all	1.48	0.70	TL1	TL1	1.00	0.20									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.46	1.06	1.55
Default			cutthroat trout	1.12	Opportunistic. Inland cutthroats feed primarily on insects (aquatic and terrestrial); often feeds in and especially downstream from riffle areas; some large individuals feed mostly on fishes; also eats zooplankton and crustaceans.		Median of all insects	in	2.14	0.50	Median of all crustaceans	crs	1.41	0.20									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.3	2.04	1.12	2.29
Default			fathead minnow	1.57	Feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990).	expected diet of small invertebrates	Median of all insects	in	2.14	0.60	Median of all crustaceans	crs	1.41	0.20	TL1	TL1	1.00	0.20											1.77	1.57	2.78
Default			flannel mouth sucker	0.98	Herbivore, Invertivore Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25															1.55	0.98	1.52
Default			flathead chub	1.20	Opportunistic; eats aquatic and terrestrial insects and some algae (Olund and Cross 1961)		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20															1.91	1.20	2.30
Default			freshwater drum	1.41	Young feed mainly on minute crustaceans; adults mostly are bottom feeders, eat insect larvae, crustaceans, fishes, and (mostly in rivers) clams and		Median of all crustaceans	crs	1.41	0.44	Median of all insects	in	2.14	0.40	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrat	f+a	2.28	0.12	1.92	1.41	2.71

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					snails (Becker 1983, Scott and Crossman 1973, Lee et al. 1980).																			es						
Default			gizzard shad	1.21	Adults primarily bottom filter-feeding detritivores		TL1	TL1	1.00	1.00																		1.00	1.21	1.21
Default			goldeye	1.21	Young-of-year eat mainly microcrustaceans, also other invertebrates. Older individuals eat mainly aquatic insects obtained at surface but also various other animals, including frogs, fishes, and small mammals.		Median insects and benthic crustaceans	in,bc	1.74	1.00																		1.74	1.21	2.10
Default			green sunfish	1.12	Feeds opportunistically on the larger, more active invertebrates that occur with them, and on small fishes. Young feed mostly on crustaceans (zooplankton) and aquatic insect larvae. Adults eat more large aquatic and terrestrial insects, crayfish, and fishes		Median of all insects	in	2.14	0.58	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	2.05	1.12	2.29
Default			Iowa darter	1.51	Eats mainly various invertebrates; commonly ingested food items of adults are midge larvae, mayfly naiads, and amphipods, and of the young, copepods and cladocerans. Apparently feeds on swimming organisms and those on bottom.	expected diet of small invertebrates	Median of all insects	in	2.14	0.70	amphipods	am	1.22	0.16	crayfish	cr	1.46	0.08	Median of planktonic crustaceans	pc	1.41	0.06						1.90	1.51	2.87
Default			kokanee salmon	1.10	Zooplankton, insects.		Median of planktonic crustaceans	pc	1.41	0.80	Median of all insects	in	2.14	0.20														1.56	1.10	1.71
Default			largemouth bass	1.39	Fry feed mainly on zooplankton. Larger young eat insects, crustaceans, and fish fry. Adults eat mainly fishes, though sometimes prefer crayfish or amphibians (Moyle 1976, Smith 1979).		Median of all insects	in	2.14	0.10	crayfish	cr	1.46	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.18	1.39	3.04
Default			longnose dace	0.71	Eats mainly benthic insects, especially Diptera and mayflies (Becker 1983, Scott and Crossman 1973); also eats algae and plant material (Sublette et al. 1990). Terrestrial insects and fish eggs common in diet of adults from Lake Michigan (see Sublette et al. 1990).		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20														1.91	0.71	1.36
Default			longnose sucker	0.90	Eats mostly bottom invertebrates (Scott and Crossman 1973).		Median of all invertebrates except bivalves	all	1.48	1.00																		1.48	0.90	1.34

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			mottled sculpin	1.38	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	Median of all crustaceans	crs	1.41	0.10	TL1	TL1	1.00	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.97	1.38	2.72
Default			mountain whitefish	1.38	Feeds actively on aquatic and terrestrial insects. Also feeds on some fish eggs and occasionally on fishes. Bottom-oriented predator (Moyle 1976), occasionally feeds at surface (Sigler and Sigler 1987).		Median of all insects	in	2.14	0.90													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.16	1.38	2.97
Default			ninespine stickleback	1.79	Eats mainly small crustaceans and aquatic insects; sometimes also fish eggs and fry (Becker 1983).		Median of all crustaceans	crs	1.41	0.48	Median of all insects	in	2.14	0.44									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	1.80	1.79	3.22
Default			northern pike	1.78	Young initially eat large zooplankton and immature aquatic insects. After 7-10 days fishes begin to enter diet and eventually dominate. Adults feed opportunistically on vertebrates small enough to be engulfed. (Scott and Crossman 1973). Sight feeder.		Median insects and benthic crustaceans	in,bc	1.74	0.05													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.95	2.25	1.78	4.02
Default			northern plains killifish	1.27	Feed effectively at all levels and food habits are generalized. Prefer aquatic insects but also feed on plants.	Montana field guide (http://fieldguide.mt.gov/detail_AFCNB04600.aspx)	Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20															1.91	1.27	2.44
Default			northern redbelly dace	1.20	Eats mainly diatoms and filamentous algae, also zooplankton and aquatic insects.		TL1	TL1	1.00	0.70	Median of all insects	in	2.14	0.15	Median insects and benthic crustaceans	in,bc	1.74	0.15											1.28	1.20	1.54
Default			northern squawfish	1.20	Small individuals feed primarily on aquatic and terrestrial insects. Adults feed on fish, insects, insect larvae, crustaceans and some plankton during spring and summer. Fishes are the major component		Median of all insects	in	2.14	0.32	Median of all crustaceans	crs	1.41	0.08									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.17	1.20	2.61

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					of the diet in winter.																									
Default			rainbow trout	1.07	In lakes, feeds mostly on bottom-dwelling invertebrates (e.g., aquatic insects, amphipods, worms, fish eggs, sometimes small fish) and plankton. In streams, feeds primarily on drift organisms. May ingest aquatic vegetation (probably for attached invertebrates).		Median of all insects	in	2.14	0.75													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.18	1.07	2.33
Default			red shiner	1.31	Eats various small invertebrates (insects, crustaceans), plant material (digestibility may be low), and microorganisms (Becker 1983). In Virgin River, diet dominated by Ceratopongidae, Simuliidae, and Chironomidae (Greger and Deacon 1988).		Median insects and benthic crustaceans	in,bc	1.74	1.00																	1.74	1.31	2.27
Default			redside shiner	1.08	Feeds mainly on aquatic and terrestrial insects; also eats molluscs, plankton, and some small fish and fish eggs. Fry eat zooplankton and algae.		Median of all insects	in	2.14	0.70	Median of planktonic crustaceans	pc	1.41	0.10	Median of all bivalves	bvs	4.29	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.30	1.08	2.48
Default			river carpsucker	1.01	Mostly a bottom feeder, browses on periphyton associated with submerged rocks and debris, ingests various small planktonic plants and animals.		TL1	TL1	1.00	0.75	Median of planktonic crustaceans	pc	1.41	0.25														1.10	1.01	1.11
Default			roundtail chub	1.20	Opportunistic; eats available aquatic and terrestrial insects, gastropods, crustaceans, fishes, and sometimes filamentous algae (Sublette et al. 1990).		Median of all insects	in	2.14	0.55	Median of all crustaceans	crs	1.41	0.15	Median of all bivalves	bvs	4.29	0.15					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.38	1.20	2.86
Default			Sacramento perch	1.12	Opportunistic; diet mainly benthic insect larvae, snails, mid-water insects, zooplankton, and fishes (Moyle et al. 1989). Young feed mainly on small crustaceans, but as they grow Sacramento perch consume more aquatic insect larvae and pupae. Large adults feed mainly on other fishes when available.		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.12	1.44
Default			sailfin molly	1.21	Eats mainly algae, vascular plants, organic detritus, and mosquito larvae (and other small invertebrates).		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.21	1.56

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Default			sand shiner	1.56	Eats various aquatic and terrestrial invertebrates (especially chironomids), algae, and (mainly) bottom particulate matter (Becker 1983). Winter diet mostly chironomids larvae and mayfly and stonefly naiads (Ohio, see Sublette et al. 1990)		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25															1.55	1.56	2.43
Default			sauger	1.60	Larvae eat microcrustaceans. Young eat zooplankton, immature and adult aquatic insects, and fish fry; adults eat small fishes and various invertebrates (Scott and Crossman 1973), or are almost exclusively piscivorous (Burkhead and Jenkins 1991). Sight feeder, adapted to low light.		Median insects and benthic crustaceans	in,bc	1.74	0.36	Median of planktonic crustaceans	pc	1.41	0.10										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.54	2.00	1.60	3.20
Default			sculpin	1.29	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	crayfish	cr	1.46	0.15										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.06	1.29	2.66
Default			shorthead redhorse	1.01	Invertivore		Median of all invertebrates except bivalves	all	1.48	1.00																			1.48	1.01	1.49
Default			smallmouth bass	0.86	Adults almost entirely piscivorous if sufficient prey available		Median of all insects	in	2.14	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.25	0.86	1.93
Default			speckled dace	0.71	An omnivorous benthic feeder, at times feeding on drift in mid-water or rarely at the surface (Schreiber and Minckley 1981). The diet consists mostly of benthic insects, also includes other invertebrates, algae, and detritus (little or no plant material or detritus in some areas) (Sublette et al. 1990, Woodbury 1933, Greger and Deacon 1988). Young feed mainly on zooplankton.		Median of all insects	in	2.14	0.70	Median insects and benthic crustaceans	in,bc	1.74	0.15	TL1	TL1	1.00	0.15											1.91	0.71	1.36

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Default			stonecat	0.77	Eats mainly bottom invertebrates (insects, crayfish); sometimes also plant material and fishes (Becker 1983, Scott and Crossman 1973).		Median insects and benthic crustaceans	in,bc	1.74	0.70	TL1	TL1	1.00	0.20										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.65	0.77	1.26
Default			sucker	1.01	White: Larvae feed near surface on protozoans, diatoms, small crustaceans, and bloodworms. Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990). Bluehead: A bottom feeder. Scrapes algae and other organisms from rocks with chisel-like ridges inside each lip; ingests fine organism-laden sediments. May feed in stream riffles, or deeper rocky pools; in lakes it may feed over rocks near shore. May eat aquatic insect larvae. Flannelmouth: Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.01	1.25
Default			tilapia	1.41	aureus: Eats mainly phytoplankton. mossambicus: Nonselective omnivore; eats planktonic algae, aquatic plants, invertebrates, and small fishes (Moyle 1976). zilli: Feeds on algae and higher plants, invertebrates, and occasionally eats dead or dying fish.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.41	1.74
Default			tui chub	1.20	Adults opportunistic. They feed on plant material, plankton, insect larvae, crustaceans, fish fry and fish eggs, etc. Young feed on zooplankton. Coarse-rakered form eats more plant material, fine-rakered form more zooplankton.		TL1	TL1	1.00	0.40	Median of planktonic crustaceans	pc	1.41	0.28	Median of all insects	in	2.14	0.28	crayfish	cr	1.46	0.04						1.45	1.20	1.75
Default			Utah sucker	1.01	Bottom feeder. Varied diet; feeds freely on both animal and plant organisms, at all depths throughout the year. Grazes on filamentous algae.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.01	1.25

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			walleye	1.60	Adults feed opportunistically on various fishes and larger invertebrates.		Median insects and benthic crustaceans	in,bc	1.74	0.50													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.01	1.60	3.21
Default			western mosquitofish	1.21	Opportunistic omnivore; eats mainly small invertebrates, often taken near water surface. Also eats small fishes and, in the absence of abundant animal food, algae and diatoms (Moyle 1976). Mosquitofish are principally carnivorous, and have strong, conical teeth and short guts (Meffe et al. 1983, Turner and Snelson 1984). They are reported to feed on rotifers, snails, spiders, insect larvae, crustaceans, algae, and fish fry, including their own progeny (Barnickol 1941, Minckley 1973, Meffe and Crump 1987). Cannibalism has been documented by several authors (Seale 1917, Krumholz 1948, Walters and Legner 1980, Harrington and Harrington 1982). Plant material is taken occasionally (Barnickol 1941) and may make up a significant portion of the diet during periods of scarcity of animal prey (Harrington and Harrington 1982). Grubb (1972) showed that anuran eggs from temporary ponds were preferentially selected over those breeding in permanent systems.		Median of all insects	in	2.14	0.75	Median of all crustaceans	crs	1.41	0.25																	1.96	1.21	2.37
Default			white bass	1.48	Eats fishes, zooplankton, aquatic insects, oligochaetes, and crayfish; fishes often dominate diet of adults; diet may vary from place to place (Moyle 1976, Sublette et al. 1990).		Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.05	crayfish	cr	1.46	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.15	1.48	3.19
Default			white crappie	2.67	Eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			white sturgeon	1.21	A bottom feeder. Young feed mostly on the larvae of aquatic insects, crustaceans, and molluscs. A significant portion of the		Median insects and benthic	in,bc	1.74	0.31	Median of all bivalves	bvs	4.29	0.09									Fish	Median all fish eating median all	f+a	2.28	0.6	2.29	1.21	2.77

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					diet of larger sturgeon consists of fish.		crustaceans																	invertebrates						
Default			white sucker	1.11	Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990).	expected common spp in benthos	TL1	TL1	1.00	0.50	Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.10						1.43	1.11	1.58
Default			wiper	1.48	Adults are predatory on fishes and larger crustaceans (Hassler 1988).		crayfish	cr	1.46	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.11	1.48	3.13
Default			yellow perch	1.42	Larvae and young primarily zooplankton feeders; older young eat mostly invertebrates associated with bottom and with aquatic plants; adults feed among plants and along bottom on larger invertebrates and small fishes (Moyle 1976).		Median insects and benthic crustaceans	in,bc	1.74	0.64	Median of planktonic crustaceans	pc	1.41	0.13	TL1	TL1	1.00	0.07					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.16	1.73	1.42	2.47
Saiki et al. 1993			bluegill	1.03	site-specific: 0.23 chironomid; 0.3 microcrustacea; 0.47 amphipod	stomach analysis	amphipods	am	1.22	0.47	Median of planktonic crustaceans	pc	1.41	0.30	midges	mg	1.90	0.23										1.43	1.03	1.47
Saiki et al. 1993			largemouth bass	1.39	site-specific: 0.73 fish; 0.27 crayfish	stomach analysis	crayfish	cr	1.46	0.27	Saiki bluegill TTFcomp.	BG	1.47	0.73														1.47	1.39	2.04
Saiki et al. 1993			western mosquitofish	1.21	site-specific: 0.89 molluscs, and insects; 0.065 chironomid; 0.045 microcrustacea	stomach contents show a large terrestrial component	Median insects and benthic crustaceans	in,bc	1.74	0.89	midges	mg	1.90	0.07	Median of planktonic crustaceans	pc	1.41	0.05										1.74	1.21	2.10
Formation 2012	Crow Creek - CC150	CC-150	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.38	2.91
Formation 2012	Crow Creek - CC150	CC-150	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.29	2.74

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Crow Creek - 1A	CC-1A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.38	2.96
Formation 2012	Crow Creek - 1A	CC-1A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.29	2.78
Formation 2012	Crow Creek - CC350	CC-350	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.38	2.97
Formation 2012	Crow Creek - CC350	CC-350	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.29	2.79
Formation 2012	Crow Creek - 3A	CC-3A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.38	2.97
Formation 2012	Crow Creek - 3A	CC-3A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.29	2.78
Formation 2012	Crow Creek - CC75	CC-75	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.38	2.87
Formation 2012	Crow Creek - CC75	CC-75	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.29	2.69
Formation 2012	Deer Creek	DC-600	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.38	3.00
Formation 2012	Deer Creek	DC-600	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.29	2.81
Formation 2012	Hoopes Spring - HS	HS	brown trout	1.38	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.38	3.86
Formation 2012	Hoopes Spring - HS	HS	sculpin	1.29	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.29	3.63
Formation 2012	Hoopes Spring - HS3	HS-3	brown trout	1.38	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.38	2.63
Formation 2012	Hoopes Spring - HS3	HS-3	sculpin	1.29	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.29	2.47

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Sage Creek - LSV2C	LSV-2C	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.38	3.01
Formation 2012	Sage Creek - LSV2C	LSV-2C	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.29	2.83
Formation 2012	Sage Creek - LSV4	LSV-4	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.38	2.88
Formation 2012	Sage Creek - LSV4	LSV-4	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.29	2.70
Formation 2012	South Fork Tincup Cr.	SFTC-1	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.38	3.05
Formation 2012	South Fork Tincup Cr.	SFTC-1	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.29	2.86

Table B-9. Calculation of site-specific invertebrate proportions using invertebrate counts in Formation 2012

| Order | Genus | Habitat / Behavior | Functional Feeding | Tolerance | Stream | SF Tincup Creek | | Crow Creek | | |

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek			Crow Creek								Deer Creek						Hoopes Spring			Sage Creek						Crow Creek								Total
					Location	SFTC1			CC75		CC150		CC350				DC600			HS			HS3			LSV2C		LSV4		CC1A			CC3A							
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008		
	sp.																																							
Trichoptera	Glossoma sp.	CN	SC	0													4																							4
Trichoptera	Helicopsyche sp.	CN	SC	3				3			5	4		5	93								2			2							19	81					214
Trichoptera	Hesperophylax sp.	CN	SH	5																		3	1	48		14	4												70
Trichoptera	Hydropsyche sp.	CN	F	4			5	47	50	23	29	17	11	74	97	41	1	14		2			2		8	9	11	53	63	91	29	105	79	151					1012
Trichoptera	Hydroptila sp.	CN	SC	6				8	9	1	1											1			16				1			2							39
Trichoptera	Lepidostoma spp.	SP/CB	SH	1		1	3		7		6	8			67	6		16												13	4		2	6					141
Trichoptera	Micrasema sp.	CN	SH	1				8		9	65	1	18	14			3			28		2	5		4			76	3	3		36							273
Trichoptera	Neothremma sp.	CN	SC	0														4																						4
Trichoptera	Oecetis disjuncta	CN	P	8		2								2	3																3		7						17
Trichoptera	Onocosmoecus sp.	CB	SH	1		1																																		1
Trichoptera	Oligophlebodes sp.	CN	SC	1				11																								2								13
Trichoptera	Parapsyche sp.	CN	P	1				16			5						7					1			2			1												32
Trichoptera	Psychoglypha sp.	SP/CB	CG	1					3																															3
Trichoptera	Rhyacophila spp.	CN	P	0		7	3	4	5	5	9	17	16	3	5	9	16	23	83				11	13		7													236
Trichoptera	Wormaldia spp.	CN	F	3			3				1	8	15	2	5	6	7						3	9				1				3	1	2	11				77
Coleoptera	Ametor sp.	SW	P	5																		1																	1
Coleoptera	Brychius sp.	CB	SC	7		2			1	3															10														16
Coleoptera	Cleptelmis sp.	CN	CG/SC	4		3	26									4											5		1			1		6				46
Coleoptera	Dubiraphia sp.	CN	CG/SC	4		3																																	3
Coleoptera	Heterlimnius corpulentus	CN/BU	CG/SC	4														30	32			5																	67
Coleoptera	Optioservus quadrimaculatus	CN	CG/SC	4		97	267	43	109	68	40	205	153	78	162	167	7	2	5	12		5	21	33	18	132	151	27	153	74	246	69	83	129					2556
Coleoptera	Oreodytes sp.	SW/DV	P	5		6		1																															7
Coleoptera	Paracymus sp.	CN	P/OM	5								1																											1
Coleoptera	Zaitzevia parvula	CN/BU	CG/SC	4		170	57	5	4		1	1	7	23	5	18						3	3		2	2	7	2	6	16	11	8	2	13					366
Megaloptera	Sialis sp.	BU/CB	P	4		1			1	3		1																											6

Order	Genus	Habitat / Behavior	Functional Feeding Groups	Tolerance	Stream	SF Tincup Creek			Crow Creek							Deer Creek							Hoopes Spring							Sage Creek							Crow Creek									Total
					Location	SFTC1			CC75			CC150			CC350				DC600				HS				HS3				LSV2C			LSV4			CC1A				CC3A				
					Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Odonata	Ophiogomphus sp.	BU	P	1																																2		7		9
Hemiptera	Sigara sp.	SW	P	10		5																																		5
Diptera	Anopheles sp.	SW	F	8									1																												1
Diptera	Antocha sp.	BU	CG	3			5	1			4			6																			18		2		1			37
Diptera	Atherix sp.	BU	P	2																																26	22	24	3	44	119
Diptera	Chelifera sp.	SP/BU	CG	6				2			1						7			4													1				5			20
Diptera	Dixa sp.	BU	CG	1																	13																				13
Diptera	Empididae	SP/BU	P	6									1						5			2																			8
Diptera	Ephydridae	BU	CG	6							1								1			1																			3
Diptera	Glutops sp.	BU	P	3				1	2									1																							4
Diptera	Hexatoma	BU	P	2		19				9		1			5	4		1							9			4				5				16	1	74
Diptera	Limnophila sp.	BU	P	4							1			3			3					5								5	3			9				29
Diptera	Muscidae	BU	P	6																						1		3											4
Diptera	Pericoma sp.							2			1																													3
Diptera	Probezzia sp.	BU	P	6						3					2		1	1								2										2			11
Diptera	Ptychoptera sp.		CG	7																															1				1
Diptera	Simulium sp.	CN	F	6			18	78	5	30	26	49	17	17	5	102	9	15	8		5	4	13	21	38	24	25	24	12	114	35	8	1	26	31	760
Diptera	Tipula sp.	BU	SH	4					7	3				1					3					3			2			2		3	3			27
Chironomidae (family)	Chironomidae	BU/SP	CG/SH/P	6				188	195	173	143	99	143	68	10	30	33	88	151	92	124	25	23	83		20	43	91	149	36	56	35	41	21	8	2168
Hirudinea (class)	Helobdella sp.		PA/P	6								1																											1
Collembola	Collembola										2																													2
Oligochaeta (class)	Oligochaeta		CG	5			5	15	7	2	6	4	7	8	3	5	3	5	19	72	101	5	3			34		9	8	9			19	56		405
Bivalvia (class)	Pisidium sp.	BU	F	8		2		2	4		2						2	6		2	5	2	2			12					3	1	1	23		69
Gastropoda (class)	Fossaria sp.	CN	SC	8			2			1					2					52	57	27	4	4			8				15		1	1		174
Gastropoda (class)	Amnicola sp.	CN	SC	5										2	2	1	1													3		1				10
Gastropoda (class)	Gyraulus sp.	CN	SC																											1							1
Gastropoda (class)	Mentus sp.	CN	SC																	6																		6
Gastropoda (class)	Physella sp.	CN	SC	8		19		3	2	1					3	1				114	55	7	2	6		14	32		1	2			3	23		288

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek				Crow Creek						Deer Creek						Hoopes Spring				Sage Creek						Crow Creek				
					Location	SFTC1		CC75		CC150			CC350			DC600			HS			HS3			LSV2C		LSV4		CC1A			CC3A			Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Gastropoda (class)	Valvata sp.	CN	SC																		1														1
Amphipoda	Gammarus sp.	SW/BU	OM	6															2			2	4	13	8	1	12				2				44
Ostracoda	Ostracoda	SW	CG	8						1												460	2	9	30	13	8	1	1						525
Tricladida	Polycelis coronata		OM	1									4																						4
Acari (subclass)	Acari		P	8				2		2	3	4		2	6	7														2	2	5			35
					% Subsampled	50	50	12.5	12.5	66.6	12.5	25	50	25	12.5	50	100	33.3	75	50	33.3	100	12.5	33.3	100	25	25	75	12.5	25	25	50	25	25	50	13872
					Total abundance	394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
					Total taxa	24	19	27	25	22	26	24	16	23	24	21	23	23	16	15	13	14	21	22	14	23	21	17	21	20	18	22	30	20	15	
					Total Counts	788	972	4128	4048	741.7417	3720	1928	1068	1908	4288	984	420	1435.435	545.3333	996	1246.246	91	4768	1411.411	280	2164	2128	593.3333	3560	1948	1808	926	1860	2012	1000	
					Density (#/1m2)	2835	3496	14849	14561	2668	13381	6935	3842	6863	15424	3540	1511	5163	1962	3583	4483	327	17151	5077	1007	7784	7655	2134	12806	7007	6504	3331	6691	7237	3597	
						394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
							880			1516			1481			1505			1307			1004			1346			1518	445			1402			1468	13872

Functional Feeding Groups (FFG): CG = Collector-Gatherer, SC = Scraper, F = Filterer, P = Predator, SH = Shredder, OM = Omnivore, Habitat/Behavior (Hab/Beh): BU = Burrower, SW = Swimmer, CN = Clinger, CB = Climber, SP = Sprawler, DV = Diver

Table B-10. Summary of Formation 2012 invertebrate data.

Phylum	Subphylum	Class	Subclass	Infraclass	Superorder	Order	Lookup ID	Common name	SFTC1		CC75		CC150		CC350		DC600		HS		HS3		LSV2C		LSV4		CC1A		CC3A	
									Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion
Arthropoda		Insecta	Pterygota		Ephemeropteroidea	Ephemeroptera	Ephemeroptera	Mayflies	36	0.04	185	0.12	231	0.16	192	0.13	444	0.34	115	0.11	325	0.24	421	0.28	56	0.13	168	0.12	136	0.09
Arthropoda		Insecta	Pterygota		Exopterygota	Plecoptera	Plecoptera	Stoneflies	107	0.12	85	0.06	40	0.03	18	0.01	195	0.15	59	0.06	20	0.01	26	0.02	30	0.07	11	0.01	22	0.01
Arthropoda		Insecta			Amphiesmenoptera	Trichoptera	Trichoptera	Caddisflies	30	0.03	268	0.18	283	0.19	539	0.36	229	0.18	34	0.03	230	0.17	324	0.21	135	0.30	325	0.23	623	0.42
Arthropoda		Insecta	Pterygota	Neoptera	Endopterygota	Coleoptera	Coleoptera	Beetles	631	0.72	234	0.15	408	0.28	457	0.30	76	0.06	18	0.02	65	0.05	327	0.22	29	0.07	507	0.36	311	0.21
Arthropoda		Insecta		Neoptera		Megaloptera	Megaloptera	Alderflies, dobsonflies and fishflies	1	0.00	4	0.00	1	0.00																
Arthropoda		Insecta	Pterygota		Odonatoptera	Odonata	Odonata	Dragonflies and damselflies																			2	0.00	7	0.00
Arthropoda		Insecta		Neoptera	Paraneoptera	Hemiptera	Hemiptera	True bugs (cicadas, aphids, planthoppers, leafhoppers, shield bugs)	5	0.01																				
Arthropoda		Insecta			Panorpida	Diptera	Diptera	True flies	42	0.05	143	0.09	103	0.07	145	0.10	55	0.04	29	0.03	89	0.07	85	0.06	36	0.08	221	0.16	166	0.11
Arthropoda		Insecta				Chironomidae (family)	Chironomidae (family)	Midges			556	0.37	385	0.26	108	0.07	272	0.21	241	0.24	106	0.08	154	0.10	149	0.33	127	0.09	70	0.05
Annelida		Clitellata	Hirudinea				Hirudinea (class)	Leeches					1	0.00																
Arthropoda		Entognatha				Collembola	Collembola	Springtails (not insects!)					2	0.00																
Annelida		Clitellata	Oligochaeta				Oligochaeta (class)	Worms	5	0.01	24	0.02	17	0.01	16	0.01	27	0.02	178	0.18	3	0.00	43	0.03	8	0.02	9	0.01	75	0.05
Mollusca		Bivalvia					Bivalvia (class)	Clams	2	0.00	6	0.00	2	0.00			8	0.01	9	0.01	2	0.00	12	0.01			4	0.00	24	0.02
Mollusca		Gastropoda					Gastropoda (class)	Snails and slugs	21	0.02	7	0.00			11	0.01	1	0.00	319	0.32	16	0.01	54	0.04	1	0.00	21	0.01	29	0.02
Arthropoda	Crustacea	Malacostraca				Amphipoda	Amphipoda	Crustaceans											2	0.00	19	0.01	21	0.01			2	0.00		
Arthropoda	Crustacea	Ostracoda					Ostracoda	Sea shrimp					1	0.00							471	0.35	51	0.03	1	0.00	1	0.00		
Platyhelminthes		Turbellaria				Tricladida	Tricladida	Flatworms							4	0.00														
Arthropoda	Chelicerata	Arachnida	Acari				Acari (subclass)	Mites and ticks			4	0.00	7	0.00	15	0.01											4	0.00	5	0.00
							Total		880		1516		1481		1505		1307		1004		1346		1518		445		1402		1468	
								Midge		0.00		0.37		0.26		0.07		0.21		0.24		0.08		0.10		0.33		0.09		0.05
								Mayfly		0.04		0.12		0.16		0.13		0.34		0.11		0.24		0.28		0.13		0.12		0.09
								Other insects		0.93		0.48		0.56		0.77		0.42		0.14		0.30		0.50		0.52		0.76		0.77
								Molluscs		0.03		0.01		0.00		0.01		0.01		0.33		0.01		0.04		0.00		0.02		0.04
								Crustaceans		0.00		0.00		0.00		0.00		0.00		0.00		0.36		0.05		0.00		0.00		0.00
								Annelids		0.01		0.02		0.01		0.01		0.02		0.18		0.00		0.03		0.02		0.01		0.05
								Other		0.00		0.00		0.01		0.01		0.00		0.00		0.00		0.00		0.00		0.00		0.00

	Total		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00
Take the top 3 that are above 1%		Insects	0.93	Insects	0.50	Insects	0.58	Insects	0.79	Insects	0.44	Midge	0.32	Insects	0.33	Insects	0.57	Insects	0.53	Insects	0.78	Insects	0.85
		Molluscs	0.03	Midge	0.38	Midge	0.27	Midge	0.07	Midge	0.21	Molluscs	0.44	Crustaceans	0.40	Midge	0.12	Midge	0.34	Midge	0.09	Midge	0.05
		Mayfly	0.04	Mayfly	0.13	Mayfly	0.16	Mayfly	0.13	Mayfly	0.35	Worms and leeches	0.24	Mayfly	0.27	Mayfly	0.31	Mayfly	0.13	Mayfly	0.12	Mayfly	0.10
			1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00

APPENDIX C: SUMMARIES OF CHRONIC STUDIES CONSIDERED FOR CRITERIA DERIVATION

White sturgeon C-2
Sacramento splittail C-12
Fathead minnow C-15
Flannelmouth & razorback suckers C-22
Northern pike C-24
Chinook salmon C-27
Rainbow trout & brook trout C-32
Cutthroat trout C-51
Dolly Varden C-65
Brown trout C-68
Desert pupfish C-86
Eastern and western mosquitofish C-103
Striped bass C-105
Bluegill sunfish C-106
Largemouth bass C-147

See Appendix E for descriptions of other, less conclusive studies with:
Rainbow trout
Fathead minnow
Sacramento splittail
White sucker

See Appendix E for descriptions of invertebrate studies.

Tashjian, D.H., S.J. The, A. Sogomoyan and S.S.O. Hung. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon *(Acipenser transmontanus*). Aquatic Toxicol.79:401-409.

Test Organism: White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Seleno-L-methionine was added to an artificial diet consisting of vitamin-free casein, wheat gluten, egg albumin, dextrin, vitamin mix, BTM-mineral mix, cellulose, corn oil, cod liver oil, choline chloride and santoquin; the measured dietary concentrations were 0.4, 9.6, 20.5, 41.7, 89.8, 191.1 mg Se/kg dw.

Test Duration: 8 weeks

Study Design: 25 juvenile white sturgeon were placed in each of 24 90-L tanks. Treatments were randomly assigned to the 24 tanks resulting in 4 replicates per dietary treatment. Four fish from each tank were sampled after 0, 4 and 8 weeks for weight, length, liver weight, condition factors, hepatosomatic indices, hemocrit, histopathology, and selenium measurement in liver, kidney, muscle and gill tissues. 8 fish after 0 and 8 weeks were sampled for whole body selenium measurement.

Effects Data: Sturgeon survival did not differ significantly among treatment groups after the 8-week exposure with a mean survival rate of 99 across all groups. Fish fed 41.7 to 191.1 mg Se/kg dw exhibited significant declines in body weight (see table). All other endpoints measured were as sensitive or less sensitive to selenium in the diet as body weight.

Mean (SE) white sturgeon moisture, lipid and whole body Se after 8-week exposure

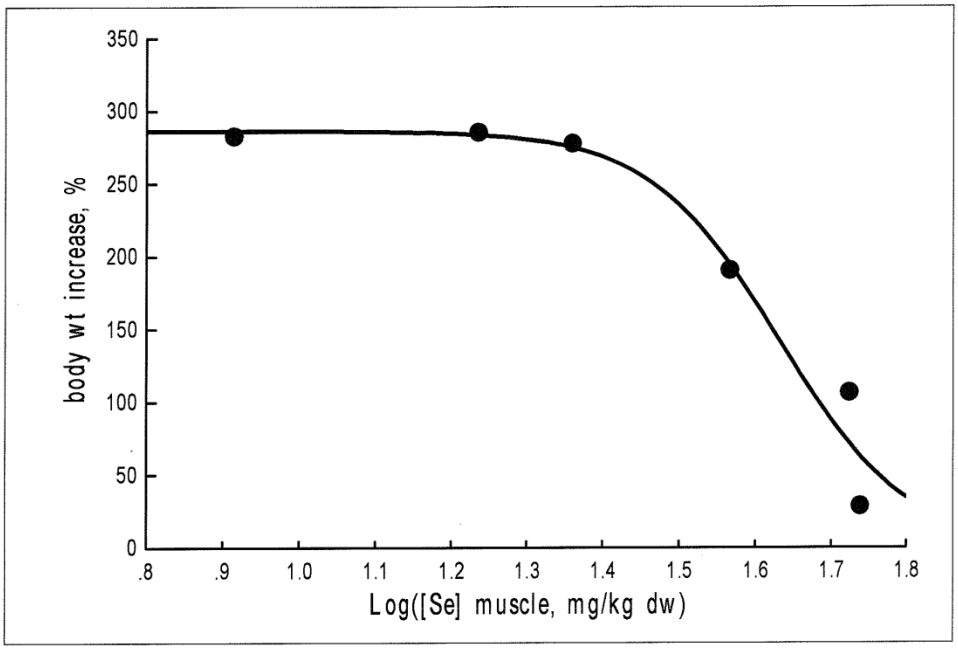
Treatment group	Moisture, % ww	Lipid, % ww	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	76.8 (0.5) b	9.5 (4) abc	8.2 (0.6) e	5.2 (0.4) c
9.6	77.0 (0.7) b	9.5 (0.9) abc	17.2 (0.7) d	11.8 (0.9) b
20.5	76.8 (0.3) b	10.1 (0.4) ab	22.9 (1.5) c	14.7 (0.8) b
41.7	77.3 (0.5) b	9.6 (0.7) abc	36.8 (1.8) b	22.5 (1.4) a
89.8	78.5 (0.3) ab	7.6 (0.4) bcd	52.9 (3.2) a	34.4 (2.3) a
191.1	80.0 (0.4) a	6.1 (0.4) cd	54.8 (2.8) a	27.5 (4.4) a

Mean (SE) white sturgeon body weight increase after 8-week exposure			
Treatment group	Body weight increase (%)	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	282.9 (4.6) a	8.2 (0.6) e	5.2 (0.4) c
9.6	285.5 (9.9) a	17.2 (0.7) d	11.8 (0.9) b
20.5	277.7 (6.1) a	22.9 (1.5) c	14.7 (0.8) b
41.7	191.0 (12.6) b	36.8 (1.8) b	22.5 (1.4) a
89.8	106.5 (5.8) c	52.9 (3.2) a	34.4 (2.3) a
191.1	28.6 (3.6) d	54.8 (2.8) a	27.5 (4.4) a

Letters denote statistical groupings among treatments within each exposure period ($p<0.05$).

Chronic Value: Using the logistic equation with a log transformation of the exposure concentrations (TRAP program), the EC_{10} and EC_{20} values for reduction in body weight are 15.08 and 17.82 mg Se/kg dw whole body and 27.76 and 32.53 mg Se/kg dw muscle tissue.

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

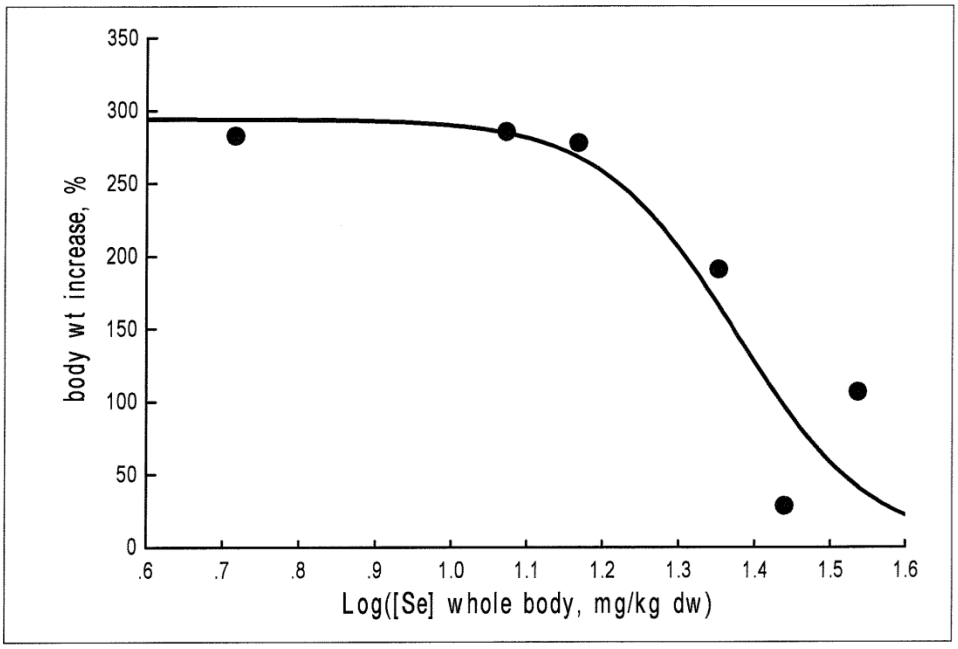
Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.6006	1.6303	0.0314	1.5304	1.7301
S	1.6574	2.938	0.925	-0.005	5.882
Y0	284.2	286.3	18.9	226.1	346.5

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	42.69	33.92	53.72
20.0	32.53	21.17	49.99
10.0	27.76	15.63	49.30
5.0	23.98	11.75	48.93

06/19/2009 10:32 MED Toxic Response Analysis Model, Version 1.03

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3403	1.3750	0.0643	1.1702	1.5797
S	2.283	2.794	1.908	-3.277	8.865
Y0	284.2	294.2	45.0	151.0	437.3

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.71	14.80	37.99
20.0	17.820	6.890	46.090
10.0	15.078	4.160	54.655
5.0	12.926	2.587	64.584

06/19/2009 10:42 MED Toxic Response Analysis Model, Version 1.03

Linville, R.G. 2006. Effects of Excess Selenium on the Health and Reproduction of White Sturgeon (*Acipenser transmontanus*): Implications for San Francisco Bay-Delta. Dissertation. University of California at Davis.

Test Organism: White Sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Selenium was added to the treatment in the form of selenized yeast. Selenized yeast (2.2%; Selenomax®, Ambi Inc.) was added to a commercial salmonid diet and pelleted with fish oil. For the control diet, the selenized yeast mixture contained 1.3% selenized yeast and 98.7 tortula yeast. Only selenized yeast was added to the treatment diet. After pelleting, the diet was allowed to air dry on drying racks.

Test Duration: Females were fed 0.3% body weight/day the experimental diet for 6 months.

Study Design: 16 adult female white sturgeon (approximately 5 years old, mean weight and fork length: 22.71 kg and 134.59 cm) were exposed in a freshwater flow through system to either the control diet (8 females in one tank fed 1.4 mg/kg Se) or treatment (8 females in a separate tank fed 34 mg/kg Se, Se from selenized yeast) for 6 months. After the 6 month dietary exposure, females were induced to spawn and fertilized with non-exposed male milt. Eggs were hatched in jars keeping eggs from each female separate. For each progeny cohort, 3000 larvae were randomly distributed into 3 reps for stage 40 (intestinal portion is void of yolk material, but stomach is not differentiated and is filled with yolk) sampling and 3 reps for stage 45 (yolk sac absorbed, start exogenous feeding) sampling. Se and biological measurements were made in each replicate.

Effects Data: No Se effects were observed for length or weight of larvae. Effects were determined for edema (Table 1), skeletal deformities (Table 2) and larval survival (Table 4). Because the mortalities for each cohort were recorded up to the time the sample was collected for abnormalities, a combined effects variable can be the total proportion of hatched larvae which were both alive and without any abnormalities at stage 45 (Table 4). This was calculated as PS·(1-PA), where PS is the proportion survival in the test chambers prior to sampling and PA is the proportion of the sample of surviving larvae with abnormalities. Binomial confidence limits are included in Table 4 for percent survival and percent abnormalities for each cohort to visualize significant differences among data points and between data points and fitted curves. Such confidence limits cannot be directly calculated for the combined effects variable, for which confidence limits were estimated by combining the lower and upper confidence limits of the individual effects variables using the same equation as above (this slightly overestimates the confidence limit range).

In Table 4, only cohort T2 is significantly different from the controls, based both on larval survival and abnormalities. That this selenium effect is also supported by the microinjection studies of Linville, which showed large abnormality frequencies for egg Se injected with >15 mg/kg, but little or no effect at lower concentrations (this is only supporting information because direct injection of a

specific form of Se is not a complete surrogate for setting effect concentrations for maternally transferred Se). For cohort T3, the data for abnormalities indicate some effects, but cannot be considered a definite effect concentration due to a combination of considerations – overlapping confidence limits with controls, no increase in mortality, limited information on within-cohort variability, and, based on egg concentrations, no effects for cohort T1 at a higher concentration.

EC_{10} Calculations: The combined effects variable is plotted versus Se concentration in the eggs in Figure 1. With only one definite partial effect, TRAP cannot be used to estimate a curve. Instead, the interpolation protocol is applied between the last two points based on specifying the highest no-effect concentration (HNOEC), 11.0 mg/kg, to be the EC_0 in the interpolation equation and specifying the upper control plateau (Y_0 in TRAP) to be average survival of the lower four points. The resultant TRAP slope is 3.0 and the interpolated EC_{10} is 15.6 mg/kg.

The egg EC_{10} of 15.6 mg/kg is slightly lower than the value of 16.3 mg/kg in the previous draft (Figure 3). The lower value was due to the inclusion of larval survival with abnormalities in the endpoint and using interpolation between the last two points rather than a TRAP model of the dataset.

Linville (2006) similarly calculated a 10% effective dose (ED_{10}) of the combined skeletal and edema data of 15.3 mg Se egg/kg dw using a logit regression. Linville (2006) also noted statistically significant differences using a Tukey Honest Significant Difference (HSD) test between Se and control treatments with respect to both the incidence of Stage 45 skeletal and total deformities, respectively, for the maternal transfer study. These author-reported results support the evidence of an effect of selenium in white sturgeon similar to the EC_{10} of 15.6 mg Se/kg egg dw interpolated by TRAP.

The combined effects variable is plotted versus Se concentration in muscle in Figure 2. Unlike for the egg concentration, the muscle concentration for cohort T3, with a small but not significant effect, is greater than that for cohort T1, with no effect, so that TRAP can be used to estimate a curve, although only barely so. This analysis was by tolerance distribution analysis with the log-triangular model. The resultant TRAP estimates are 100% for the control value and 8.8 for the EC_0 (about 11% below the T1 concentration); the standard deviation is 0.14 log units, equivalent to a slope of 3.7. The EC_{10} estimate is 11.9 mg/kg.

Chronic Value: The chronic value for combined deformities and larval survival using egg Se is an EC_{10} of 15.6 mg egg/kg dw. The chronic value for this same endpoint in muscle tissue is an EC_{10} of 11.9 mg muscle/kg dw.

Table 1. Edema deformities.

	Control			Treatment		
	Cohort	**Edema (%)**	**Larval Se (mg/kg dw)**	**Cohort**	**Edema (%)**	**Larval Se (mg/kg dw)**
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	6.67 (1)	7.75
Stage 40	C4	0.00 (3)	1.8	T1	0.00 (3)	11.6
	C5	0.00 (3)	2.88	T2	4.44 ± 2.22 (3)	20.4
				T3	1.67 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	15.56 ± 1.11 (3)	19.4
				T3	0.00 (2)	7.61

Table 2. Skeletal deformities.

	Control			Treatment		
	Cohort	**Skeletal (%)**	**Larval Se (mg/kg dw)**	**Cohort**	**Skeletal (%)**	**Larval Se (mg/kg dw)**
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	10.00 (1)	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.8	T1	0.00 (3)	11.6
	C5	1.11 ± 1.11 (3)	2.88	T2	14.44 ± 1.11 (3)	20.4
				T3	8.33 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	21.11 ± 1.11 (3)	19.4
				T3	13.33 ± 3.33 (2)	7.61

Table 3. Combined edema and skeletal deformities.

Control					Treatment			
	Cohort	Affected (%)	Egg Se (mg/kg)	Larval Se (mg/kg)	Cohort	Abnormal (%)	Egg Se (mg/kg)	Larval Se (mg/kg)
Stage 36	C3	0.00 (1)	2.46	2.43	T1	0.00 (1)	11	11.6
	C4	0.00 (1)	1.61	1.69	T2	0.00 (1)	20.5	18.4
	C5	0.00 (1)	2.68	2.67	T3	16.67 (1)	7.61	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.61	1.8	T1	0.00 (3)	11	11.6
	C5	1.11 ± 1.11 (3)	2.68	2.88	T2	18.89 ± 1.11 (3)	20.5	20.4
					T3	10.00 ± 0 (2)	7.61	7.22
Stage 45	C4	0.00 (3)	1.61	1.96	T1	0.00 (3)	11	12
	C5	0.00 (3)	2.68	2.59	T2	27.78 ± 2.94 (3)	20.5	19.4
					T3	13.33 ± 3.33 (2)	7.61	7.61

Table 4. Stage 45 data combined abnormalities and percent larval survival.

Cohort	Egg Se (mg/kg)	Muscle Se (mg/kg)	% Survival (95% Binomial CL)	% Abnormal (95% Binomial CL) [# Abnormal][1]	% Alive & w/o Abnormalities (95% Binomial CL)
C4	1.61	1.22	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
C5	2.68	1.48	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T3	7.61	11.1	>99.6 (98.7-99.8)	13.3 (3.7-24.6) [3,5]	86.4 (74.4-96.3)
T1	11	9.93	>99.6 (98.7-99.8)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T2	20.5	15.3	91.6 (90.1-92.8)	27.8 (18.8-38.3) [7,8,10]	66.2 (55.6-75.4)

[1] Bracketed numbers denote abnormal larvae in each of the 2-3 replicates of n=30.

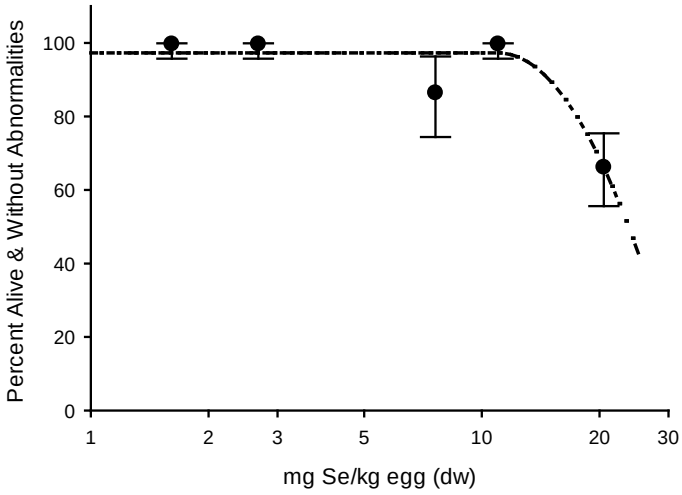


Figure 1. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in eggs. TRAP is used to interpolate between the last two points; EC10 = 15.6 mg Se/kg egg dw.

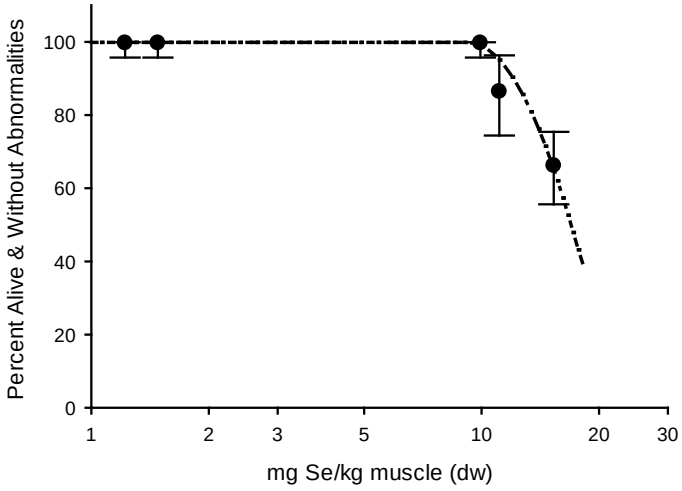
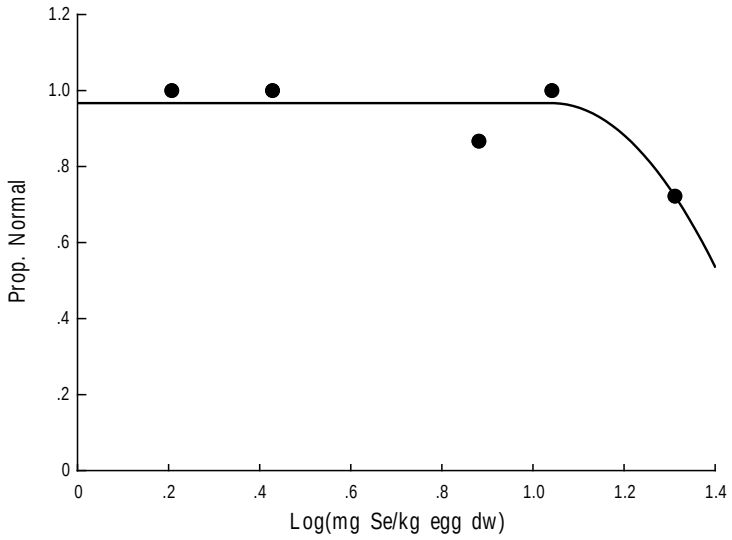


Figure 2. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in female muscle. TRAP tolerance distribution analysis with the log-triangular model; EC10 = 11.9 mg Se/kg muscle dw.



Parameter	Initial	Final
LogX50	1.416	1.4215
S	**2**	**2.6306**
Y0	0.96667	0.96667

Figure 3. TRAP analysis from previous draft. Initial estimate for slope set equal to or less than 2.645 (set to 2 for this figure). EC_{10} = 16.3 mg/kg.

Teh, S.J., X. Deng, D-F Deng, F-C Teh, S.S.O. Hung, T.W. Fan, J. Liu, R.M. Higasi. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (*Pogonichthys macrolepidotus*). Environ. Sci. Technol. 38: 6085-6593.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*); juveniles 7-mos.old

Exposure Route: Dietary only

Dietary Treatments: 8 graded levels of dietary Se; dietary levels obtained by combining selenized yeast with Torula (non-active) yeast. Selenized yeast contained approximately 21% of Se as selenomethionine and proteinaceous Se forms. Diet was formulated as pellets by mixing dry ingredients with water and oil, fan-dried, crumbled and sieved. Analyzed levels: 0.4 (no selenized yeast), 0.7, 1.4, 2.7, 6.6, 12.6, and 57.6 mg/kg.

Fish were fed twice daily with a daily feeding rate of 3% BW in first 5 months and then adjusted to 2% BW thereafter.

Test Duration: 9 months

Study Design: A flow-through system with 40 fish/tank (24 total tanks) was used; each tank held 90 L. Flow rate was 4 L/min. Water temperature was maintained at 23°C for 6 months and then 18°C for last 3 months due to failure of water heating system. 5 fish were sampled from each tank at 5 and 9 months and measured for gross deformities, length, weight, Se in liver and muscle. Sections of the liver were kept for histopathology. Condition factor (100 x BW/length), heptatosomatic index (100 x liver weight/BW), BCF (total organ Se/dietary Se) were determined.

Effects Data: Mortality was observed in the two highest dietary treatments: 10 and 34.3%, respectively. No mortalities were observed in fish fed diets # 12.6 mg/kg. No significant difference in growth of fish fed 12.6 mg/kg Se in diet, but there was in the fish fed 26.6 mg/kg Se. See table below for levels of Se in fish at 9 months and associated effects.

Authors determined prevalence of deformities was higher in fish fed 6.6 and 12.6 mg/kg Se in their diet, however a dose-response relationship did not occur (e.g., no deformities in high concentration). Gross pathology was a more sensitive endpoint than growth.

Summary of effects and assoc. dietary and tissue concentrations in Sacramento splittail after 9 month exp.								
Dietary conc'n mg/kg	0.4	0.7	1.4	2.7	6.6	12.6	26.0	57.6
Se in liver, mg/kg dw	20.1	18.6	20.0	23.0	26.8	31.3	40.4	73.7
Se in muscle, mg/kg dw	6.6	6.9	9.2	10.1	15.1	18.9	29.4	38.7
Liver histopathology (mean lesions scores, N=15)								
Macrophage aggregate	0.13	0.07	0.2	0.27	0.40	0.20	0.20	0.85
Glycogen depletion	0	0	0.2	0	0.4	0.2	0	1.38
Single cell necrosis	0	0	0	0.07	0.13	0	0.07	0.46
Fatty vacuolar degeneration	0	0	0	0.2	0.53	0.07	0.2	0.08
Eosinophilic protein droplets	0	0	0	0	0	0	0.07	0.85
Sum of mean lesion scores	0.13	0.07	0.4	0.54	1.46	0.47	0.54	3.62
Gross Pathology (No. of deformities, N=15)								
Facial deformities (eye, jaw, and mouth)	0	1	0	1	5	3	0	0
Body deformities (kyphosis, lordosis, scoliosis)	0	0	4	2	3	1	1	0
Prevalence of deformity (%)	0	6.7	26.7	20	53.3	26.7	6.7	0

Chronic Value: Using gross pathology as the endpoint (prevalence of deformities, %), the NOAEC is 10.1 mg Se/kg dw and the LOAEC is 15.1 mg/kg Se dw in muscle tissue; MATC or CV = 12.34 mg/kg Se in muscle dw.

The above concentrations in juvenile muscle tissue cannot be exactly translated into an equivalent egg-ovary or whole-body concentration in adult splittail. But using the median egg-ovary to muscle ratio of 1.59 for the family Cyprinidae, the NOEC and MATC would represent 16.1 and 19.6 mg Se/kg egg-ovary. Using the median muscle to whole-body ratio of 1.26 for the family Cyprinidae, the NOEC and MATC would represent 8.04 and 9.83 mg Se/kg whole body. However, appropriateness of these conversion estimates rests upon uncertain assumptions that the muscle concentrations in juvenile splittails equal those of adult splittails under the same exposure conditions, and that splittail tissue ratios are those typical of the family Cyprinidae.

Comments: The authors observed deformities including spinal deformities using fish that were 7-months-old at test initiation. This is the only study in which deformities were observed in fish that were not exposed maternally.

Deng et al. (2008) exposed Sacramento splittail juveniles (21-day post hatch) to dietary selenium and dietary methylmercury in a two factorial design for four weeks. No adverse effects (growth, condition factor, lethargy or abnormalities) were observed in the selenium only exposures. The splittail accumulated approximately 3.5 mg Se/kg ww muscle in the highest dietary exposure (35 mg

Se/kg. Using the average percent moisture in fish muscle of 78.4% (May et al. 2000), the dw Se concentration is 16.2 mg Se/kg muscle indicating the recommended CV does not over-estimate an effect concentration.

Rigby et al. (2010) re-analyzed the juvenile Sacramento splittail data generated in the Teh et al. (2004) study. The authors used logistic regression to estimate EC values for deformities on a culled data set which eliminated the three highest dietary treatments due to their departure from a standard concentration-response relationship. The EC_{10} value for the culled data set was 7.9 mg Se/kg dw muscle which is lower than the recommended CV of 12.3 mg Se/kg dw muscle. Due to the lack of a concentration-response relationship across the entire dietary range and the lack of effects in the Deng et al. (2008) study, an EC_{10} of 7.9 mg Se/kg dw muscle is too uncertain for a recommended CV. Although the recommended CV of 12.3 mg Se/kg dw muscle is based on deformities (an uncertain response), it is considered representative of an effect level for this species because of the significant reductions in growth at the two highest test concentrations.

Bennett, William N., Arthur S. Brooks, and Martin E. Boraas. 1986. Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. Arch. Environ. Contam. Toxicol. 15:513-517.

Test Organism: Fathead minnow (*Pimephales promelas*; 2 to 8 day-old larvae).

Exposure Route: Dietary only
Green alga, *Chlorella pyrenoidosa* were exposed to Se ($H_2{}^{75}SeO_4$) in culture water for 3 days. Rotifers, *Brachionus calyciflorus*, were cultured in chambers with selenium containing green algae at the ratio of 25 μg algae/ml to 50 μg rotifer/ml for 5 hr. The rotifers were filtered to separate them from the algae and immediately heat-killed. The Se concentration in the rotifers was measured for ^{75}Se activity.

Test Duration: 9 to 30 days

Study Design: Selenium uptake by larval fathead minnows was measured in three experiments. Se-contaminated and control rotifers for feeding to larval fish were prepared in advance using the low algae:rotifer ratio. Daily equal volumes of rotifers were divided among five 800 mL polypropylene larval chambers. Three chambers received Se-contaminated rotifers and two received control rotifers. The rotifers were dead at the time of feeding (heat killed).

Larval fish were hatched from eggs spawned in the laboratory. After hatching, active larvae were divided equally among the larval test chambers (daily renewal exposures using dechlorinated Lake Michigan water). Larvae were initially fed rotifers raised on control algae (no selenium). The age of the larvae when first fed Se-contaminated rotifers was 4, 9, and 3 days post-hatch for experiments 1, 2, and 3, respectively. Larval fish were fed Se-contaminated rotifers for 7, 9, and 7 days in the 3 experiments. A post-exposure observation period of 19 and 2 days was used for experiments 1 and 2, respectively. During this time the larvae were fed control rotifers. Daily, larvae from a replicate were removed from the test chamber, washed, placed in a 20 ml vial, and counted for ^{75}Se activity for 20 min. All larvae were then placed in test chambers with fresh food rations. At the end of the study all fish were individually dried and weighed.

	Experiment 1	Experiment 2	Experiment 3
Initial feeding of control diet (days)	3	8	2
Day Se diet first fed	4	9	3
Day Se diet last fed	11	17	9
Observation days on control diet	19	2	0
Age at study termination (days)	30	19	9

Effects Data:

	Experiment 1	Experiment 2	Experiment 3
Mean food Se concentration (mg/kg)	>70	68	55
Food intake (μg rotifers/larva)	50	1330	1190
Initial larvae mean dry wt. at start of Se-laden food (μg)	90	400	100
Final larvae mean dry wt. (μg) at end of test	1470 (Control) 800 (Treatment)[a]	1888 (Control) 1354 (Treatment)[a]	475 (Control) 416 (Treatment)
Final mean larval Se content (μg Se/larva)[b]	0.0062	0.0700	0.0248
Final mean larval Se concentrations (mg Se/kg dw)	43.0	51.7	61.1

[a] Significantly different from the control.
[b] Values when Se-laden feeding was ended.

Selenium was measured in the test water during the feeding exposures, but the concentrations were insignificant (0.84 μg/L). Survival was not affected by the selenium exposures. Preliminary tests showed that fathead minnow larvae would reach plateau concentrations of selenium within the 7- to 9-day exposure periods. The food supply was sufficient to sustain growth of the larvae during the study, according to the authors. The authors state that selenium uptake and higher selenium content in experiment 2 larvae was due to their larger size and ability to consume more rotifers/unit time. Se-exposed larvae were significantly smaller ($p<0.05$) in mass than controls for experiments 1 and 2.

Chronic Value: GM of mean larval Se concentrations measured in the three experiments, i.e., 43.0, 51.7, and 61.1 mg/kg dw WB, respectively, is 51.40 mg Se/kg dw.

Dobbs, M.G., D.S. Cherry, and J. Cairns, Jr. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. Environ. Toxicol. Chem. 15:340-347.

Test Organism: Rotifer (*Brachionus calyciflorus*), and fathead minnow (*Pimephales promelas*) 12 to 24 hr-old at start.

Exposure Route: Dietary and waterborne

Water
Filtered and sterilized natural creek water supplemented with nutrients (Modified Guillard's Woods Hole Marine Biological Laboratory algal culture medium) for algal growth. Sodium selenate (Na_2SeO_4) was added to test water to obtain nominal concentrations of 100, 200, or 400 µg Se/L. Concentrations remained stable and equal in each trophic level.

Control Diet
No selenium was added to the water medium for the alga; green alga was free of selenium for the rotifer; and rotifers were free of selenium for the fathead minnow.

Selenium Diet
Sodium selenate was added to the culture medium for the alga; green alga thereby contained a body burden for the rotifer; and rotifers thereby contained a body burden for the fathead minnow.

Dietary Treatments: Each trophic level had a different treatment. The green alga was exposed directly from the water (1, 108.1, 204.9, 397.6 µg total Se/L); rotifers were exposed from the water (1, 108.1, 204.9, 393.0 µg total Se/L) and the green alga as food (2.5, 33, 40, 50 mg Se/kg dry wt.); and the fathead minnow were exposed from water (1, 108.1, 204.9, 393.0 µg total Se/L) and the rotifer as food (2.5, 47, 53, 60 mg Se/kg dry wt.).

Test Duration: 25 days

Study Design: A flow-through system utilizing a stock solution of filtered and sterilized creek water controlled at 25°C was used to expose three trophic levels of organisms. Approximately one liter of media was pumped from the algal chamber into the rotifer chamber each day. A cell density between 3 and 6×10^6 cells/ml was delivered to the rotifer chambers. Rotifers were started at a density of 151.4 ± 7.7 females/ml and one liter/day of rotifers containing culture water was intermittently pumped into the minnow chamber. (*B. calyciflorus* has a life span of about 7 days at 25°C.) The pump was necessary to overcome the swimming ability of rotifers to avoid an overflow tube. Larval fathead minnows (35/chamber) were prevented from escaping by a screened overflow. Chambers were cleaned daily and aeration was provided. All chambers were duplicated for test replication and water was measured for selenium on days 0, 2, 6, 7, 11, 14, 17, 20, and 24. All algal and rotifer biomass and selenium samples were made on these days. Fathead minnow chambers were measured for biomass, dissolved selenium, and tissue selenium concentrations of days 0, 7, 11, 14, 20, and 24.

Additional measurements were made in the 200 µg Se/L test chambers on the fathead minnow on day 16. Selenium concentrations were maintained near the nominal concentrations and the standard deviation of mean concentrations was less than 4 percent.

Effects Data:

Rotifers. Rotifers did not grow well and demonstrated reduced survival at all selenium exposure concentrations during the 25 day test. By test day 7 only the lowest test concentration (108.1 µg/L) had surviving rotifers which showed a decrease in selenium content from test days 18 through 25. A reduction in rotifer biomass was discernable by test day 4 in the selenium treatments and since all test concentrations had viable rotifer populations present, the effect level was calculated using these data.

Effect of Dietary and Waterborne Selenium on Rotifers after 4 Days Exposure			
Se in water, µg/L	**Se in diet, mg/kg dw**	**Se in rotifer tissue, mg/kg dw**	**rotifer biomass, mg/ml dw**
1	2.5	2.5	0.028
108.1	33	40	0.025
202.4	40	54	0.011
393	50	75	0.003

Fathead minnows. Due to the reduction of rotifer biomass in the higher test concentrations, fish mortality and reduction in fish growth observed in the latter days of the test was difficult to discern between effects from starvation and selenium toxicity. The data from test day 8 was selected for determining the effect of selenium on fathead minnows because starvation could be excluded as a variable.

Effect of Dietary and Waterborne Selenium on Larval Fathead Minnows after 8 Days Exposure			
Se in water, µg/L	**Se in diet, mg/kg dw**	**Se in fathead minnow tissue, mg/kg dw**	**Average fish weight, mg dw**
1	2.5	2.5	0.8
108.1	47	45	0.7
202.4	53	75	0.4
393	60	73	0.2

Chronic Value:

Rotifers	42.36 mg Se/kg dw (EC_{20})
Fish	< 73 mg Se/kg dw (LOAEC) - not amenable to statistical treatment; the LOAEC was based on the observation that a >50 percent reduction in mean fish weight occurred at this tissue concentration.

Schultz, R. and R. Hermanutz. 1990. Transfer of toxic concentrations of selenium from parent to progeny in the fathead minnow (*Pimephales promelas*). Bull. Environ. Contam. Toxicol. 45:568-573.

Test Organism: Fathead minnow (*Pimephales promelas*; Adults)

Exposure Route: Dietary and waterborne
Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Study Design: Four Monticello artificial streams were used for the study which lasted from September 1987 to September 1988. For each study, two streams (treated) were dosed continuously to achieve 10 µg/L and two streams served as controls. Mean selenium concentrations at the head of the treated streams were 9.8 ± 1.2 and 10.3 ± 1.7 µg/L, respectively. The concentrations of selenium measured in the water from controls streams were all less than the detection limit, i.e., 2 µg/L. Spawning platforms were submerged into each stream. One subset of six embryo samples (n = 2000 embryos per sample) were collected from the streams for selenium analysis. Another subset of ten embryo samples were reared in incubation cups receiving the same stream water dosed with sodium selenite via a proportional diluter. The treated embryos in egg cups received an average 9.7 ± 2.6 µg Se/L. Samples of hatched larvae were analyzed for selenium content while others were inspected for occurrence of edema and lordosis. Prior to test termination, female parents were seined. The mean selenium content in the ovaries of seven to eight females from the treated and control streams was reported.

Effects Data: Edema and lordosis occurred in approximately 25 percent of the fish spawned and reared in 10 µg Se/L. Corresponding occurrence in control fish incubated in the egg cups was only 1 and 6 percent, respectively. Table 1 provides the abnormality observations and the selenium residues in the embryos and ovaries from the control and treated streams. Although a case can be made that the Se treatment had a higher rate of edema and lordosis, there are some problems that add uncertainty to the estimation of an effect concentration (R. Erickson, pers. comm.). Heavy mortality/loss of embryo/larvae during monitoring and the erratic occurrence of the abnormalities (e.g., there is a significant incidence of edema in only 3 of 10 replicates for the Se treatment) led to the conclusion that results should not be used for criterion derivation. However, the data from this study support the range of reproductive effect levels determined in other studies. The Se concentration in embryos from the 10 µg/L treatment stream of 3.91 mg/kg ww converts to 25.6 mg/kg dw using 15.3% dw (N=3 range 14.7 – 15.6%) for fathead minnow eggs (R. Erickson, pers. comm). The previous draft used the Se concentrations in the ovaries collected at the end of the study for the effect concentration estimate. However, it was determined that the embryos are a more direct representation of Se exposure and toxicity to the larvae.

Chronic Value: The LOEC for embryos is <25.6 mg Se/kg dw.

Table 1. Percent Abnormalities in Fathead Minnow Larvae and the Associated Selenium Concentrations in Embryos and Ovaries.

Treatment	[Se] embryos, mg/kg ww (SD)	[Se] ovaries, mg/kg ww (SD)	Edema, % (SD)	Lordosis, % (SD)
Control	0.31 (0.01)	0.77 (0.14)	0.9 (2.2)	5.6 (8.8)
10 µg/L	3.91 (1.87)	5.89 (2.21)	24.6 (36.1)	23.4 (20.8)

SD = standard deviation

Beyers, D.W. and Sodergren, C. 2001a. Evaluation of interspecific sensitivity to selenium exposure: Larval razorback sucker versus flannelmouth sucker. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval flannelmouth sucker (*Catostomus latipinnis*) and larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage) Continuous flow diluter supplied a range of aqueous test concentrations <1, 25.4, 50.6, 98.9, and 190.6 µg/L selenate. Well water was used as the dilution water. Across the range of aqueous exposure concentrations, each test chamber was fed the same daily ration of living rotifers containing selenium at <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively. Rotifers accumulated selenium from algae (*Chlorella vulgaris*) exposed to 0, 25, 50, 100, and 200 :g/L selenate.

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1^{st} factor - selenium; 2^{nd} factor - species). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data : No survival effects were observed and there were no decreases in fish weight or length. Fish mass was found to increase as a function of selenium concentration.

Chronic Value: The chronic values for the flannelmouth sucker and razorback sucker were >10.2 and >12.9 mg Se/kg dw, respectively, based on the concentrations of selenium measured in whole-body tissue of larval fish at the highest water and dietary selenium concentrations.

Beyers, D.W. and Sodergren, C. 2001b. Assessment of exposure of larval razorback sucker to selenium in natural waters and evaluation of laboratory-based predictions. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage)
Larvae were exposed in a daily static-renewal system to control water (reconstituted very hard) and site waters: De Beque, Orchard Mesa, North Pond diluted 50%, and North Pond. Each water type received either a control diet (rotifers) or a diet previously exposed to the site water (site food: rotifers fed algae exposed to respective site water).

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1^{st} factor - test water type; 2^{nd} factor - rotifers cultured in control water or in site water). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data: No survival effects were observed. There were no significant decreases in growth of fish exposed to both site water and site food compared to fish exposed to control water and control food. There was a significant increase in growth of fish exposed to site water and control food relative to fish exposed to control water and control food ($p<0.0001$). There were reductions in the growth of fish (14%) exposed to site water and site food compared to site water and control food ($p<0.0001$). Due to the lack of a dose-response relationship in both the concentration of selenium in the food (rotifers) and growth, and the concentration of selenium in the fish larvae and growth, the authors did not attribute the effect of site food on the growth of fish to selenium.

Chronic Value: The NOAEC for the razorback sucker larvae in the four site water types based on selenium in whole-body tissue were: De Beque >5.45 mg Se/kg dw; Orchard Mesa >11 mg Se/kg dw; North Pond 50% dilution >41.1 mg Se/kg dw; North Pond >42 mg Se/kg dw. Because no significant effects were observed in larvae exposed to North Pond water at >42 mg Se/kg dw whole-body tissue, this value was selected as the chronic value for the study.

Muscatello, J.R., P.M. Bennett, K.T. Himbeault, A.M. Belknap and D.M. Janz. 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environ. Sci. Technol. 40:6506-6512.

Test Organism: Northern pike (*Esox lucius*)

Exposure Route: Dietary and waterborne - field exposure

Test Duration: Eggs were collected in the field and incubated in the laboratory. The test was terminated when the majority of the fry exhibited swim-up and had absorbed the yolk.

Study Design: The study area was Key Lake uranium milling operation in north-central Saskatoon. Spawning northern pike were collected from four sites, one reference (Davies Creek) and three exposure sites, David Creek near-field (high exposure), Delta Lake (medium exposure), and David Creek far-field (low exposure). The exposure sites were located approximately 2, 10 and 15 km downstream of the effluent discharge. Milt and ova were stripped from ripe fish and eggs were fertilized in the field. Females were saved for metal analysis and age determination. Subsamples of ova (prior to fertilization) were collected for metal analysis.

Although the study sites represent open systems where fish can potentially migrate among sites, radiotelemetry data from tagged adult pike (Muscatello and Janz, unpublished data) indicate high site fidelity at the “high” and “medium” exposure sites (lakes). In contrast, the “low” exposure site likely represents pike that migrated from further downstream sites that were likely of similar Se exposures as the reference site.

Eggs were incubated using a two-way ANOVA experimental design using water collected from reference or exposure sites. So, embryos originating from reference or exposure site females were incubated in either reference or appropriate exposure water. In addition, embryos from reference site females were incubated in water from all four study sites. 50 viable embryos from each individual female were transferred to each of four replicate incubation chambers. Cumulative time to 50% eyed, 50% hatch and 50% swim-up were determined. When the majority of the fry exhibited swim-up and had absorbed the yolk, the remaining fry were preserved and examined for deformities.

Effects Data: Mean egg diameter and fertilization success did not differ among sites. Cumulative embryo mortality throughout incubations was not significantly different among the sites ranging from 45 to 60%. There were no significant differences in the cumulative time to reach 50% eyed embryos, 50% hatch or 50% swim-up among treatments. Differences in the percent total deformities between test waters used during embryo incubation exposures were not significant, so the data were combined for each site (see Table below).

Selenium concentrations in eggs and muscle from female northern pike collected from reference and exposed sites and associated total deformities in embryos					
Site	**Site ID**	**Female**	**[Se] mg/kg dw**		**Total deformities %**
			Egg	**Muscle**	
Davies Creek	Reference	1	3.45	0.86	17
Davies Creek	Reference	2	2.72	1.89	2.5
Davies Creek	Reference	3	3.39	2.56	15.51
Davies Creek	Reference	4	3.72	1.34	7.13
Davies Creek	Reference	5	2.69	1.04	10.41
David Creek (far field)	Low	1	3.39	1.95	20.32
David Creek (far field)	Low	2	4.07	2.04	13.19
David Creek (far field)	Low	3	4.07	1.26	15.33
David Creek (far field)	Low	4	4.07	2.48	18.83
David Creek (far field)	Low	5	3.4	1.26	11.8
Delta Lake	Medium	1	43.19	17	37.8
Delta Lake	Medium	2	24.53	16.52	31.71
Delta Lake	Medium	3	26.14	16.52	26.29
David Creek (near field)	High	1	48.23	47.82	39.5
David Creek (near field)	High	2	N/A*	28.72	N/A*

*female had no eggs

Significant increases in total deformities (edema, skeletal deformities, craniofacial deformities and fin deformities) were observed in fry originating from pike collected at the medium exposure site. Determination of an effect level for the percent total deformities relative to the concentration of selenium in eggs or in female muscle tissue was not amenable to analysis by TRAP. One requirement of TRAP is to have a response greater than 50%, which was not satisfied with the available data.

When data are not amenable to determining an effect level using a software program, such as TRAP, one way to estimate the effect level is to make a direct measurement of effect at an exposure or tissue concentration. For example, if only a control and one exposure concentration, 10 μg/L, were tested in an acute toxicity test and there was 100% survival in the control and 35% in the 10 μg/L, the effect level would be an EC_{35} of 10 **μg**/L. Such an approach was used to estimate effect in the Muscatello et al. data. Because no significant differences were observed in either selenium concentrations in eggs or percent total deformities between the reference and low exposure site, the data from these 10 sites were combined. Similarly, the egg **and muscle** selenium and total deformity data were combined for the 4 medium and high exposure sites. These means, geometric for the selenium concentrations and arithmetic for the percent total deformities, are given in the following table.

	Mean selenium in northern pike egg and muscle and effect values for reference and exposure sites			
Sites	**[Se] in eggs, mg/kg dw (geometric mean)**	**[Se] in muscle, mg/kg dw (geometric mean)**	**Total deformities, % (arithmetic mean)**	**Total deformities, % (accounting for reference deformities and transformed to new scale)[a]**
Reference sites (includes low exposure)	3.462	1.570	13.20	0
exposure sites	34.00	21.70	33.82	23.76

[a] The % total deformities in the reference and exposed sites were normalized to the reference effect (13.2%) and then transformed to a new scale (100%). i.e, Abbott's formula.

The percent affected becomes 24% or an EC_{24} and the effect level is 34.00 mg Se/kg dw in eggs and 21.70 mg Se/kg in muscle**.**

Chronic Value: EC_{24} = 34.00 mg Se/kg dw in eggs. Note: an EC_{10} cannot be estimated with the data.

Hamilton, S.J., K.J. Buhl, N.L. Faerber, R.H. Wiedermeyer and F.A. Bullard. 1990. Toxicity of organic selenium in the diet of chinook salmon. Environ. Toxicol. Chem. 9:347-358.

Test Organism: Chinook salmon (*Oncorhynchus tshawytscha* Walbaum; swim-up larvae)

Exposure Route: Dietary only

Control Diet

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish (1.0 mg Se/kg dw) collected from a reference site.

Selenium Diet #1

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from high-selenium mosquitofish (35.4 mg Se/kg dw) collected from the San Luis Drain, CA, termed SLD diet.

Selenium Diet #2

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish same as in the control diet, but fortified with seleno-DL-methionine (35.5 mg Se/kg dw), termed SeMet diet.

Dietary Treatments: Each selenium diet was formulated to contain about 36 mg Se/kg dw as the high exposure treatment. The remaining treatments were achieved by thoroughly mixing appropriate amounts of high-exposure treatment diet with control diet to yield the following nominal concentrations (3, 5, 10, and 18 mg Se/kg dw).

Test Duration: 90 days

Study Design: Each dietary treatment was fed twice each day to swim-up larvae (n=100) in each of two replicate aquaria that received 1 L of replacement water (a reconstituted experimental water that simulated in quality a 1:37 dilution of water from the San Luis Drain, CA minus the trace elements) every 15 minutes (flow-through design). Mortality was recorded daily. Growth was evaluated at 30-day intervals by measuring the total lengths and wet weights of two subsets of individual fish (n=10x2) held in separate 11.5 L growth chambers within each replicate aquarium. Tissue samples were collected for whole-body selenium determinations (dw basis) at 30-day intervals throughout the study; 10, 5, and 2 fish were sampled from each duplicate treatment after 30, 60, and 90 days of exposure, respectively. Concentrations of selenium measured in water were below the limit of detection (1.5-3.1 μg/L) in all dietary selenium exposure concentrations.

Effects Data: The magnitude of reduced growth was most evident in the weight of the fish, although total length was significantly reduced in fish fed high Se-laden diets as well. The effect of increasing dietary selenium on mean larval weight was similar in both the SLD and seleno-methionine diets.

Effect of San Luis Drain Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	3.3	2.68	97.3
5.3	4.5	2.76	93
9.6	8.4	2.8	95
18.2	13.3	2.62	92.4
35.4	29.4	1.4	89

Effect of Seleno-methionine Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	2	3.08	100
5.3	3.1	3.22	95
9.6	5.3	3.07	94.1
18.2	10.4	2.61	92.4
35.4	23.4	1.25	62.5

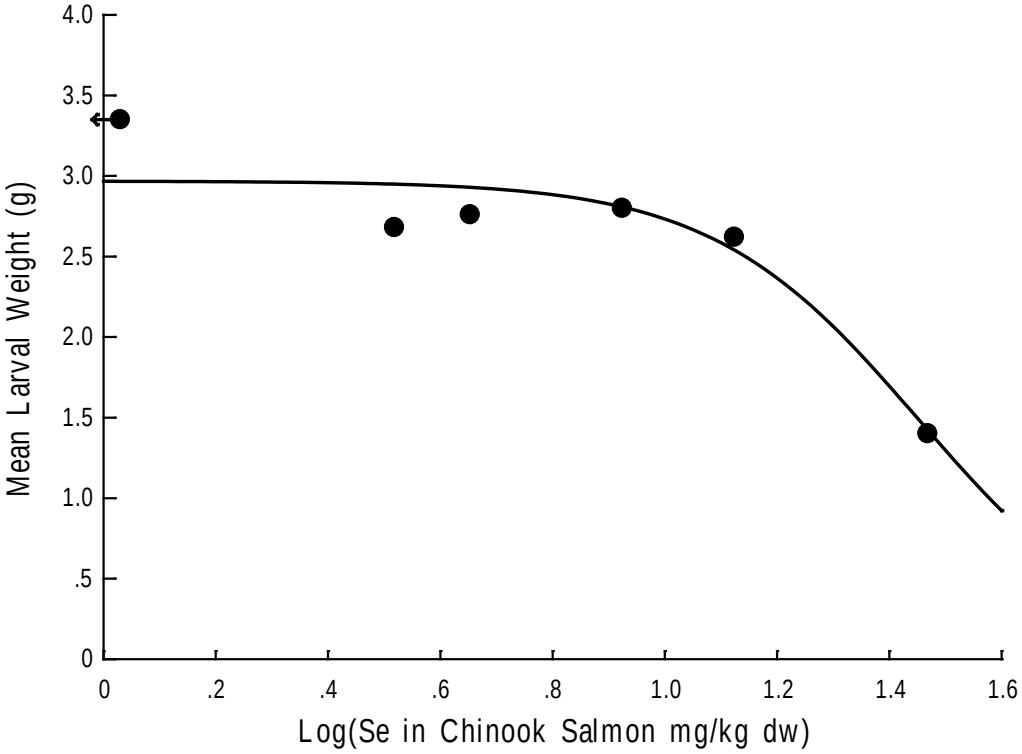
Chronic Value: Due to unacceptable control mortality of swim-up larvae in control treatments after 90 days (33.3 percent - SLD diet; 27.5 percent - SeMet diet), chronic values had to be determined from respective values reported after 60 days (tables above).

Analysis of the elemental composition of the SLD diet indicated that B, Cr, Fe, Mg, Ni and Sr were slightly elevated compared to the control and SeMet diets. No additional analyses were performed to determine the presence of other possible contaminants, i.e., pesticides.

	EC_{20} values		**EC_{10} values**
	Survival (after 60 d of exposure)	**Growth (after 60 d of exposure)**	**Growth (after 60 d of exposure)**
Diet type	**Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**
SLD	NA[a]	15.73	11.14
SeMet	NA[a]	10.47	7.355

[a] The EC_{20} and EC_{10} values for survival of swim-up larvae versus levels of selenium for the SLD and SeMet dietary exposure could not be estimated using non-linear regression.

Hamilton et al (1990) Chinook Salmon fed SLD Diet
Logistic Equation, Three Parameter Model, Se concentrations log_{10} transformed

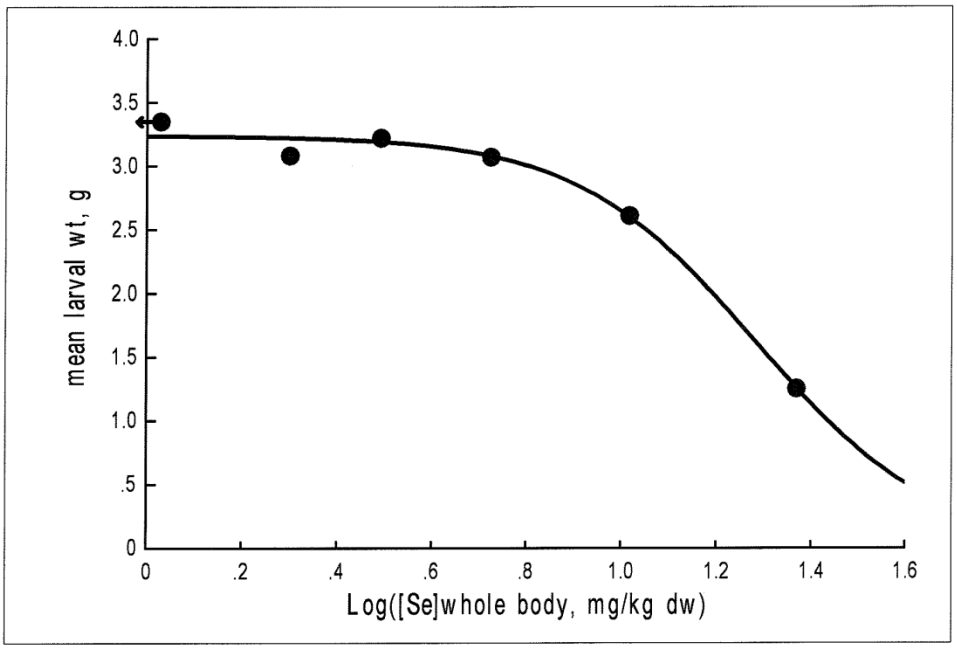


	Guess	FinalEst	SE	95%LCL	95%UCL
LogX50	1.453	1.453	7.30E-02	1.2206	1.6854
StDev	1.353	1.353	6.67E-01	-7.71E-01	3.4769
Y0	2.968	2.968	1.89E-01	2.3651	3.5709

%Effect	Xp Est	95% LCL	95% UCL
50	28.379	16.62	48.458
20	15.734	5.7003	43.431
10	11.143	2.4771	50.127
5	8.1085	1.1213	58.637

	DF	SS	MS	F	P
Total	5	2.0749	0.41498		
Model	2	1.8202	0.91009	10.719	0.95699
Error	3	0.2547	8.49E-02		

Chinook salmon SeMet diet (Hamilton et al. 1990)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3148	1.2823	0.0242	1.2053	1.3593
S	0.6971	1.3214	0.1826	0.7404	1.9025
Y0	3.217	3.239	0.067	3.027	3.452

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	19.156	16.045	22.870
20.0	10.472	7.516	14.591
10.0	7.355	4.595	11.775
5.0	5.312	2.899	9.733

06/19/2009 13:52 MED Toxic Response Analysis Model, Version 1.03

Hilton, J.W. and P.V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). J. Nutr. 113:1241-1248.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only

Low carbohydrate diet (LCD)
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler.

High carbohydrate diet (HCD)
This diet contained cerelose at 25 percent of the diet with cellulose as the filler.

For both diets, the selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The two diets were supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the six different dietary selenium treatments (n = 3 low carbohydrate diet; n= 3 high carbohydrate diet). The six diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw, and the measured concentrations of selenium in the high carbohydrate diet were: 0.7 (control), 6.6, and 11.8 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 weeks

Study Design: Body weights, feed: gain ratios, and total mortalities were determined after each 28-day interval. After 16 weeks, approximately 20 fish were randomly removed from each tank, weighed, and blood was collected for hemoglobin, hematocrit, and plasma glucose, protein, and calcium determination. The livers and kidneys were then dissected. The livers were assayed for glycogen content, and samples of both liver and kidney were assayed for selenium content. Additional subsamples of fish were sacrificed and assayed for selenium content and for ash, crude protein, and moisture content (n=6 per treatment). Finally, 30 fish were killed, their livers and kidneys dissected, and analyzed for Ca, Cu, Fe, Mg, P, and Zn content.

Effects Data: The only overt sign of selenium toxicity was food avoidance observed in trout fed the highest selenium content in both low and high carbohydrate diets, which led to significantly reduced body weight after 16 weeks. There were no significant differences detected between treatment groups in hematological parameters. Kidney, liver, and carcass selenium levels increased with increasing selenium content of the diet, however, only the liver selenium concentrations were significantly affected by dietary selenium level, dietary carbohydrate level, and the interaction between the two treatments. Mineral analysis of the kidney showed significantly higher levels of calcium and phosphorous in trout reared on the two highest levels of dietary selenium. Concentrations of copper in the liver increased significantly with increasing dietary selenium levels and decreasing dietary carbohydrate levels.

Effect of Selenium in Low carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.6	0.8	3.3
6.6	38.3	3.3
11.4	49.3	1.8

Effect of Selenium in High carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.7	0.6	2.7
6.6	21.0	2.3
11.8	71.7	1.4

Chronic Value: The following table lists the NOAEC, LOAEC and MATC for both diets in liver tissue. EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Diet	**NOAEC, mg Se/kg dw liver**	**LOAEC, mg Se/kg dw liver**	**MATC, mg Se/kg dw liver**
Low carb	38.3	49.3	43.5
high carb	21.0	71.7	38.8

Hicks, B.D., J.W. Hilton, and H.W. Ferguson. 1984. Influence of dietary selenium on the occurrence of nephrocalcinosis in the rainbow trout, *Salmo gairdneri* Richardson. J. Fish Diseases. 7:379-389.

(Note: These data are the exact same as reported for the low carbohydrate diet in **Hilton and Hodson 1983**, with the addition of prevalence of nephrocalcinosis occurring in trout after 16 to 20 weeks of consuming the contaminated test diets).

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The test diet was supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the three different dietary selenium treatments. The three diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 to 20 weeks

Study Design: See Hilton and Hodson (1983). After 20 weeks on the test diets, ten fish were randomly removed from each treatment. Tissues for histopathological examination included the stomach, intestine and pyloric ceca (including pancreas), spleen, liver, heart, kidney, skin, muscle, and gills.

Effects Data: Only effects of selenium on kidney tissue are included in the article. The kidneys of the 10 trout fed the highest selenium content in the diet exhibited normal appearance. Five of these trout exhibited precipitation of calcium in the tubules with some epithelial necrosis, but no loss of epithelial continuity. Extensive mineralized deposition of Ca within the tubules, tubular dilation and necrosis of tubular epithelium, ulceration of tubules, and intestinal Ca mineralization was observed in four of the ten fish.

Chronic Value: Same as for growth of rainbow trout reported by Hilton and Hodson (1983). The MATC estimated for growth of rainbow trout relative to final concentration of selenium in liver tissue of trout reared on the low carbohydrate diet is the GM of 38.3 (NOAEC) and 49.3 (LOAEC) mg/kg dw, or 43.45 mg/kg dw.

EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Hilton, J.W., P.V. Hodson, and S.J. Slinger. 1980. The requirements and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J. Nutr. 110:2527-2535.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 1.28 g each)

Exposure Route: Dietary only
A casien-torula yeast diet was formulated to contain geometrically increasing levels of selenium from 0 to 15 mg/kg dw. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Duration: 20 weeks

Study Design: Six test diets were fed to triplicate groups of 75 fish. The trout were fed to satiation 3-4 times per day, 6 days per week, with one feeding on the seventh day. Measured concentrations of selenium in the diet were: 0.07 (control), 0.15, 0.38, 1.25, 3.67, and 13.06 mg/kg dw. The tanks received a continuous flow of dechlorinated tap water from the City of Burlington, Ontario municipal water supply. The waterborne selenium content of this water was 0.4µ g/L. During the experiment, the fish were weighed every 2 weeks with the feeding level adjusted accordingly. Mortalities were noted daily and the feed consumption for each treatment was recorded weekly. After 4 and 16 weeks, three to six fish were randomly removed from each tank, sacrificed, and their livers and kidneys removed and weighed. An additional three to six fish were then obtained from each treatment, killed, and prepared for tissue analysis. Organs and carcasses were freeze-dried for determination of selenium concentration. After 16 weeks, three more fish were removed. Kidney, liver, spleen and dorsal muscle tissue was dissected for examination of histopathology. At the end of 8 and 16 weeks, four to five fish were removed, sacrificed, and a blood sample was taken for hematological measurements (hematocrit, red blood cell count, and blood iron concentration). After 20 weeks, three to four more fish were removed, sacrificed, and a blood sample was taken for measurement of glutathione peroxidase activity.

Effects Data: There were no significant differences detected between treatment groups in histopathology, hematology, or plasma glutathione peroxidase activity. Trout raised on the highest dietary level of selenium (13.06 mg/kg dw) had a significantly lower body weight and a higher number of mortalities (10.7; expressed as number per 10,000 fish days) than trout from the other treatments levels after 20 weeks of exposure.

Effects on Juvenile Rainbow Trout			
Se in diet, mg/kg dw	**Se in Liver, mg/kg dw**	**Weight, g/fish**	**Mortality***
0.07	0.6	3.2	0
0.15	0.95	3.5	0
0.38	2.4	3.7	0.6
1.25	11	4.1	0.6
3.67	40[a]	4.1	0
13.06	100[b]	1.4	10.7

* expressed as number per 10,000 fish-days
[a] NOAEC
[b] LOAEC

Chronic Value: NOAEC = 40 mg Se/kg dw
LOAEC = 100 mg Se/kg dw
MATC = 63.25 mg Se/kg dw

Holm, J. 2002. Sublethal effects of selenium on rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Masters Thesis. Department of Zoology, University of Manitoba, Winnipeg, MB.

Holm, J., V.P. Palace, K. Wautier, R.E. Evans, C.L. Baron, C. Podemski, P. Siwik and G. Sterling. 2003. An assessment of the development and survival of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) exposed to elevated selenium in an area of active coal mining. Proceedings of the 26th Annual Larval Fish Conference 2003, Bergen, Norway. ISBN 82-7461-059-B.

Holm, J., V.P. Palace, P. Siwik, G. Sterling, R. Evans, C. Baron, J. Werner, and K. Wautier. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. Environ. Toxicol. Chem. 24: 2373-2381.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; spawning adults) and brook trout (*Salvelinus fontinalis*; spawning adults)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the high selenium site ranged from 6 to 32 μg/L. Selenium was not measured at the reference streams; selenium concentrations at reference locations in the area ranged from <0.5 to 2.2 μg/L.

Study Design: Spawning fish were collected at low selenium or reference streams (Deerlick Creek, Wampus Creek and Cold Creek), a slightly elevated selenium stream (Gregg Creek), and an elevated selenium stream (Luscar Creek) in the Northeastern slopes region of Alberta, Canada. An active coal mine is the source of selenium in the elevated streams. Eggs and milt from the spawning trout were expressed by light pressure from abdomen. Individual clutches of eggs were fertilized from a composite volume of milt derived from 3-5 males. Fertilized eggs from individual females were reared to swim-up stage and examined for a number of parameters including percent fertilization, mortality, edema, and deformities (craniofacial, finfold, and spinal malformations). Similar studies were conducted in 2000, 2001 and 2002. One notable difference is that the embryos were incubated at 8°C in 2000 and at 5°C in 2001. The authors noted that 5°C is a better representation of the actual stream temperature during embryo development.

Effects Data : Other than selenium, there were no significant differences in the concentrations of other elements (Al, As, Sb, Ba, Be, Ni, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ag, Sr, Tl, Th, Sn, Ti, U, V, Zn) in trout eggs between the low level and elevated selenium streams. There are two ways to approach determination of effects due to selenium in this study and both are presented here. The first approach determines effects based on a comparison of average conditions between streams (*between streams approach*). For example, if there is a significant difference between the average frequency of deformities in a contaminated stream and reference stream, the effect level for the *between streams approach* would be the average concentration of selenium in the tissue from the contaminated stream. The second approach evaluates individual response variables (e.g., edema, deformities) against the individual selenium tissue concentrations for the combined contaminated and reference stream data set with each year (*within streams approach*). This approach, which results in an

EC estimate (e.g., EC_{10}) if the data meet the model assumptions, is explained below.

Between streams approach: For each sampling location (stream), data for the three years (Tables 1 and 2) were combined in the between streams analysis of variance (ANOVA). For rainbow trout embryos, there were no significant differences in fertilization, time to hatch and mortality between the streams with elevated selenium and the reference streams. ANOVA indicated significant differences in the frequency of embryonic effects between streams (Table 3). The analysis did not prove useful; however, due to a higher occurrence of effects in some of the reference streams relative to the exposed streams (Tables 3 and 4). The between streams analysis, therefore, was not used to determine effect concentrations for rainbow trout.

ANOVA of brook trout data indicated the only significant difference in embryonic abnormalities among sites was craniofacial deformities (Tables 5 and 6). Significant differences were also found for fertilization and larval weight. The highest average percent fertilization was observed at the site with the greatest concentration of selenium in eggs, which indicates that the differences in fertilization among sites were not caused by variation in selenium concentrations. Because the percent of embryos with craniofacial deformities in Luscar Creek was 7.9% (2.1% in Cold Creek), it was not considered biologically meaningful. Likewise the significantly lower larval weights at the exposed sites was not large (16% lower than Cold Creek larvae) and again coupled with the low occurrence of abnormalities by the brook trout, a signature of selenium effects, the lower larval weights were not considered biologically meaningful.

Within streams approach: As with the *between streams* analysis, data were combined for the three years of study in the *within streams* analysis (Tables 1 and 2). Craniofacial deformities, skeletal deformities and edema in rainbow trout embryo, as a function of selenium in egg ww, were fitted to a curve using a weighted regression and threshold sigmoidal equation from which EC_{10} values were calculated (see Figures 1, 2 and 3). EC estimates for finfold deformities, length and weight of rainbow trout embryos could not be made because of inadequate dose-response. The brook trout data were not suitable for fitting logistic curves (Figure 5).

Table 1. Rainbow trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference sites (Deerlick Creek and Wampus Creek) in northeastern Alberta over three consecutive years.

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2000	Luscar	11	6.84	7.18	13.26	1.66	4.97
2000	Luscar	12	6.66	1.48	4.43	0.74	1.85
2000	Luscar	14	11.6	14.43	23.71	7.22	85.57
2000	Deerlick	16	1.78	0.63	1.9	0.63	0.63
2000	Deerlick	17	1.39	0	0	0	0
2000	Deerlick	18	1.00	0	0.86	0	0
2000	Deerlick	15	5.01	0	0	0	0
2001	Luscar	1	5.39	7.35	6.76	3.53	2.94
2001	Luscar	3	8.39	6.29	4.97	2.98	6.95
2001	Luscar	4	6.48	22.22	22.22	33.33	26.67
2001	Luscar	8	4.47	12	9.33	2.67	10.67
2001	Luscar	14	10.4	34.55	44.85	4.24	43.64
2001	Luscar	32	5.64	8.24	5.97	3.13	9.09
2001	Luscar	33	3.88	5.26	6.58	9.21	3.95
2001	Luscar	39	5.14	1.91	3.18	0	1.27
2001	Luscar	40	3.36	11.62	7.05	5.39	6.64
2001	Luscar	41	11.7	37.67	83.41	3.59	87
2001	Deerlick	8	3.68	9.55	5.45	1.36	5.45
2001	Deerlick	9	3.08	5.39	4.98	0.41	2.07
2001	Deerlick	10	1.62	7.89	7.89	5.26	10.53
2001	Deerlick	16	2.62	24.24	48.48	3.03	12.12
2001	Deerlick	17	2.79	14.13	15.22	4.35	20.65
2001	Deerlick	21	1.96	13.27	35.71	7.14	25.51
2001	Deerlick	22	3.13	1.09	2.17	0	1.09
2001	Deerlick	23	3.03	9.65	14.04	3.51	7.89
2001	Deerlick	25	3.32	9.25	13.29	7.51	8.09
2001	Deerlick	39	2.43	11.89	9.09	7.69	14.69
2001	Gregg	2	4.57	11.97	7.75	15.49	7.04
2001	Gregg	3	4.49	5.58	9.3	2.33	4.65
2001	Gregg	5	4.05	4.95	5.45	2.48	5.94
2001	Gregg	9	5.09	20	13.85	15.38	16.15
2001	Gregg	18	5.97	16.13	19.35	41.94	35.48
2001	Wampus	9	2.66	16.07	0	1.79	7.14
2001	Wampus	13	2.04	7.84	9.8	1.31	7.84
2002	Luscar	3	5.4	60.47	27.9	93	14
2002	Luscar	8	18.3	94.12	23.5	4.4	97.1
2002	Luscar	10	22	100	64.3	3.6	100
2002	Luscar	12	15.7	82.35	47.1	66.7	52.9

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2002	Luscar	22	20.5	100	42.1	2.1	100
2002	Luscar	23	6.3	5.59	6.6	1.6	2.7
2002	Luscar	24	26.8	100	100	0	100
2002	Luscar	26	6.5	1.72	1.7	4.3	0.9
2002	Deerlick	10	5.9	5.65	7.26	7.26	3.23
2002	Deerlick	18	7.8	10.77	1.54	9.23	3.08
2002	Deerlick	21	5	6.9	6.9	20.69	1.72
2002	Deerlick	24	4.3	2.88	2.88	21.58	0.72
2002	Deerlick	25	4.4	5.3	5.3	6.82	3.03
2002	Deerlick	26	6.6	2.95	1.85	1.11	1.85
2002	Gregg	1	5.8	4.76	3.81	3.81	3.81
2002	Wampus	1	3	18.84	14.49	72.46	11.59
2002	Wampus	2	4	0	0	100	100
2002	Wampus	3	4.6	4.1	3.28	7.58	0.61
2002	Wampus	4	4.7	25	20	70	12.5
2002	Luscar	28	7	19.23	0	76.9	0

Table 2. Brook trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference site (Cold Creek) in northeastern Alberta over three consecutive years.

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2000	Luscar	1	4.78	15.38	0	0	15.38
2000	Luscar	2	4.83	38.06	1.49	3.73	1.49
2000	Luscar	3	5.98	7.39	3.03	0.34	0.5
2000	Luscar	5	3.86	25	5.7	8.77	4.82
2000	Luscar	12	6.06	16.77	1.83	0.7	0
2000	Luscar	13	5.8	4.06	1.42	0.2	0
2000	Luscar	14	5.17	4.13	0.49	0.36	0.12
2000	Luscar	15	9.92	16.22	0.54	0.54	0
2000	Luscar	16	5.03	5.61	0	0.27	0.27
2000	Luscar	17	6.01	9.44	5.83	0.83	1.11
2000	Luscar	18	12.7	14.34	0.72	0	0.36
2000	Cold	21	1.15	3.26	1.48	0.89	0
2000	Cold	22	1.83	4.83	1.38	1.38	0.69
2000	Cold	24	0.97	1.67	0	0.72	0
2000	Cold	25	No data	3.31	1.1	1.66	1.1
2000	Cold	26	0.59	3.45	4.83	6.9	0.69
2000	Cold	33	1.35	6.15	0	1.54	0
2000	Cold	34	2.18	6.45	0	0.81	0
2001	Cold	6	1.79	0	0	0	0
2001	Cold	7	1.36	1.61	0.69	0.46	1.38
2001	Cold	8	0.94	1.36	0	0.27	0.54
2001	Cold	21	1.07	0.43	0	0	0
2001	Cold	51	1.09	0	2.13	0	6.38
2001	Luscar	3	8.4	0	0.93	0	0.46
2001	Luscar	7	7.26	1.35	1.62	0.81	0.27
2001	Luscar	17	14.6	2.22	0.63	0.32	0
2001	Luscar	19	9.79	7.55	2.11	2.42	0.3
2001	Luscar	59	5.8	2.28	0.46	0.91	0.46
2001	Luscar	60	9.03	3.16	0	1.05	1.05
2001	Luscar	61	7.29	0	0	9.09	0
2001	Luscar	64	7.08	1.54	2.19	0	0
2001	Luscar	76	7.1	36.71	13.29	19.65	1.16
2001	Luscar	82	6.06	1.11	0.22	0.88	0.44
2001	Luscar	83	5.82	6	2	5.6	0.8
2001	Gregg	3	7.08	6.32	1.58	20.53	1.58
2001	Gregg	22	7.95	0	0	1.08	0
2001	Gregg	23	9.23	0.5	0.5	2.51	0
2001	Gregg	25	6.46	0.56	0	0.56	0

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2001	Gregg	31	7.35	0.51	1.7	0.17	0
2001	Gregg	32	4.91	7.21	0.48	3.37	0.48
2001	Gregg	33	7.02	1.88	1.88	4.38	0
2001	Gregg	34	5.01	0	0.37	0	0
2002	Luscar	17	6.28	1.7	12.74	0.85	0.21
2002	Luscar	23	5.27	7.34	0.46	0	0.46
2002	Luscar	26	6.36	1.81	0.52	0.26	0.26
2002	Luscar	38	18.9	0.9	0.54	0	0.18
2002	Luscar	42	4.95	2.79	0.44	0.15	0.15
2002	Luscar	44	6.47	0	0.25	0	0
2002	Luscar	54	7.96	0.33	0.33	0	0
2002	Luscar	56	18.8	3.99	0.75	0.5	0.75
2002	Gregg	25	6.27	1.23	1.23	0	0
2002	Gregg	37	4.58	2.99	0	0	0
2002	Gregg	39	6.67	3.57	1.19	1.19	1.19
2002	Cold	32	0.42	0	0.6	0	0
2002	Cold	26	0.89	0	0	0	0.29
2002	Cold	2	0.94	0.96	0.32	0	0
2002	Cold	5	1	0.25	0.5	0.25	0
2002	Cold	29	1.02	0.72	1.09	0.36	0.72
2002	Cold	23	1.2	0.35	0.35	0.35	0.35
2002	Cold	48	1.25	9.52	4.76	2.38	0
2002	Cold	42	1.6	0	0	0	0
2002	Cold	22	1.74	0	0	1.09	1.09
2002	Cold	51	2.11	2.17	2.17	0	2.17

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites

% fertilization

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	77.60	25.8653	0.06336703	0.978935
Residuals	51	20817.33	408.1829		

% mortality

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3751.51	1250.504	1.848008	0.1502207
Residuals	51	34510.50	676.676		

% craniofacial deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8093.97	2697.989	4.430272	0.007732133
Residuals	50	30449.48	608.990		

% skeletal deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3279.30	1093.101	2.773923	0.05094422
Residuals	50	19703.16	394.063		

% finfold deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	6273.17	2091.056	3.888612	0.01417887
Residuals	50	26886.93	537.739		

% edema

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8902.51	2967.502	3.449597	0.0233558
Residuals	50	43012.30	860.246		

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites (continued)

Fry length

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	5.0847	1.694896	0.5694271	0.6377436
Residuals	50	148.8246	2.976493		

Fry weight

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	1721.104	573.7012	3.563888	0.02080915
Residuals	48	7726.859	160.9762		

Table 4. Rainbow trout means (standard deviation) for measurements made in eggs, embryos and larvae spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference sites (Deerlick and Wampus Creeks).

Parameter	Site			
	Luscar Cr.	Gregg Cr.	Deerlick Cr.	Wampus Cr.
egg Se, mg/kg ww	9.93 (6.77)	6.52 (4.11)	3.49 (1.90)	3.5 (1.09)
fertilization, %	77.8 (20.3)	81.2 (12.7)	77.5 (20.9)	77.5 (24.1)
mortality, %	35.0 (29.5)	34.2 (32.5)	18.1 (14.6)	37.3 (34.5)
craniofacial, %	33.3 (37.2)	10.6 (6.5)	7.1 (6.1)	12.0 (9.6)
skeletal, %	25.0 (27.9)	9.9 (5.8)	9.2 (12.3)	7.9 (8.2)
finfold, %	15.0 (27.1)	13.6 (15.2)	5.4 (6.2)	42.2 (43.7)
edema, %	34.5 (40.3)	12.2 (12.3)	6.1 (7.3)	23.3 (37.8)
larval length, mm	18.5 (2.0)	19.4 (1.6)	19.0 (1.5)	19.2 (0.9)
larval weight, mg	53.3 (16.3)	44.6 (10.4)	41.2 (9.3)	40.6 (8.4)

Table 5. Brook trout means (standard deviation) for measurements made in eggs, embryos and larva spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference site (Cold Creek).

Parameter	Site		
	Luscar Cr.	**Gregg Cr.**	**Cold Cr.**
egg Se, mg/kg ww	7.78 (3.80)	6.59 (1.39)	1.26 (0.47)
fertilization, %	92.8 (7.2)	78.4 (18.2)	89.1 (19.6)
mortality, %	6.5 (8.9)	2.9 (2.3)	6.9 (12.1)
craniofacial, %	7.9 (10.1)	2.3 (2.5)	2.1 (2.6)
skeletal, %	2.0 (3.3)	0.8 (0.7)	1.0 (1.4)
finfold, %	1.9 (4.1)	3.1 (6.0)	0.9 (1.5)
edema, %	1.0 (2.9)	0.3 (0.6)	0.7 (1.4)
larval length, mm	17.4 (1.1)	17.9 (0.9)	18.5 (1.2)
larval weight, mg	31.7 (8.6)	31.3 (5.4)	37.8 (7.2)

Table 6. Results of ANOVA comparing brook trout endpoints among sites

% fertilization					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	1683.3	841.67	3.9128	0.0253
Residuals	60	12906.4	215.11		
% mortality					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	131.4	65.72	0.7257	0.4882
Residuals	60	5433.6	90.56		
% craniofacial deformities					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	519.1	259.54	4.9427	0.0103
Residuals	60	3150.6	52.51		

Table 6. Results of ANOVA comparing brook trout endpoints among sites (continued)

% skeletal deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	19.2	9.58	1.5631	0.2179
Residuals	60	367.6	6.13		
% finfold deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	37.5	18.74	1.2562	0.2921
Residuals	60	895.1	14.92		
% edema					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	4.6	2.32	0.4966	0.6110
Residuals	60	280.6	4.68		
Fry length					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	16.1	8.04	6.5265	0.0027
Residuals	60	73.9	1.23		
Fry weight					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	546.2	273.10	4.6644	0.0131
Residuals	60	3512.9	58.55		

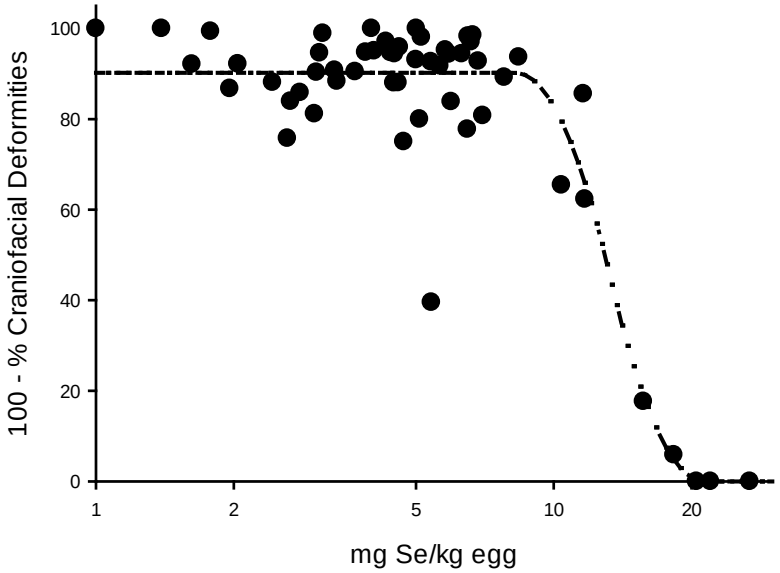


Figure 1. Rainbow trout percent normal (100 - % craniofacial deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 90.2%, the slope 4.8%, and the EC_{10} 10.2 mg Se/kg egg ww.

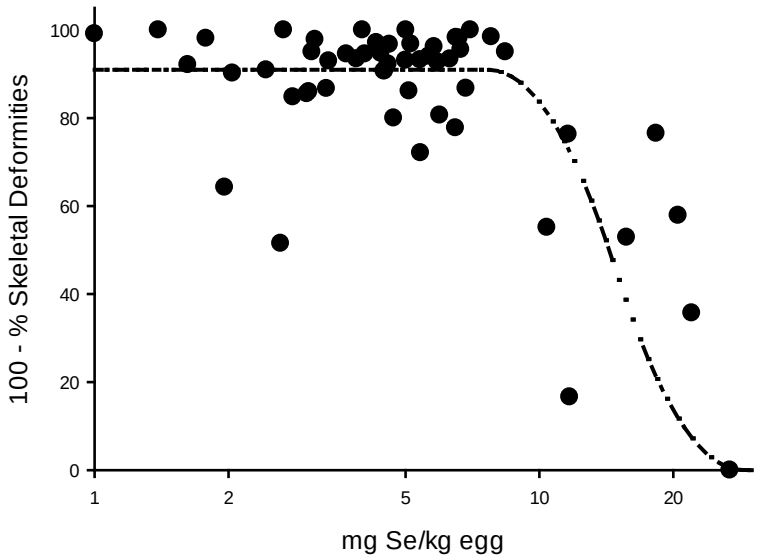


Figure 2. Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 91%, the slope3.5%, and the EC_{10} 10.3 mg Se/kg egg ww.

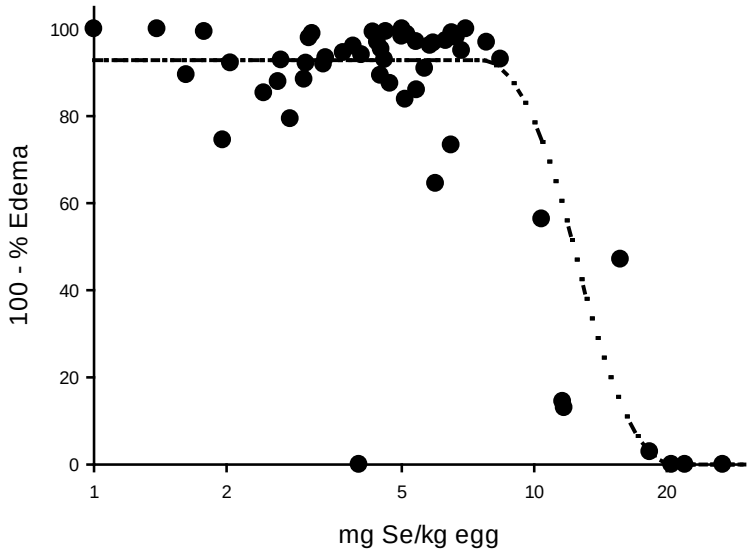


Figure 3. Rainbow trout percent normal (100 - % edema) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoidal equation excluding the one outlier with 100% edema at 4 mg/kg. The background value was estimated to be 92.8%, the slope 4.6%, and the EC_{10} 9.5 mg Se/kg egg ww.

The previous draft used a TRAP logistic regression (Figure 4). A weighted regression using a threshold sigmoidal equation (Figures 1-3) is a better application of these data.

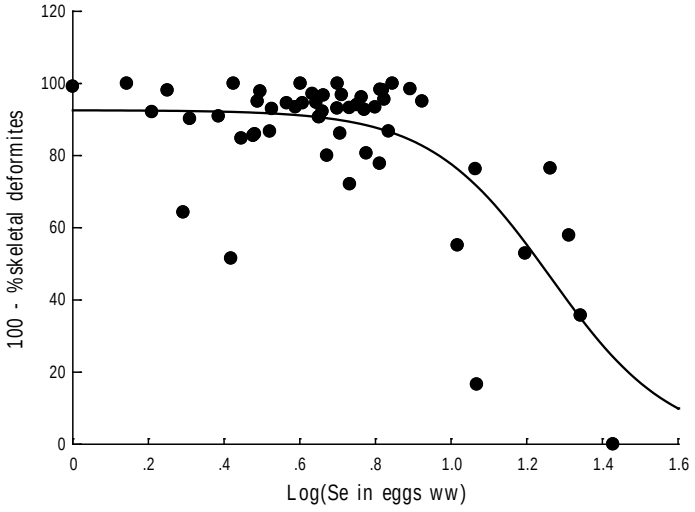
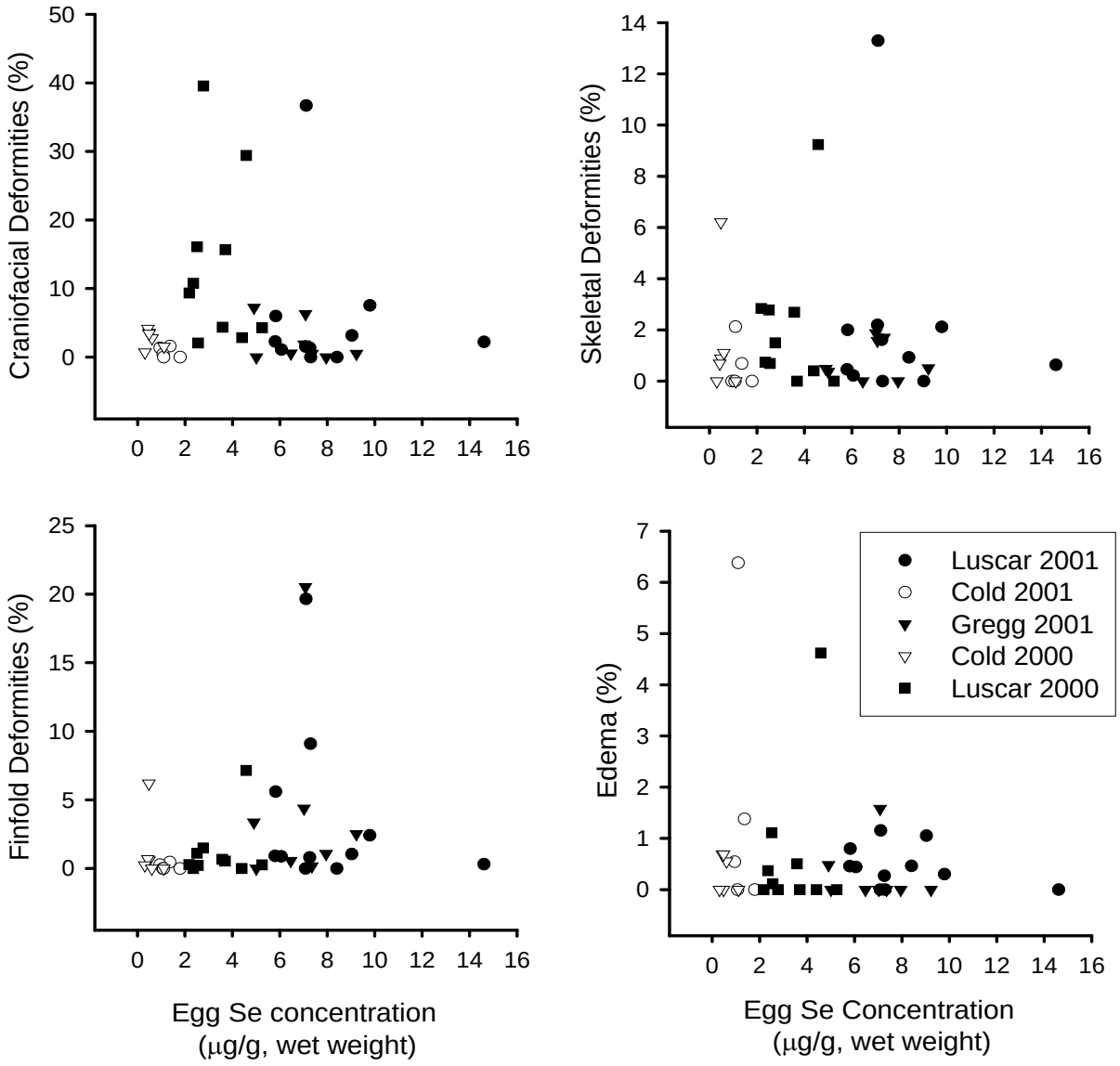


Figure 4. (From Previous Draft) Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). EC_{10} = 8.2 mg/kg ww.

Figure 5. Plot of percent abnormal for craniofacial, skeletal and finfold deformities and edema against selenium concentration in brook trout eggs ww, 2000 and 2001 data.



The effect levels determined using the *within streams* approach resulted in values based on ww in eggs. The primary tissue for which the reproductive effect levels were based, eggs, was converted from ww to dw using the average percent moisture of 61.2% for rainbow trout eggs reported by Seilor and Skorupa (2001).

Chronic Values:

Brook trout: *Between streams approach*
No effects at EC_{10} level at 7.78 mg Se/kg eggs ww or 20.05 mg Se/kg eggs dw; egg. **Chronic value is >20.05 mg Se/kg eggs dw.** Table 3 data, converted to dry weight, suggest no effects at least up to 25-35 mg Se/kg eggs dw.

Rainbow trout: *Within streams approach*
EC_{10} value (edema) at 9.5 mg Se/kg egg ww or 24.5 mg Se/kg egg dw. **Chronic value is 24.5 mg Se/kg eggs dw.**

Kennedy, C.J., L.E. McDonald, R. Loveridge, M.M. Strosher. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarkii lewisi*). Arch. Environ. Contam. Toxicol. 39:46-52.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii lewisi*; spawning adults, 3-6 years)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the time the eggs were taken were <0.1 μg/L from the reference site and 13.3 to 14.5 μg/L at the exposed site.

Study Design: At reference and exposed site (Fording River, BC, Canada which receives drainage from open-pit coal mining), eggs were stripped from females (n=20 from reference site; n=17 from exposed site) and fertilized from milt from one male collected at each site. Fertilized eggs were reared in well water and examined for time to hatch, deformities (craniofacial, finfold, skeletal and yolk sac malformations), and mortalities. Inspection of deformities in eggs was performed using 40X magnification.

Effects Data : No significant correlations between the selenium concentrations in the eggs from either site and: hatching time (reference, 25.5-26.5 days; exposed, 22-25.5 days); percent deformities preponding (reference, 0-2.4%; exposed, 0-0.34%); percent deformities after ponding (reference, 0-0.26%; exposed, 0-0.09%); percent mortalities preponding (reference, 1.5-70.3%; exposed, 1-100%); percent mortalities after ponding (reference, 0.3-4.3%; exposed, 1.5-43.7%); total percent mortalities (reference, 2.8-55.8%; exposed, 3.7-100%). The average selenium residues in tissues were as follows:

Site	Adult fish liver, mg Se/kg dw	Adult fish muscle, mg Se/kg dw	eggs, mg Se/kg dw
Reference	8.2; Range: 3.4-14.6	2.4; 1.4-3.8	4.6
Exposed	36.6; Range:18.3-114	12.5; Range: 6.7-41	21.2

Chronic Value: >21.2 mg Se/kg dw in eggs
>12.5 mg Se/kg dw in muscle

Hardy, R.W. 2005. Effects of dietary selenium on cutthroat trout (*Oncorhynchus clarkii*) growth and reproductive performance. Report for Montgomery Watson Harza. December 14, 2005.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii*, 0.9 g)

Exposure Route: Dietary only
Six experimental dietary treatments were produced by cold extrusion. The formulation of the diet was designed to be similar to commercial trout diets and had a proximate composition of 45% protein and 16% lipid. Seleno-methionine diluted in distilled water (100 µg/L) was added in appropriate volumes to each batch of feed to facilitate pelleting. Measured dietary selenium concentrations were 1.2 (control), 3.8, 6.4, 9.0, 11.5, and 12 mg Se/kg dw. Fry were fed initially at a rate of 10 times per day 6 days each week to apparent satiation. Feeding frequency decreased as fish grew.

Test Duration: 124 weeks (865 days, 2.5 yrs)

Study Design: Groups of 50 fish were placed into triplicate tanks (145 L) receiving 4-15 L/min of hatchery water at 14.5EC and fed one of the six experimental diets. The fish in each tank were bulk-weighed and counted every 14 days for the first 12 weeks of the experiment, and then every 4 weeks until 48 weeks. Samples of fish for whole-body selenium analysis were taken at each sampling date for the first 12 weeks followed by every 3 months thereafter. After six months of feeding, the fish were transferred to 575 L tanks and the number of replicate tanks per dietary treatment was reduced to two. After 80 weeks of feeding, the fish were transferred to 1050 L outdoor tanks each supplied with 70 L/min of constant temperature (14.5°C) spring (hatchery) water. After 2.5 years of the feeding trial, fish were spawned and whole body selenium level , egg selenium level, % eyed eggs, % hatched eggs, and % deformed larvae were examined.

Effects Data: No signs of toxicity (reduced growth or survival relative to controls) were observed in fish fed the highest dietary selenium treatment (12 mg Se/kg dw) after the first 80 weeks of exposure just prior to transfer outdoors. No signs of clinical disease were evident, and no relationship was found between feed conversion ratios and the level of selenium added to the feed. Average whole body selenium levels of female Henry's Lake cutthroat trout at spawning at 2.5 to 3 years of age were 5.87, 9.10, 11.37 and 5.61 mg Se/kg dw in the four highest dietary treatments. Average egg selenium levels in the same four dietary treatments were 6.61, 5.05, 5.18, and 16.04 mg Se/kg dw. Percent survival from the eyed stage to hatching varied among treatment groups, with the control and the highest Se dietary treatment having the second highest survival (85%) and the fifth dietary treatment group the highest (93%). Percent deformed larvae ranged from a low of 5.6% in controls to a high of 20.2% in the 6.4 mg Se/kg dw dietary treatment group; larvae in the two highest dietary treatment groups only exhibited 7 and 6.8 %, respectively.

Chronic Value: The chronic value for embryo/larval deformity is a NOAEC of >11.37 mg Se/kg dw whole-body parent tissue and >16.04 mg Se/kg dw egg.

Rudolph, B-L, I. Andreller, CJ. Kennedy. 2008. Reproductive success, early life stage development, and survival of Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) exposed to elevated selenium in an area of active coal mining. Environ. Sci. Technol. 42: 3109-3114.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)

Exposure Route: Field collected.
In June, 2005, eggs were collected from 12 females from Clode Pond (exposed site) and 16 females from O'Rourke Lake (reference site). Milt was obtained from 3-5 males at each site. Clode Pond is on the property of Fording River Coal Operations in Southeast British Columbia with reported selenium concentrations of 93 µg/L. O'Rourke Lake is an isolated water body into which Westslope cutthroat trout were stocked in 1985, 1989 and 1992 and has selenium levels reported <1 µg/L.

Test duration: Through the end of yolk sac absorption (at swim-up) by the alevins.

Study Design: Individual batches of eggs were fertilized in the field with 2 ml composites of milt. Water-hardened eggs were transported to the rearing laboratory. Eggs and alevins were monitored daily for fertilization, hatching and mortality. After the yolk sacs were absorbed, alevins were sacrificed and preserved in Davidson's solution.

All viable fry (n = 4,922) after yolk absorption were observed for the frequency and severity of skeletal (lordosis, kyphosis, and scoliosis), craniofacial (head, eyes or jaw), and fin malformations as well as edema. The authors used a graduated severity index (GSI) for deformities in which fry were scored 0 (normal) to 3 (severe) based on the level of defect.

Effects Data: Eggs with the four highest Se concentrations (86.3 to 140 mg/kg dw) collected from Clode Pond fish died before reaching the laboratory (Table 1). Excluding the eggs that died from females CP1, CP3, CP4 and CP5, fertilization (total eggs reaching the eyed stage/total eggs x 100) was not related to Se concentrations in the eggs. The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs (Table 1). Note: The data used to estimate the EC_{10} value excluded the variable from OL1 and OL2 (shaded areas in Table 1). These are data from the reference lake in which only 57% of the larvae survived (OL1) or where the % dead eggs plus % hatch did not add up to %100. Alevin survival was meaningfully higher in the other 15 clutches of eggs from the reference site (85.1 to 99.8%). Because there were insufficient partial effects, a TRAP model was not used to estimate the EC_{10} value. The data consist of a cluster background data and a cluster of 100% mortality (Figure 1). With no way to fit a credible curve, the interpolation method is applied here with the EC_0 set to 20.6 mg/kg with background % survival of 95.75% (not including the one low outlier) and the second extrapolation point being 46.8 mg/kg with 0.3% survival. The resultant slope is 5.6 (similar to slopes in other datasets where it was estimated) and the EC_{10} is 24.7 mg/kg. Note: TRAP was used in the previous draft to derive a similar EC_{10} of 24.1 mg/kg, however as stated above, it

was determined that the data are not amenable to a TRAP model because of insufficient partial effects.

An EC_{10} based on Se in maternal muscle was estimated using the same approach as was used for Se in eggs, that is, by interpolation between an EC_0 and a high EC_P. An EC_{10} of 16.6 mg Se/kg muscle dw was interpolated from an EC_0 (HNOEC) of 13.4 mg/kg and the average background survival of 95.75 and the EC_{100} set to 34.7 mg/kg muscle (Figure 2).

Deformity analysis was not performed on the alevins that died prior to the swim-up stage. Therefore, due either to dead eggs or dead alevins, the occurrence and severity of deformities were assessed on four clutches of eggs from Clode Pond (CP2, CP6, CP11 and CP12) with a range of 11.8 to 20.6 :g Se/g dw and 15 of the 16 clutches (all eggs died in OL8) from O'Rourke Lake (Table 1). There was no correlation between egg Se concentration and frequency of deformity or edema. Statistical differences between sites were observed ($p < 0.05$) for skeletal deformities and edema for both the frequency of the occurrence and the severity score (Table 2). Note: the percent and severity score of skeletal deformities were greater in the reference site than in the exposed site.

The effect level for this study was based on the alevin mortality data and not the deformity measurements. Although edema occurred statistically more often at the exposed site (87.7% at Clode Pond, 61.2% at O'Rourke Lake), it was not correlated with selenium levels in the eggs. Also the greater occurrence of skeletal malformations in the reference site confounded the use of statistical differences between sites to determine effect levels for this study.

Effect Concentration: 24.7 mg Se/kg dw in eggs; 16.6 mg Se/kg dw in muscle.

Table 1. Fertilization, egg mortality and alevin mortality for offspring from individual fish collected in Clode Pond and O'Rourke Lake.

Fish ID	Muscle [Se] mg/kg dw	Egg [Se] mg/kg dw	Hatch %	Dead eggs, %	Dead alevins, %	% Survival[1]
Clode Pond (exposed site)						
CP1	38.8	88.3	0	100	NA	
CP2	11.8	16.1	98.2	1.8	0.9	99.1
CP3	40.4	86.3	0	100	NA	
CP4	46.1	121	0	100	NA	
CP5	50.4	140	0	100	NA	
CP6	34.7	51	92.6	7.4	92.6	0.0
CP7	39	65.3	91.1	8.9	91.1	0.0
CP8	7	11.8	63.9	36.1	0.8	98.7
CP9	35.4	46.8	63.4	36.6	63.2	0.3
CP10	35.5	75.4	82.4	17.6	82.4	0.0
CP11	11.3	16.9	77.9	22.1	1.3	98.3
CP12	13.4	20.6	97	3	5.1	94.7
avg	30.3	61.6	55.5	44	42	20.0
SD	15.1	42.4	42.5	42	44	0.0
O'Rourke Lake (reference site)						
OL1	8.28	12.9	71.4	28.6	42.9	39.9
OL2	7.7	13.9	27.7	53.1	6.9	75.1
OL3	8.16	12.5	96.1	3.9	2.4	97.5
OL4	8.03	15	85.5	14.5	12.7	85.1
OL5	8.12	14.9	80.7	19.3	5.3	93.4
OL6	6.61	15.2	68	32	4	94.1
OL7	8.52	12.9	97.9	2.1	0.2	99.8
OL8	7.22	12.3	0	100	NA	
OL9	7.25	16.7	87.2	12.8	4.5	94.8
OL10	7.64	13.1	79.6	2.5	5.5	93.1
OL11	8.74	15.6	89.2	10.8	2.4	97.3
OL12	8.2	13.9	83.6	16.4	3	96.4
OL13	7.86	15.1	74.1	25.9	2.8	96.2
OL14	8.5	13.1	77.8	22.2	0.5	99.4
OL15	7.62	12.3	88.2	11.8	2.6	97.1
OL16	8.13	12.7	54.8	45.2	4.8	91.2
avg	7.9	13.9	72.6	25	7	
SD	0.6	1.4	25.8	25	10	

[1] % Survival based on % hatch

Table 2. Deformity results (frequency and severity) for offspring from O'Rourke Lake and Clode Pond. Values are presented as mean ± SE. * indicates a significant difference ($p < 0.05$) between means from the two sites.

Frequency of deformity, %	**O'Rourke Lake**	**Clode Pond**
Skeletal*	37.4 ± 3.6	16.5 ± 2.2
Craniofacial	10.2 ± 2.0	5.7 ± 1.0
Finfold	10.6 ±3.1	7.5 ± 3.84
Edema*	61.2 ± 4.9	87.7 ± 2.0
Severity of deformity, score		
Skeletal*	0.47 ± 0.07	0.18 ± 0.02
Craniofacial	0.12 ± 0.03	0.06 ± 0.01
Finfold	0.15 ± 0.05	0.09 ± 0.05
Edema*	0.61 ± 0.05	0.88 ± 0.02

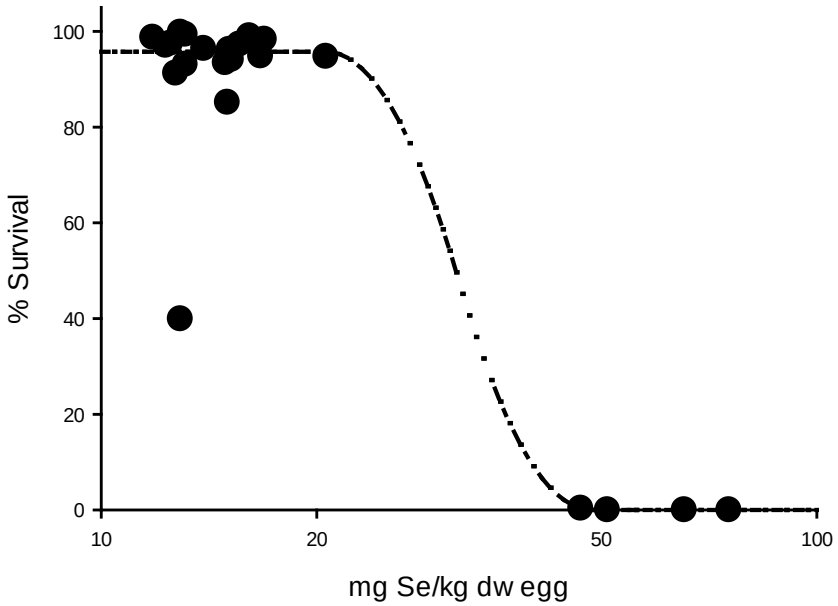


Figure 1. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in eggs.

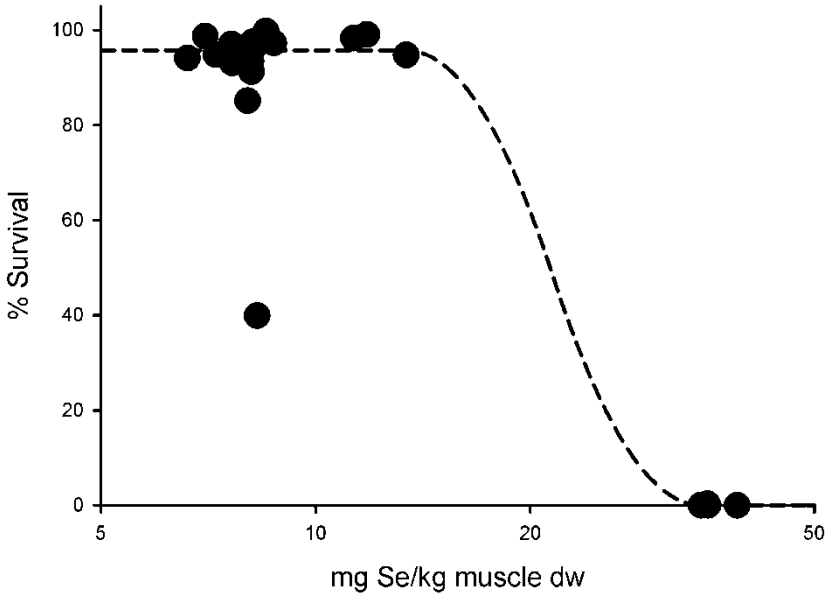


Figure 2. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in maternal muscle.

Nautilus Environmental. 2011. Evaluation of the Effects of Selenium on Early Life Stage Development of Westslope Cutthroat Trout from the Elk Valley, BC. Report to Elk Valley Selenium Task Force, November 24, 2011.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi)*

Exposure Route: Field collected. Adult fish were collected and spawned from lentic and lotic environments in areas proximate to Teck Coal's Fording River Operations. Eggs were also obtained from fish collected from Connor Lake, a lake located within the Elk valley watershed not exposed to mine discharges and considered a reference site and a methodological control.

Test Duration: Fertilized eggs were reared in the laboratory until they reached swim-up fry stage. A subset of fry surviving at swim-up were reared for an additional 28 days.

Study Design: Gametes were stripped from the ripe adults in the field during June and July 2008 and transported immediately to the laboratory in coolers containing wet ice. Eggs were fertilized in the laboratory. After stripping the eggs, female fish were sacrificed and the whole body stored on ice for later Se analysis. For a given female, approximately 240 fertilized eggs were divided into four replicates of 60 eggs. In cases when fewer eggs were available three replicates of 60 eggs were used. If less than 180 eggs were available, either 3 or 4 replicates of 30 were used. Females with less than 90 eggs were not used. The fertilized eggs were maintained in the laboratory until the fry reached swim-up at which point deformities were assessed. Survival was also assessed up to swim-up. In test chambers in which there were at least 40 surviving fish at swim-up, one-half of the surviving fish were maintained for an additional 28 days. Survival, length, weight and deformities were assessed in the 28-day post swim-up test.

The number, type and severity of deformities were measured at swim-up and at the end of the 28-day post swim-up test. Deformity assessments were conducted on recently killed fresh fish to avoid artifacts caused by preservation. A graduated severity index (GSI) was assigned to each of four types of deformity/abnormality: skeletal, craniofacial, finfold and edema. Graduated Severity Index (GSI) methods followed those described in Holm et al. (2003) and Rudolph et al (2006; 2008).

Effects Data: Survival of the larvae from hatch through swim-up spawned from the four fish collected from the reference site, Connor Lake, ranged from 73 to 92% (egg Se 4.32 to 7.31 mg/kg dw) (Table 1). Larval survival at swim-up was also generally high for fish collected in the Se exposed sites up to egg Se concentration 29.6 mg/kg dw (Table 1, Figure 1). Larvae exposed above this egg Se concentration had poor to no survival. Larvae from one fish (P00811) below this threshold did have poor survival (11.7%). The authors noted that the many of the eggs from this fish displayed an unusual distribution of lipid vesicles which resulted in greater than 50% mortality in the first 24 hours due to egg breakage. The remaining eggs may have been compromised due to the organic material released during the egg breakage.

The rate of deformities in larvae at swim-up showed no relationship with Se in egg through 29.6 mg/kg dw (Table 2).

The results of the 28-day post swim-up test showed no relationships between larval survival or deformities and egg Se (Table 3). The authors also measured the length and weight of larvae at the end of the 28 day test; neither of which showed a relationship with egg Se concentration.

Se Tissue Concentrations. Two analytical laboratories (A and B) measured Se in the eggs. The mean difference in egg Se concentrations between the two laboratories was 34.2%. To better understand the difference between the two laboratories, five egg samples (i.e., from five different fish) from this study were sent to both laboratories in 2010. Both laboratories digested the eggs using the methods they used in their own 2008 original analysis. The respective digestates were split and then shared between laboratories. Both labs then measured selenium in their own digestates and the digestate received from the other lab. The results of this follow-up study showed that when each lab used their own digestion procedures Laboratory A had on average 43% higher measurements in the 2008 analysis and 23% higher in the follow-up 2010 analysis. When each lab measured selenium using the same digestate the difference in the Se measurements between labs was on average only 1 to 8%. The authors concluded that although both laboratories employed acceptable and approved practices, Laboratory A used a more efficient digestion process resulting in higher Se measurements. To compensate for the reduced Se measurements in Laboratory B, its values were increased by 34.2%. The measurements made by Laboratory A are marked in Table 1; unmarked values are Laboratory B measurements increased by 34.2%.

Effect Concentration: The most sensitive endpoint determined by TRAP was larval survival at swim-up. Interpolation was used to estimate an effect concentration for larval survival with the entire egg Se dataset that included egg Se measurements from Laboratory A and adjusted measurements from Laboratory B (EC_{10} = 31.1 mg/kg egg dw; Figure 1) and using only the egg Se measurements from Laboratory A (Figure 2). Because the Laboratory A dataset estimated slightly lower EC values, the EC_{10} of 27.7 mg/kg egg dw is the selected effect concentration for this study. Note: In the previous draft, a TRAP model was used to estimate the EC_{10}. However, because of insufficient partial effects, TRAP was determined not appropriate so the EC_{10} was estimated using an interpolation between the HNOEC and the LOEC (see Figure 3 for the TRAP analysis used in the previous draft).

Table 1. Summary of westslope cutthroat trout larvae surviving to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

				Proportion surviving				
Fish ID	Location	Se egg, mg/kg dw	Replicates	Replicate mean	Replicate min	Replicate max	Number survivors	Total number
YO93	Lentic	3.88*	4	0.8125	0.6667	0.9167	195	240
CL1	Reference	4.32	4	0.9167	0.8833	1	220	240
R082	Lotic	5.21	3	0.9056	0.8333	0.95	163	180
CL4	Reference	5.96*	4	0.7333	0.6	0.8	176	240
CL2	Reference	6.82	4	0.8333	0.7	0.9167	200	240
CL3	Reference	7.31	4	0.8542	0.8167	0.8833	205	240
P00815	Lotic	7.6	3	0.8222	0.7167	0.95	148	180
R026	Lotic	12.53	4	0.5792	0.5	0.65	139	240
P00823	Lotic	12.71	4	0.8875	0.85	0.95	213	240
R039	Lotic	12.9	4	0.6042	0.55	0.65	145	240
R086	Lotic	13.4*	4	0.9417	0.85	0.9833	226	240
R077	Lotic	14.29	3	0.6444	0.6167	0.6667	116	180
R042	Lotic	16.44	3	0.8	0.7	0.9	72	90
R055	Lotic	16.5	4	0.8792	0.7833	0.9667	211	240
R043	Lotic	16.85	4	0.8667	0.7667	0.9667	104	120
R074	Lotic	17.8*	4	0.9375	0.8833	0.9833	225	240
P00811	Lotic	19.25	1	0.1167	0.1167	0.1167	7	60
P00809	Lotic	19.72	4	0.7667	0.65	0.8833	184	240
P00803	Lotic	24.8*	4	0.9375	0.9333	0.95	225	240
R078	Lotic	29.61	4	0.8825	0.8333	0.9333	105	119
GO99	Lotic	34.2*	4	0.2083	0.1667	0.2667	50	240
O087	Lentic	54.7*	4	0.07083	0.01667	0.2	17	240
O085	Lentic	56.8*	4	0	0	0	0	240
WO52	Lentic	61.1*	4	0	0	0	0	240
R069	Lotic	65.61	4	0	0	0	0	240
R071	Lotic	72.9	4	0	0	0	0	240
WO94	Lentic	73.1	4	0	0	0	0	240
UT101	Lentic	74.67	4	0	0	0	0	240

*Laboratory A dataset

Table 2. Summary of westslope cutthroat trout larval deformities to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Se egg, mg/kg dw	Skeletal combined	Craniofacial combined	Finfold combined	Edema combined	Deformities combined
YO93	Lentic	3.88*	4.5%	0.9%	4.4%	1.9%	7.7%
CL1	Lentic	4.32	7.6%	1.9%	1.0%	1.0%	9.5%
R082	Lotic	5.21	1.2%	1.3%	2.5%	0.0%	3.7%
CL4	Lentic	5.96*	4.3%	7.3%	1.7%	0.7%	12.6%
CL2	Lentic	6.82	11.1%	3.7%	0.8%	3.0%	15.9%
CL3	Lentic	7.31	5.0%	2.0%	1.0%	0.0%	7.0%
P00815	Lotic	7.6	0.0%	2.7%	0.0%	2.9%	5.6%
R026	Lotic	12.53	2.1%	2.1%	0.7%	1.4%	2.1%
P00823	Lotic	12.71	1.9%	2.9%	1.8%	5.6%	7.4%
R039	Lotic	12.9	2.1%	1.9%	2.9%	4.9%	9.9%
R086	Lotic	13.4*	2.7%	1.0%	0.0%	0.0%	2.7%
R077	Lotic	14.29	1.7%	10.4%	0.9%	12.2%	15.5%
R042	Lotic	16.44	1.2%	0.0%	0.0%	2.6%	2.6%
R055	Lotic	16.5	0.0%	2.8%	1.0%	2.9%	4.7%
R043	Lotic	16.85	0.9%	2.6%	1.8%	1.7%	4.4%
R074	Lotic	17.8*	2.7%	1.8%	0.9%	0.9%	3.6%
P00809	Lotic	19.72	3.9%	2.8%	3.3%	4.7%	9.0%
P00803	Lotic	24.8*	2.7%	0.9%	0.0%	0.9%	4.5%
GO92	Lotic	26.1	0.0%	1.9%	1.9%	4.4%	4.4%
R078	Lotic	29.61	1.8%	0.0%	1.0%	2.9%	5.7%
GO99	Lotic	34.2*	14.5%	53.9%	6.8%	28.2%	64.7%

*Laboratory A dataset

Table 3. Summary of larval survival and rates deformities after the 28-day post swim-up test per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Sample size (n)	Egg Se (mg/kg dw)	Survival (%)	Skeletal (%)	Craniofacial (%)	Finfold (%)	Total (%)
CL1	Reference	112	4.3	99.1	0	0	0	0
CL2	Reference	93	6.8	99	0	0	0	0
CL3	Reference	96	7.3	91.7	0	1	1	2
CL4	Reference	68	6	98.6	0	0	4.3	4.3
Y093	Lentic	93	3.9	95.6	0	0	2	2
R082	Lotic	71	5.2	87.4	0	2.9	0	2.9
P00815	Lotic	69	7.6	91.1	0	1.2	1.4	2
P00823	Lotic	105	12.7	96.3	0	0	0	0
R086	Lotic	112	13.4	97.2	0	0.9	0	0.9
R077	Lotic	36	14.3	92.4	2.8	2.8	2.8	4.2
R055	Lotic	101	16.5	95.9	0	4.6	0	4.6
R074	Lotic	106	17.8	93.1	0	0	0	0
P00809	Lotic	65	19.7	91.7	0	0	0	0
P00803	Lotic	108	24.8	95.7	0	0	1	1

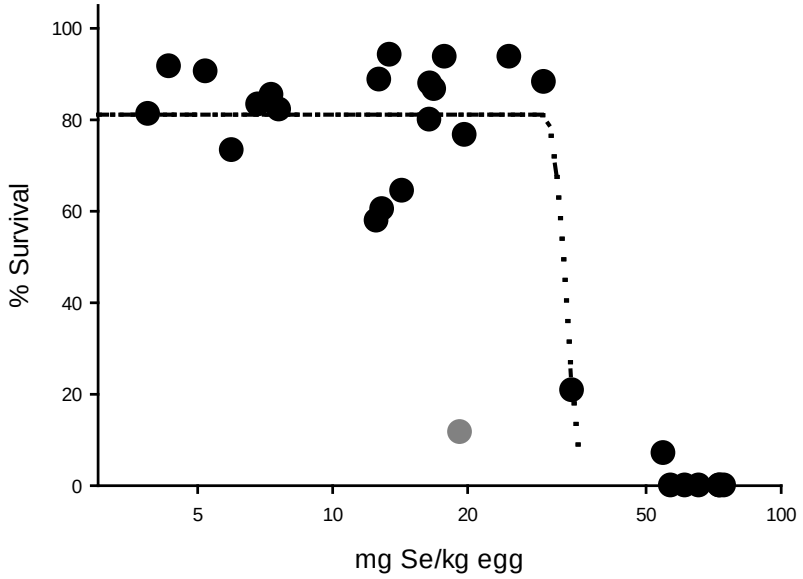


Figure 1. Labs A and B datasets. EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (29.6 mg/kg and 81.1%). The slope is 20.5 and the EC10 is 31.1 mg/kg. Note: the gray point denotes egg batch with quality problems noted by authors and was not used in the analysis.

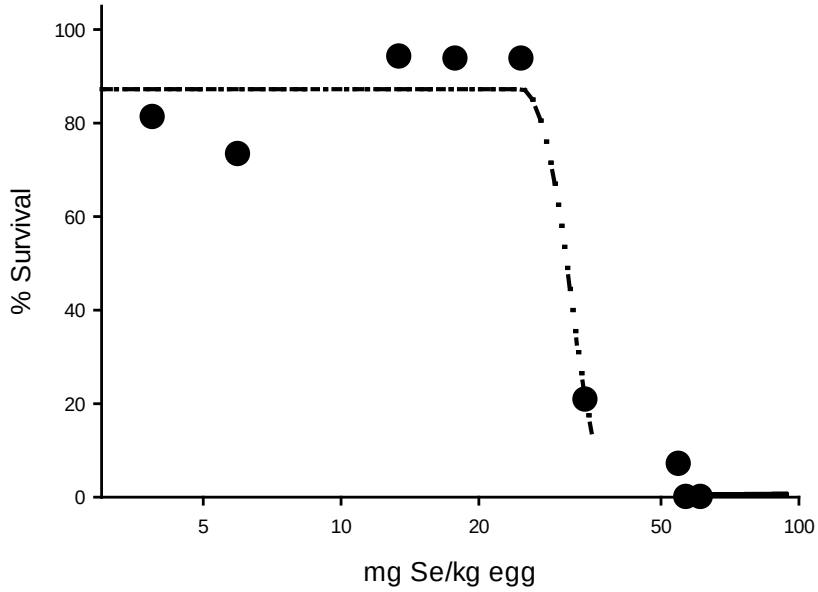


Figure 2. Lab A dataset.* EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (24.8 mg/kg and 87.25%). The slope is 9.4 and the EC10 is 27.7 mg/kg.

*Although some scientists have attempted to explain certain occurrences of improved response with increasing concentration in terms of nutrient selenium sufficiency-deficiency, the concentrations involved in this study are too high to for selenium deficiency to be an explanation. The figure's apparent bi-phasic measured response is thus best explained as being a chance outcome of noise.

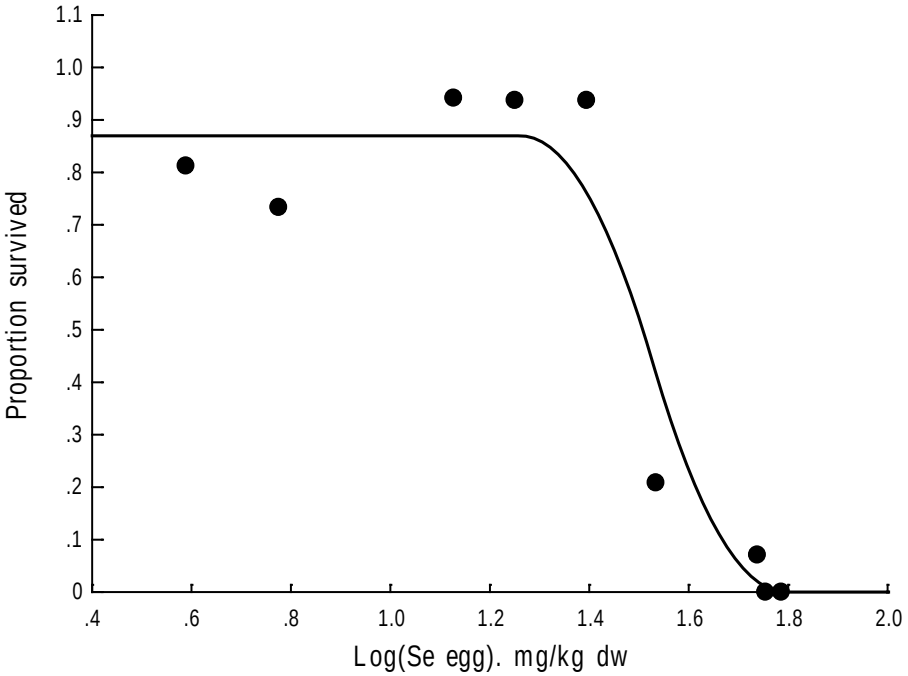


Figure 3. (From previous draft) Tolerance distribution; Model option – Triangular distribution (3 parameter). Includes Laboratory "A" dataset only TRAP EC_{10} estimate = 24.0 mg/kg.

Golder Associates. 2009. Development of a Site-specific Selenium Toxicity Threshold for Dolly Varden Char. Report to Northgate Minerals Corporation, PO Box 3519, Smithers, British Columbia. Report Number 04-1421-101/2000.

Test Organism: Dolly Varden (*Salvelinus malma*)

Exposure Route: Field collected.

Adult Dolly Varden char were collected from reference (North Kemess Creek), high Se exposure (Upper Waste Rock Ponds and Creek) and moderate Se exposure (lower Waste Rock Creek) sites during September 22 to 24, 2008. Eggs were stripped from females and fertilized with milt from males collected from the reference site. Fertilized eggs were taken to the laboratory for testing.

Test duration: The test was terminated when 90% of the larvae reached swim-up, approximately 5 months after fertilization.

Study Design: Approximately 30 fertilized eggs were added to each replicate rearing container. The number of replicates per female parent ranged from one to four depending on the number of eggs available. Embryos were maintained in 4 L containers with 3.5 L dechlorinated tap water in a static-renewal system (3 renewals times/week) at 5°C. The condition of the embryos and alevins were observed daily and any dead individuals were counted and removed. Test termination occurred over a 3-day period during February 11 to 13, 2009. The hatched larvae were sacrificed using an overdose of the anesthetic, clove oil. Individual length and weight were measured on each fry, and deformity analysis was performed on fresh unpreserved larval fish using 40X magnification.

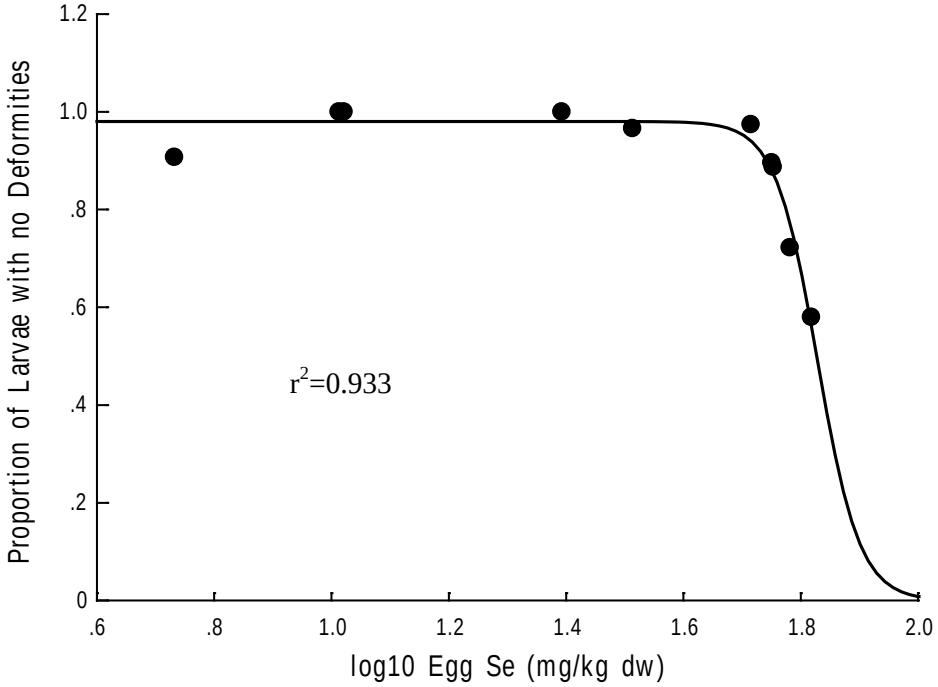
A graduated severity index (GSI) was used for deformity assessment (skeletal, craniofacial, and finfold as well as edema). The narrative criteria were the same as used by Holm et al. (2005) and Rudolph et al. (2008).

Effects Data: Alevin survival was not related to Se concentration in the eggs (Table 1). Almost all of the mortality occurred during the egg stage. Only 4 alevins died during the study, 1 from Fish #19 and 3 from Fish #2, both females collected at an exposed site. The prevalence of deformities increased sharply after the selenium egg concentration exceeded 50 mg/kg dw (Table 1, Figure 1). The proportion of Dolly Varden larvae with any type of deformity (skeletal, craniofacial, and finfold as well as edema) as a function of the log of the selenium concentration in the eggs using TRAP (logistic equation) produced an EC_{10} value of 56.22 mg/kg dw eggs (Figure 1).

Table 1. Selenium concentration in the eggs of Dolly Varden char and the survival of alevins to the swim-up stage and the proportion of larvae without any type of deformity.

Fish #	Sample ID	Location	[Se] eggs mg/kg dw	Survival of eggs to swim-up			Proportion of larvae without any type of deformity
				Initial	End	%	
1	WRC-F105	Waste Rock Creek	56.6	120	71	59	0.89
2	WRC-F61	Waste Rock Creek	65.8	120	81	68	0.58
5	WRC-F103	Waste Rock Creek	32.6	29	29	100	0.97
6	WRC-F83	Waste Rock Creek	51.9	120	115	96	0.97
15	WRC-F104	Waste Rock Creek	56.3	60	48	80	0.90
19	WRC-F86	Waste Rock Creek	60.5	120	115	96	0.72
9	NK-F30	North Kemess Creek	11	30	1	3	a
12	NK-F29	North Kemess Creek	10.5	46	15	33	1.00
17	NK-F21	North Kemess Creek	5.4	90	86	96	0.91
SCD1	Redd #1	Southern Collection Ditch	10.3	30	18	60	1.00
SCD2	Redd #2	Southern Collection Ditch	24.7	40	32	80	1.00

Figure C-1. Proportion of Dolly Varden alevin without any type of deformity as a logistic function of the logarithm of the selenium concentration in eggs (TRAP).



	Guess	Final	SE	95% LCL	95% UCL
LogX50	1.844	1.829	0.007	1.812	1.845
Slope	4.152	6.963	1.252	4.003	9.924
Y0	0.975	0.980	0.017	0.939	1.021

ECx	EC	95% LCL	95% UCL
50	67.42	64.92	70.01
20	60.12	57.96	62.35
10	56.22	53.00	59.64
5	52.85	48.64	57.43
1	46.11	40.13	52.99

	DF	SS	MS	F	P
Total	9	1.74E-01	1.93E-02		
Model	2	1.63E-01	8.13E-02	51.429	0.99993
Error	7	1.11E-02	1.58E-03		

AECOM. 2012. Reproductive success study with brown trout (*Salmo trutta*). Data quality assurance report. Final. December 2012.

Formation Environmental. 2011. Brown Trout Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion. Prepared for J.R. Simplot Company by Formation Environmental. Revised October 2011.

Test Organism: Brown trout (*Salmo trutta*)

Exposure Route: Field collected.

Adult female and male brown trout were collected at three field sites from two streams downstream of the Smokey Canyon mine. In addition, brown trout eggs were obtained from two hatcheries as method controls.

Test duration: Embryo-larval monitoring to 15 days post swim-up.

Study Design: Eggs were collected from 26 ripe female brown trout at three field sites downstream of the Smokey Canyon mine. These included one site on the highly impacted Sage Creek (LSV2C) as well as two sites along Crow Creek (CC-150 and CC-350) downstream of the conflux with Sage Creek. The downstream – most station along Crow Creek (CC-150) was intended to be a field control. Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. Selenium was measured in adult fish (whole body) and in eggs of field collected females. In addition, eggs were collected from 8 ripe females obtained from the Saratoga National Fish Hatchery (SC) to serve as method controls. Similar to field-caught fish, SC hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. As a result of lower than expected hatch rates and fungal contamination in some SC hatchery samples, additional hatchery fish were obtained (as already fertilized eyed embryos) from the Spring Creek Trout Hatchery (SPC), which were divided into four treatments.

Approximately 600 fertilized eggs from each female (or 600 eyed embryos for SPC treatments) were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15-day post swim up feeding trial. Test termination ranged from 83 to 88 days after hatch for all but the Spring Creek Hatchery egg treatments, which occurred 50 days after the arrival of fertilized, eyed embryos from that hatchery.

Endpoints measured in the laboratory study were fecundity, hatch, growth, survival/mortality, and feeding success (growth) post swim up. Larval brown trout were also evaluated for deformities (craniofacial, vertebral, fin) and edema. For this study, deformities were combined and assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Se concentrations in eggs ranged from 6.2-12.8 mg Se/kg dw at CC150, 6.9-14.0 mg Se/kg dw at CC350, and 11.2-40.3 mg Se/kg dw at LSV2C. Se concentrations in hatchery eggs ranged from 0.76-1.2 mg Se/kg dw at the SC hatchery, and were 0.73 mg Se/kg dw at the SPC hatchery. The Se whole body concentration in field collected fish ranged from 7.2-22.6 mg/kg dw at LSV2C, 4.7-8.4 mg/kg dw at CC150, and 5.5-9.2 mg/kg dw at CC350. Se whole body concentrations in SC hatchery fish ranged from 2.5-4.3 mg/kg dw. Hatchery data were combined with field data and included in all analyses.

Three endpoints were considered for purposes of calculating an EC_{10}. These were percent survival, percent fully free from deformities, and percent surviving and normal. Initially, data for these endpoints were combined and analyzed for both portions of the test: hatch through swim up and the 15-day post swim feeding trial. Data for these endpoints over both portions of the test are shown in Tables 1-3.

A U.S. Fish and Wildlife (2012) review of the Formation Environmental (2011) report suggested that fish lost due to an overflow even resulting from a drain the became clogged with food during the 15-day post swim up portion of the test were more likely to have been dead or deformed, and proposed that all treatments that lost fish to the overflow event should be excluded from the EC_{10} calculation. In the 2014 and 2015 draft Se documents, endpoints assessed for the hatch through 15-day post swim up test were analyzed using two scenarios. In the "worst-case" scenarios, the hypothesis from the USFWS review was examined, by treating all fish lost to overflow as either dead or deformed, rather than excluding those treatments altogether. In the "optimistic" scenario, the overflow event was treated as a random technician error unrelated to selenium toxicity, and any lost fish were removed from the calculation. In other words, fish lost to overflow were assumed to be equally likely to have been dead or deformed compared to fish that were not lost.

Because of the importance of these data for the numeric criterion calculation, and because of several experimental factors that resulted in the calculation of several reasonable EC_{10}s, such as the loss of fish due to an overflow event described above, EPA conducted a careful and thorough reanalysis of the study data and subjected the reanalysis to independent, external peer review (ERG 2012) to confirm the validity and scientific robustness of the approach taken by EPA in the reanalysis and use of the reanalyzed data. Those assessments were then superseded by a reanalysis of a more complete enumeration of the deformity counts provided by AECOM (2012). All analyses reported in the 2014 and 2015 draft Se documents and the current Se document used values from the updated dataset provided by AECOM (2012).

Hatch Through 15-Day Post Swim Up Combined Data

In the 2014 and 2015 draft Se documents, data for three endpoints, survival, deformities, and combined survival+deformities were considered for both portions of the test. The first portion of the test was from hatch through swim up, lasting 88 days (on average). The second portion was the 15-day post-swim up

feeding trial. None of the fry from the five treatments with Se concentrations of 26.8 mg/kg and higher reached swim-up. However, surviving fry from those treatments were included in the post-swim up feeding trial.

Combined Survival and Deformity Endpoint

Selenium concentrations and counts of total larvae, and counts of proportions of fully normal larvae (alive and normal) are included in Table 1. Background percentages of live and normal individuals were extremely variable and often low (Figure 1). In the 2014 draft document, EC_{10}s for the optimistic (21.16 mg/kg) and worst case (20.65 mg/kg) scenarios were calculated, and these were also reported in the 2015 draft document. Although there is a clear demarcation between treatments equal to or less than 20.5 µg/L and treatments equal to or greater than 26.8 µg/L, suggesting an effect level between these concentrations, a careful reanalysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} cannot be calculated because of the high background variability.

Table 1. Brown trout selenium concentrations and survival + deformity data (combined endpoint) from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
SC-001	3.6	0.76	63		63	115	8		123	0.512
SC-002	4.1	0.94	72		72	113	4		117	0.615
SC-003	3.7	0.83	131		131	302	7	9	309	0.424
SC-004	4.3	0.92	46		46	140	28		168	0.274
SC-005	3	1.2	23		23	42	6		48	0.479
SC-006	3.1	1.2	457		457	535	8		543	0.842
SC-007	2.7	1	93		93	137	30		167	0.557
SC-008	2.5	0.96	283		283	359	6	10	365	0.775
SPC-001[c]		0.73	427		427	570	8		578	0.739
SPC-002[c]		0.73	371		371	545	20		565	0.657
SPC-005[c]		0.73	400		400	561	8		569	0.703
SPC-006[c]		0.73	427		427	556	17		573	0.745
CC-150-009	8.4	12.8	106		106	142	11		153	0.693
CC-150-011	5.6	8.4	87		87	266	2		268	0.325
CC-150-012	6.7	8.5	156		156	282	12		294	0.531
CC-150-013	5.9	8.4	137		137	310	46	26	356	0.385
CC-150-015	6	9.1	210		210	445	14		459	0.458
CC-150-016	7	7.5	13		13	23	3	43	26	0.500
CC-150-017	5.6	6.6	99		99	163	7	33	170	0.582
CC-150-018	4.7	6.9	195		195	486	16		502	0.388
CC-150-020	7.2	6.2	453		453	558	6		564	0.803
CC-350-006	9.2	14	120		120	386	26		412	0.291
CC-350-007	5.5	6.9	68		68	131	10	20	141	0.482
CC-350-008	8.5	9.5	269		269	338	21	28	359	0.749
LSV2C-002	8.9	12.8	483		483	544	4	16	548	0.881
LSV2C-003	13.8	40.3	2	2	0	0	395		395	0.000
LSV2C-004	17.9	36	16	16	0	0	289		289	0.000
LSV2C-005	13.6	26.8	8	8	0	0	267		267	0.000
LSV2C-008	9.6	17.7	147		147	194	4	45	198	0.742

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
LSV2C-010	22.6	38.8	5	5	0	0	97		97	0.000
LSV2C-012	7.2	13.2	217		217	554	17		571	0.380
LSV2C-016	9.2	13.4	440		440	530	20		550	0.800
LSV2C-017	13.2	20.5	110		110	150	28	19	178	0.618
LSV2C-019	8.6	12.5	267		267	390	22	39	412	0.648
LSV2C-020	11.3	11.2	240		240	296	5	36	301	0.797
LSV2C-021	20	28.1	8	8	0	0	404		404	0.000

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek
[b] Test end was 15 days after swim up.
[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

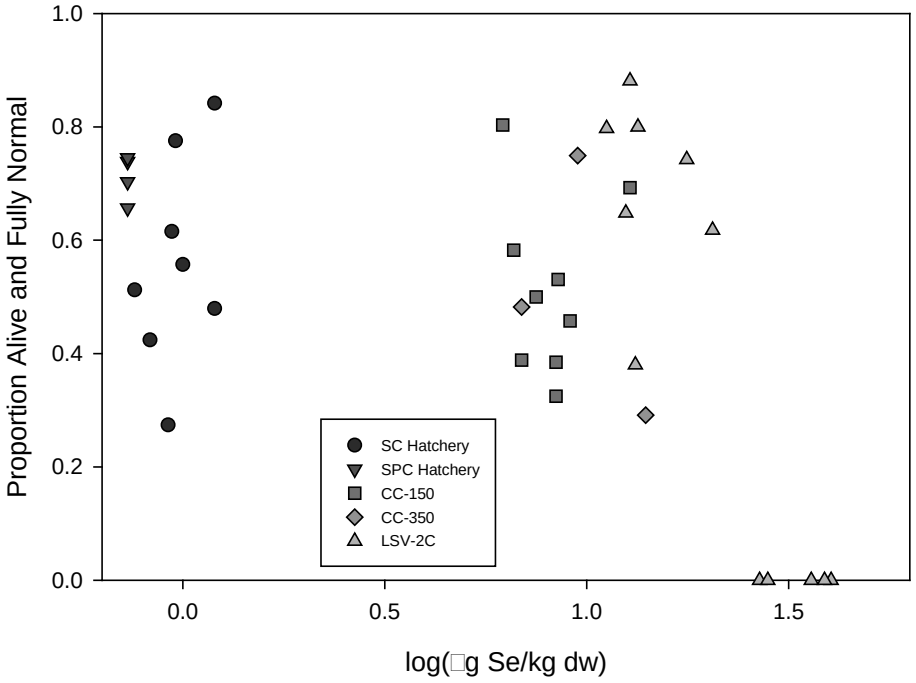


Figure 1. Proportion of alive and normal larvae plotted against Se concentrations in eggs. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

Deformity Endpoint

Selenium concentrations, counts of larvae assessed for deformities, and counts and proportions of normal larvae are included in Table 2. As with the combined endpoint, background (at or below 20.5 mg/kg) proportions of deformities were highly variable (Figure 2). In the 2014 draft document, EC_{10}s were calculated for both the optimistic and worst case scenarios, and the EC_{10} of 15.91 mg/kg for the worst case scenario was used as the EC_{10} for Salmo. During the review phase following the release of the 2014 draft Se document, several public commenters noted that because of the high variability, more than one EC_{10} could be calculated by TRAP for both the optimistic and the worst case scenarios depending on the initial model conditions, in particular the slope of the falling limb of the concentration-response curve. For the optimistic scenario, EC_{10}s based on initial conditions ranged from 16.36-21.95 mg/kg, and for the worst case scenario, EC_{10}s based on initial conditions ranged from 15.91-21.58 mg/kg. In order to evaluate the most appropriate EC_{10} for the deformity endpoints, models were evaluated based on residual sum of squares, and the EC_{10} for the model with the lowest residual sum of squares was selected as the most appropriate. For the worst case scenario deformity endpoint, the model with the lowest residual sum

of squares was the EC_{10}=21.58 mg/kg model, and for the optimistic deformity endpoint, the model with the lowest residual sum of squares was the EC_{10}=21.94 mg/kg model.

These variable EC_{10}s were the result of large variability in background concentration, with several treatments at low Se concentrations experiencing greater than 60% deformities (Figure 2). Although there is clear evidence of an effect between the 20.5 and 26.8 mg/kg concentrations, because of this high background variability, a careful re-analysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} could be calculated for the deformity endpoint.

Some of the background variability in deformities appears to be the result of differences among field sites. For example, deformity rates among field samples appear to be greater for fish hatched from eggs collected in the two Crow Creek sites (CC-150, CC-350) compared to Sage Creek (LSV-2C) (Figure 2). If the result of higher background deformities among Crow Creek sites is not a random artifact, it suggests a confounding factor, unrelated to selenium exposure. Whether the higher deformity rates represent random variation, population differences, other environmental quality differences (unrelated to Se), or methodological issues is unclear**.**

Table 2. Brown trout selenium concentrations and deformity data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
SC-001	3.6	0.76	63	115		0.548
SC-002	4.1	0.94	72	113		0.637
SC-003	3.7	0.83	131	302	9	0.434
SC-004	4.3	0.92	46	140		0.329
SC-005	3	1.2	23	42		0.548
SC-006	3.1	1.2	457	535		0.854
SC-007	2.7	1	93	137		0.679
SC-008	2.5	0.96	283	359	10	0.788
SPC-001[c]		0.73	427	570		0.749
SPC-002[c]		0.73	371	545		0.681
SPC-005[c]		0.73	400	561		0.713
SPC-006[c]		0.73	427	556		0.768
CC-150-009	8.4	12.8	106	142		0.746
CC-150-011	5.6	8.4	87	266		0.327
CC-150-012	6.7	8.5	156	282		0.553
CC-150-013	5.9	8.4	137	310	26	0.442
CC-150-	6	9.1	210	445		0.472

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
015						
CC-150-016	7	7.5	13	23	43	0.565
CC-150-017	5.6	6.6	99	163	33	0.607
CC-150-018	4.7	6.9	195	486		0.401
CC-150-020	7.2	6.2	453	558		0.812
CC-350-006	9.2	14	120	386		0.311
CC-350-007	5.5	6.9	68	131	20	0.519
CC-350-008	8.5	9.5	269	338	28	0.796
LSV2C-002	8.9	12.8	483	544	16	0.888
LSV2C-003	13.8	40.3	2	100		0.020
LSV2C-004	17.9	36	16	142		0.113
LSV2C-005	13.6	26.8	8	149		0.054
LSV2C-008	9.6	17.7	147	194	45	0.758
LSV2C-010	22.6	38.8	5	80		0.063
LSV2C-012	7.2	13.2	217	554		0.392
LSV2C-016	9.2	13.4	440	530		0.830
LSV2C-017	13.2	20.5	110	150	19	0.733
LSV2C-019	8.6	12.5	267	390	39	0.685
LSV2C-020	11.3	11.2	240	296	36	0.811
LSV2C-021	20	28.1	8	172		0.047

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

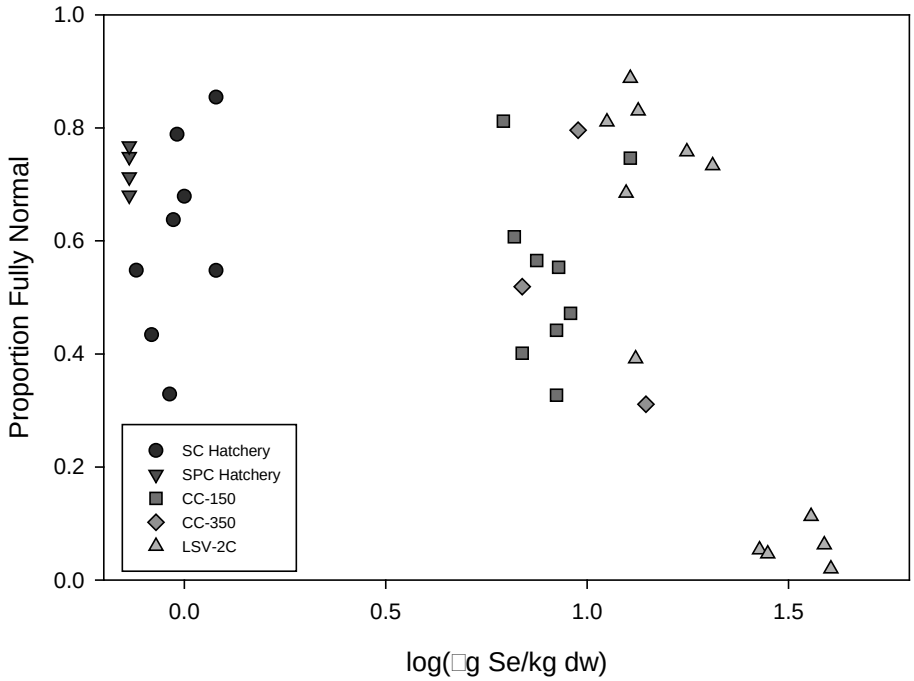


Figure 2. Proportion of normal (free from deformities) larvae plotted against Se concentrations in eggs, hatch through 15-days post swim up. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

Survival Endpoint

Selenium concentrations and estimated counts and proportions of larvae surviving from hatch through 15 days post swim up are included in Table 3. Estimated counts and proportions were reported for survival through the 15-day post swim up test because larvae were thinned to a target of 100 individuals/treatment prior to the onset of the post swim up test, and final full test survival is calculated as the product of survival from hatch to swim up and survival during the 15-day post swim up test. In the 2014 draft document, EC_{10}s were calculated for the worst case (16.78 mg/kg) and optimistic (20.40 mg/kg) survival scenarios, and these were also reported in the 2015 draft document. For both scenarios, the assumption was made that fry that failed to swim up would not have survived, and so the survival for the post swim up portion of the test in the 5 treatments with the highest selenium concentrations (26.8 mg/kg and above) was set to zero. The EC_{10} of 16.78 mg/kg for the optimistic is nearly identical to the EC_{10} for the worst case survival scenario of 16.76 mg/kg presented in the response to the FWS review of the Formation Environmental study (Taulbee et al. 2012), peer reviewed by ERG (2012).

In contrast to the deformity and combined deformity+survival endpoints, background survival (concentrations up to and including 20.5 mg/kg) was much less variable. Despite the lower variability among background effect levels, a careful re-examination of these data following the release of the 2015 draft Se

document determined that a meaningful EC_{10} cannot be calculated by TRAP so long as the assumption is made that fry failing to reach swim up are assumed to be dead. This is because TRAP requires at least 2 partial effects to calculate an EC_{10}, and this dataset has no partial effects, but rather, a background range with high and relatively stable survival through 20.5 mg/kg, and then no survival at concentrations of 26.8 mg/kg and above (Figure 3). In order to calculate an EC_{10} for survival, the assumption regarding fry that failed to swim up was removed. In addition, in order to remove the uncertainty introduced by the clogged drain leading to the overflow and loss of fish from some of the treatments in the post swim up test, the EC_{10} for larval survival was calculated for the much longer hatch through swim up portion of the test, as described below.

Table 3. Brown trout selenium concentrations and survival data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
SC-001	3.6	0.76	144	0.951	0.990	0.942
SC-002	4.1	0.94	138	0.978	0.990	0.968
SC-003	3.7	0.83	340	0.982	0.989	0.971
SC-004	4.3	0.92	189	0.868	0.971	0.842
SC-005	3	1.2	70	0.914	1.000	0.914
SC-006	3.1	1.2	564	0.988	0.990	0.978
SC-007	2.7	1	188	0.856	0.970	0.830
SC-008	2.5	0.96	396	0.985	1.000	0.985
SPC-001[c]		0.73	598	0.987	1.000	0.987
SPC-002[c]		0.73	20	1.000	1.000	1.000
SPC-003[c]		0.73	585	0.966	1.000	0.966
SPC-004[c]		0.73	21	1.000	1.000	1.000
SPC-005[c]		0.73	589	0.986	1.000	0.986
SPC-006[c]		0.73	593	0.971	1.000	0.971
CC-150-009	8.4	12.8	173	0.942	0.990	0.933
CC-150-011	5.6	8.4	288	0.993	1.000	0.993
CC-150-012	6.7	8.5	314	0.965	0.990	0.955
CC-150-013	5.9	8.4	402	0.891	0.973	0.866
CC-150-015	6	9.1	479	0.971	1.000	0.971
CC-150-016	7	7.5	89	0.966	1.000	0.966
CC-150-017	5.6	6.6	223	0.969	1.000	0.969
CC-150-018	4.7	6.9	522	0.969	1.000	0.969
CC-150-020	7.2	6.2	584	0.990	1.000	0.990
CC-350-006	9.2	14	432	0.944	0.980	0.926
CC-350-007	5.5	6.9	181	0.950	0.988	0.938
CC-350-008	8.5	9.5	407	0.951	0.986	0.938
LSV2C-002	8.9	12.8	584	0.993	1.000	0.993
LSV2C-003[d]	13.8	40.3	404	0.079	0.281	0.022
LSV2C-004[d]	17.9	36	309	0.414	0.477	0.197
LSV2C-005[d]	13.6	26.8	287	0.387	0.622	0.240
LSV2C-008	9.6	17.7	263	0.989	0.982	0.971
LSV2C-010[d]	22.6	38.8	108	0.231	0.440	0.102
LSV2C-012	7.2	13.2	591	0.971	1.000	0.971

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
LSV2C-016	9.2	13.4	570	0.965	1.000	0.965
LSV2C-017	13.2	20.5	217	0.885	0.963	0.852
LSV2C-019	8.6	12.5	471	0.953	1.000	0.953
LSV2C-020	11.3	11.2	357	0.986	1.000	0.986
LSV2C-021[d]	20	28.1	424	0.288	0.730	0.210

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.[d] Survived but failed to reach swim up. Assumed dead in all hatch to 15-day post swim up analysis.

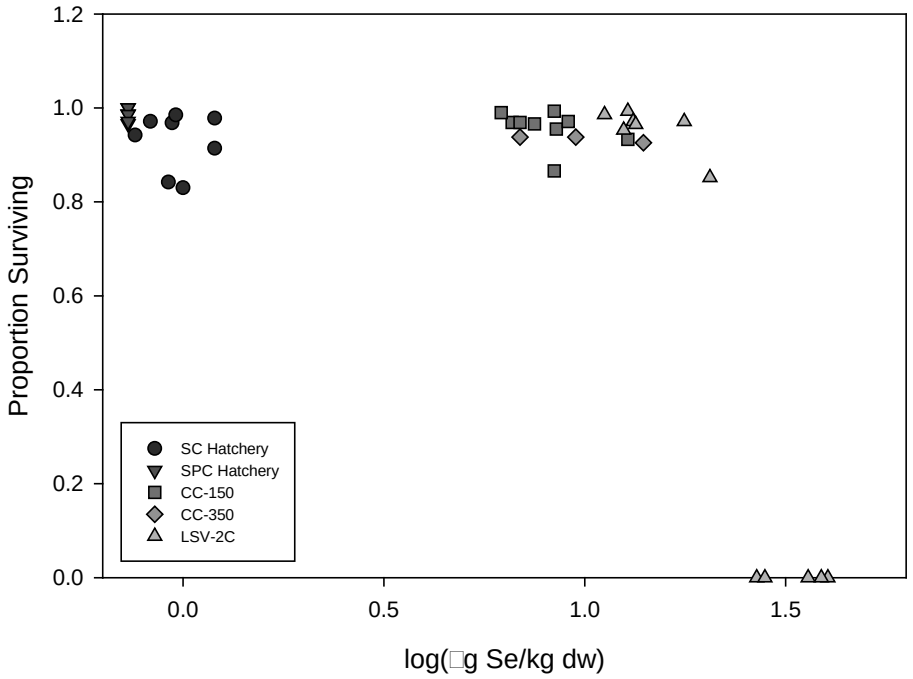


Figure 3. Proportion of larval survival plotted against log transformed Se concentrations in eggs, hatch through 15-day post swim up. Larvae from the five highest Se concentration treatments failed to reach swim up and were assumed to not have survived in the wild.

<u>Assessment of Overflow Loss During 15-day Post Swim Up Feeding Trial</u>

In the 2015 draft Se document, an assessment was made to determine whether the loss of fish from the overflow event during the 15-day post swim up portion of the test was related to survival or to Se treatment concentration measured during the first portion of the test. In this assessment, data were examined from the perspective of whether the overflow loss of brown trout during the second stage of the test could reflect dead, dying, or weak organisms. This was done to examine the hypothesis proposed in the U.S. FWS review that fish lost to overflow were either dead or dying.

First, the relationship between larval survival in the first and second stages of the test (hatch to swim up, 15 days post swim up) were compared for all treatments where larvae successfully reached the swim up stage (Figure 4). Overall, survival in the second stage tracks survival in the first stage (r^2=0.6), but survival in the second stage was noticeably higher in than in the first stage. This result is consistent with the following statement made by the principle scientist of the brown trout study in the public comments to the 2014 selenium draft document submitted for external peer review: "escaped fry were observed swimming in the water bath where the treatment containers were being held. These fry congregated near the treatment cells. Dead or dying fish were not observed."

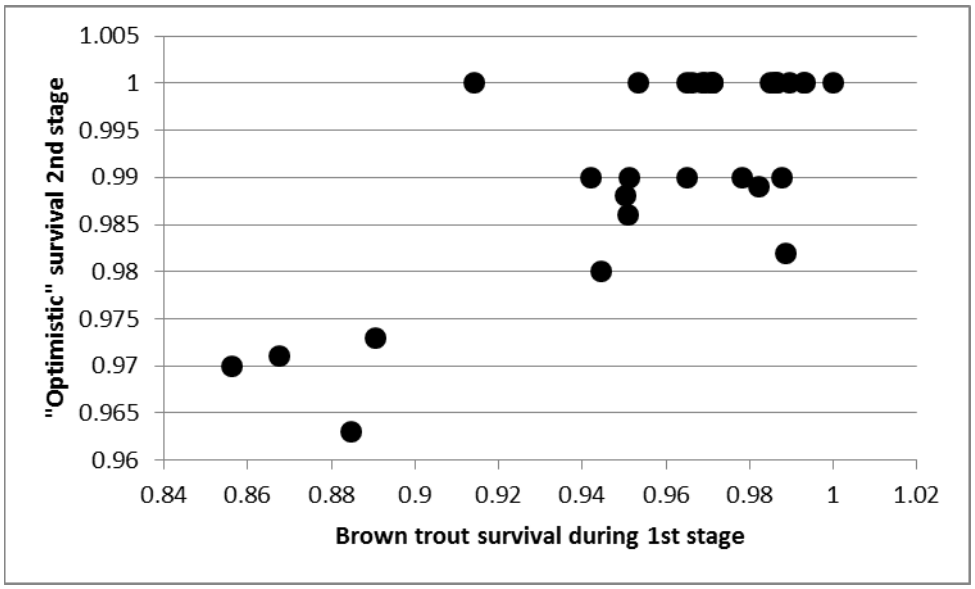


Figure 4. Relationship between survival during the first and second portions of the test. All treatments where larvae successfully reached swim up (Se concentrations of 20.5 mg/kg and lower).

Second, the relationship between larval mortality in the first stage and overflow loss in the second stages of the test (hatch to swim up, 15 days post swim up) were compared separately for all treatments (field and hatchery) and for all field collected treatments (Figure 5). As with figure 4, these correlations were made for treatments where larvae successfully reached the swim up stage. In these instances, there is no apparent relationship between health, as reflected by mortality in the first stage, and overflow loss in the second stage, whether considering all individuals or wild-only: r^2 for both graphs is 0.0. The lack of a relationship in these correlations suggests that overflow loss has a likelihood of being a random noise variable.



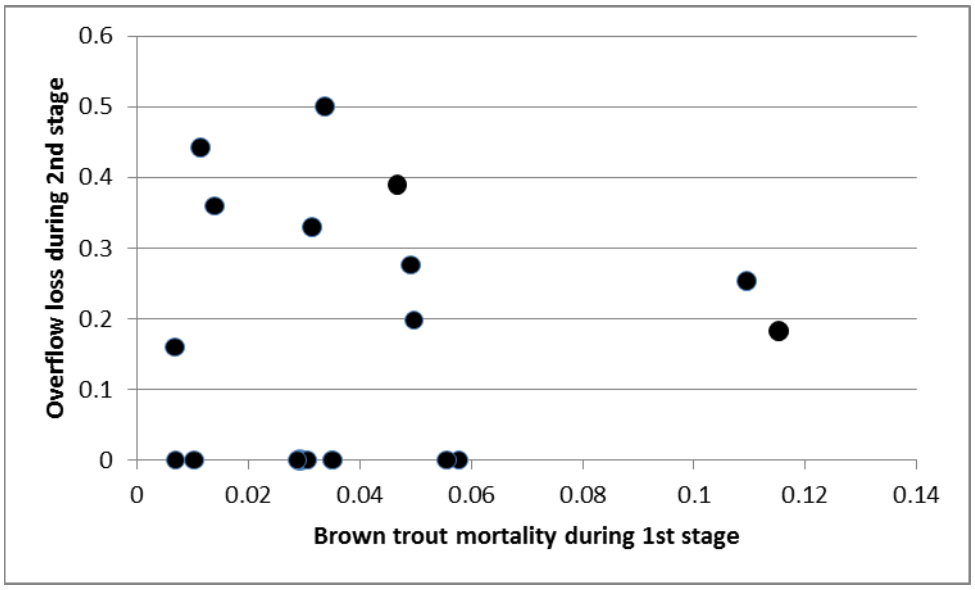


Figure 5. Relationship between mortality during the first stage of the test and overflow loss during the second stage of the test. Upper figure – all hatchery and field treatments. Lower figure – field treatments only. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

Finally, the relationship between overflow loss and selenium concentrations in eggs was examined (Figure 6). As with previous correlations, only larvae from treatments where individuals reached swim up were considered.

Figure 6 shows a clear difference between hatchery (far left) and field treatments, but across the concentration range for the offspring of field collected fish there is no apparent relationship between overflow loss and Se concentration. Within the field treatments, the r^2 of the correlation between Se concentration and overflow loss is 0.01. Although there are no known genetic differences between hatchery

and wild fish, if leaving the aquarium required swimming over the rim, one might speculate that previous generations of hatchery fish might have developed a tolerance to remaining in conditions that might seem crowded to wild organisms. (That is, however, purely speculative.) Otherwise, the difference between hatchery and wild fish would seem only to reflect a random artifact, since the Se concentrations at which the wild fish displayed high overflow losses are low.

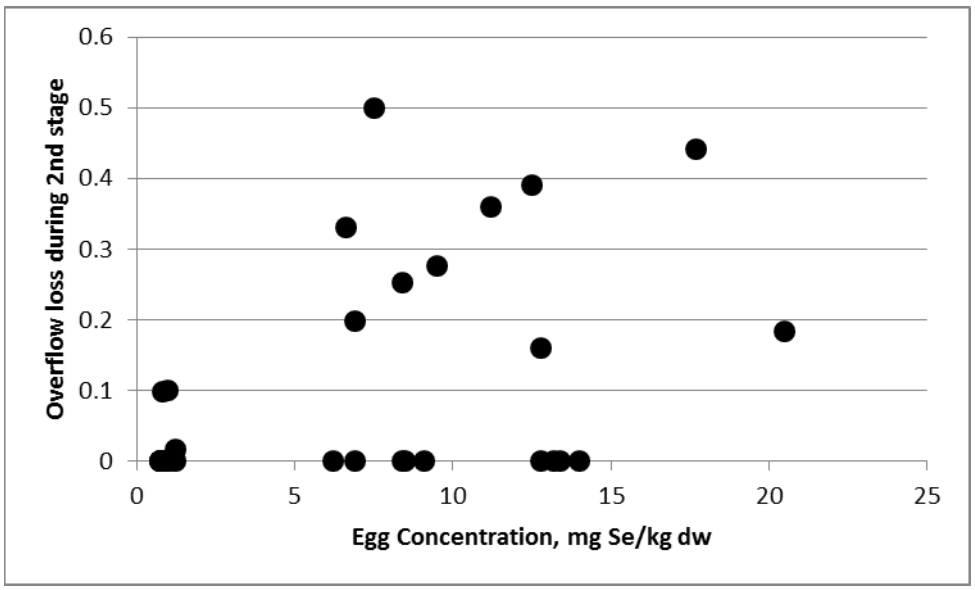


Figure 6. Relationship between egg Se concentration and overflow loss during the second stage of the test. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

In summary, the positive correlation between survival during the hatch to swim up portion of the test and survival during the 15-day post swim up portion of the test, combined with the lack of a correlation between mortality during the hatch to swim up portion of the test and overflow loss during the second stage of the test, suggests that the overflow loss likely represents a random technician error not related to the health of the individuals lost. The relationship between selenium egg concentrations and overflow loss was lower for the larvae hatched from hatchery fish compared to the larvae hatched from field collected fish; however, among field treatments ranging from 6.0-20.5 mg/kg there was no correlation, further supporting the hypothesis that the overflow event was a random occurrence unrelated to the health of larval fish.

The results of the above assessment of the overflow event strongly suggest that the overflow event was a random technician error unrelated to selenium toxicity, and that the "optimistic" scenario is also likely more realistic.

Survival Endpoint – EC_{10} for the first portion of the test

Because larval survival was measured at the end of the first portion of the test (hatch to swim up), an alternative approach to measuring survival would be to

calculate the brown trout EC_{10} for survival for only the first portion of the test. Selenium concentrations and counts of total larvae and larvae that survived the first portion of the test are included in Table 4. The hatch to swim up portion of the test was much longer than the second portion (88 days on average compared to 15 days), and more importantly, it avoids the experimental confound introduced by the loss of fish during the overflow event. With this approach, the second portion of the test would be rejected as inconclusive due to the laboratory accident.

Unlike survival, deformities could not be analyzed for the first portion of the test because of a bias introduced during the thinning process prior to the initiation of the 15-day post swim up portion of the test. During the thinning process, visibly deformed larvae were selectively removed, so that the fish used in the 15-day post swim up test were less likely to have been deformed. Because of this selection bias, only survival could be evaluated from hatch to swim up. Nevertheless, survival appears to be as sensitive an endpoint as deformities or survival+deformities, as all endpoints exhibit background effects (with differing levels of variability) through 20.5 mg/kg, and severe effects at concentrations between 26.8-40.3 mg/kg.

In contrast to survival endpoints measured from hatch through 15 days post swim up, survival for all treatments were included, including larvae from the five treatments of 26.8 mg/kg and higher, where larvae failed to reach swim up. This avoids any potential inconsistency stemming from not knowing whether small percentages of individuals did not swim up in other treatments. In contrast to the previous EC_{10} calculations, this approach is free from all assumptions about individuals lost in the lab accident. In the 2015 draft document, an EC_{10} of 18.09 mg/kg was calculated for this endpoint in TRAP, and this EC_{10} was used as the GMCV for Salmo. During a subsequent review, this EC_{10} was determined to be inappropriate, because it is lower than the 20.5 mg/kg concentration, which with 88.5% survival falls within the variability of the 32 data points at lower concentrations. Compared to the average survival for all 33 background concentration treatments, the survival at 20.5 mg/kg represents an approximately 8% effect.

In order to calculate an EC_{10} that would not fall below the background concentration of 20.5 mg/kg, a weighted least squares linear regression was calculated in TRAP, using a threshold sigmoid model (Figure 7). The model was weighted using the standard deviation of the 33 background concentrations (all concentrations between 0.73-20.5 mg/kg), and the residual standard deviation of the five concentrations between 26.8-40.3 mg/kg. This was done to provide less weight to the more variable, and more uncertain, high Se treatments relative to the less variable background treatments. The EC_{10} for survival using the weighted regression model is 21.0 mg/kg.

One issue with the above TRAP analysis is that to fit the 5 higher effects data well, the EC_0 estimate is pushed down to 16.4 mg/kg, below two of the points in the background range. Also, the fitted curve goes through the data point at 20.5 mg/kg, so that this point is considered to be an EC_8. This is not unreasonable because the response is so steep at concentrations above this point that some effect at this point is plausible. Nevertheless, this point is within the range of the

background and there are insufficient data to say that this concentration is an effect level. Thus, to accept this analysis and use the EC_{10} from this curve requires making a slightly conservative risk management decision that the point at 20.5 mg/kg should be treated as having some effect.

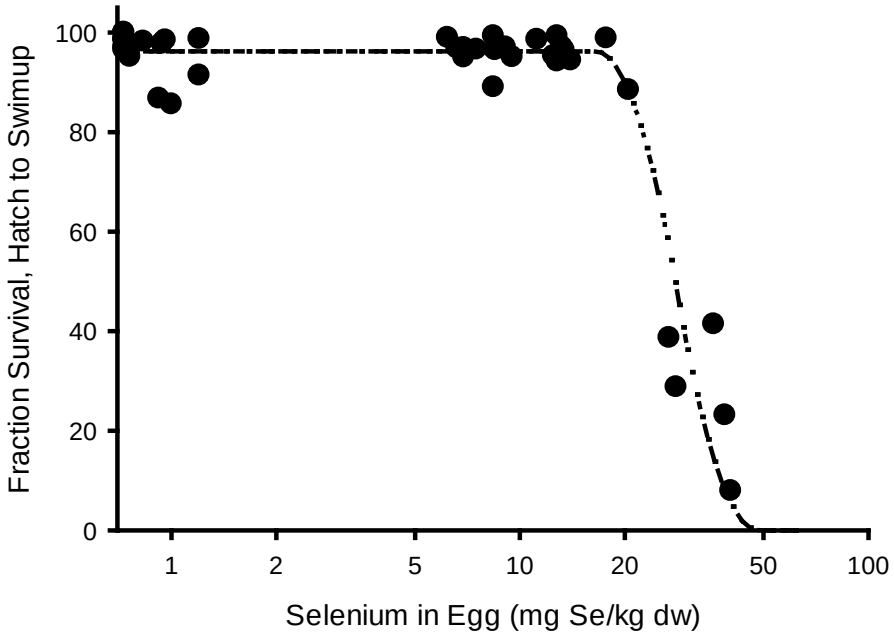


Figure 7. Brown trout survival, hatch to swim up. EC_{10} of 21.0 mg/kg calculated using a weighted nonlinear regression model.

Table 4. Brown trout selenium concentrations and survival data from hatch to swim up (first portion of the test).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SC-001	3.6	0.76	144	137	95.1
SC-002	4.1	0.94	138	135	97.8
SC-003	3.7	0.83	340	334	98.2
SC-004	4.3	0.92	189	164	86.8
SC-005	3	1.2	70	64	91.4
SC-006	3.1	1.2	564	557	98.8
SC-007	2.7	1	188	161	85.6
SC-008	2.5	0.96	396	390	98.5
SPC-001[b]		0.73	598	590	98.7
SPC-002[b]		0.73	20	20	100
SPC-003[b]		0.73	585	565	96.6
SPC-004[b]		0.73	21	21	100
SPC-005[b]		0.73	589	581	98.6

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SPC-006[b]		0.73	593	576	97.1
CC-150-009	8.4	12.8	173	163	94.2
CC-150-011	5.6	8.4	288	286	99.3
CC-150-012	6.7	8.5	314	303	96.5
CC-150-013	5.9	8.4	402	358	89.1
CC-150-015	6	9.1	479	465	97.1
CC-150-016	7	7.5	89	86	96.6
CC-150-017	5.6	6.6	223	216	96.9
CC-150-018	4.7	6.9	522	506	96.9
CC-150-020	7.2	6.2	584	578	99
CC-350-006	9.2	14	432	408	94.4
CC-350-007	5.5	6.9	181	172	95
CC-350-008	8.5	9.5	407	387	95.1
LSV2C-002	8.9	12.8	584	580	99.3
LSV2C-003	13.8	40.3	404	32[c]	7.9
LSV2C-004	17.9	36	309	128[c]	41.4
LSV2C-005	13.6	26.8	287	111[c]	38.7
LSV2C-008	9.6	17.7	263	260	98.9
LSV2C-010	22.6	38.8	108	25[c]	23.1
LSV2C-012	7.2	13.2	591	574	97.1
LSV2C-016	9.2	13.4	570	550	96.5
LSV2C-017	13.2	20.5	217	192	88.5
LSV2C-019	8.6	12.5	471	449	95.3
LSV2C-020	11.3	11.2	357	352	98.6
LSV2C-021	20	28.1	424	122[c]	28.8

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

[c] Survived, but failed to reach swim up.

Whole Body Concentration

The whole-body concentration response curve for survival, hatch to swim up is shown in Figure 8. These data are not amenable to TRAP modeling, and Figure 8 shows the interpolation procedure, the first interpolation point being an EC_0 at 13.2 mg/kg and 96% survival and the second point an LOEC at 13.6 mg/kg and 39% survival. Because the HNOEC (13.2 mg/kg) and LOEC (13.6 mg/kg) are so close, the chronic value for whole body selenium is the HNOEC of 13.2 mg/kg dw.

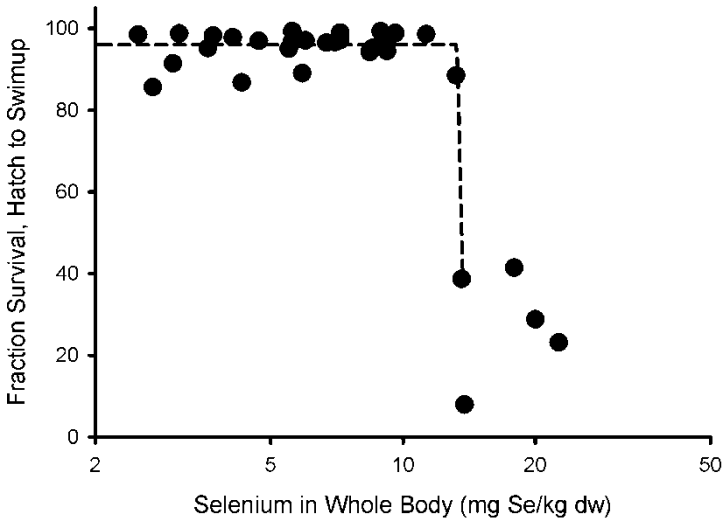


Figure 8. Fraction survival of brown trout larvae as a function of selenium in eggs.

Effect Concentration: For this study the most appropriate, least confounded endpoint is survival, hatch to swim up. For egg selenium, EC_{10} is 21.0 mg Se/kg egg dw, calculated for survival from hatch to swim up using a weighted nonlinear regression model. Expressed as whole body, the chronic value is 13.2 mg Se/kg WB dw.

Besser, J.M., W.G. Brumbaugh, D.M. Papoulias, C.D. Ivey, J.L. Kunz, M. Annis, and C.G. Ingersoll. 2012. Bioaccumulation and toxicity of selenium during a life-cycle exposure with desert pupfish (*Cyprinodon macularius*): U.S. Geological Survey Scientific Investigations Report 2012–5033, 30 p. with appendixes.

Test Organism: Desert pupfish (*Cyprinodon macularius*)

Exposure Route: Dietary and waterborne. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, which had been grown on a diet of selenized yeast.

Test Duration: 180 days life cycle, 21 days F1 larvae, 58 days F1 juveniles and adults.

Study Design: Desert pupfish (*Cyprinodon macularius*), a federally-listed endangered species, were exposed simultaneously to waterborne and dietary selenium at six exposure levels (controls and five selenium treatments) in a three-phase life cycle exposure study. Aqueous exposures were prepared using sodium selenate and sodium selenite salts at an 85%-15% proportion, respectively. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, daily to satiation (25 to 30% rations based on wet weights). Prior to being fed to the pupfish, the oligochaetes were exposed to aqueous selenium and fed selenized yeast at appropriate concentrations to attain the target dietary tissue concentrations. The measured concentrations in water, oligochaetes (pupfish diet), and pupfish tissues for the control and five treatments during the life cycle exposures.

Treatment	water µg/L	oligochaetes mg/kg dw	pupfish, mg/kg dw		
			F_0 WB	eggs	F_1 WB
Control	nd	1.6	0.75	1	1.2
Se–1	3.4	5.1	2.5	3	3.4
Se–2	6.2	7.3	3.4	4.4	3.7
Se–3	14	14	6.7	8	6.7
Se–4	26	24	12	13	12
Se–5	53	52	24	27	31

The 85-day Phase 1 exposure was initiated with approximately five week old juvenile pupfish (F_0). Phase 1 consisted of two separate groups with one group (started two weeks prior to the second group) used for determining survival, growth and whole body selenium concentrations, and the other group used for survival assessment and to provide adults for the main reproduction exposure. Both groups in Phase 1 were similarly exposed to all six treatments, with each treatment having 8 replicates and 10 fish in each replicate.

At the end of the 85-day Phase 1 exposure, the pupfish were reproductively mature and were used for the Phase 2 exposure, the main reproduction study. A preliminary reproduction study was conducted with adults from the first exposure group of F_0 pupfish. These fish were divided into two spawning groups and eggs were collected on four dates during a 9-day period. The main purpose of the preliminary study was to confirm the reproductive maturity of the pupfish, but samples of larvae from this study were used for assessment of deformities. The main reproduction study in Phase 2 was started with adults from the second F_0 exposure. These fish were sorted into spawning groups (1 male and 3 females) in

7-L exposure chambers, with eight replicate spawning groups per selenium treatment. Spawning activity was monitored by removing (and replacing) spawning substrates from each chamber three times a week (Monday-Wednesday-Friday). There were 23 egg collection dates during a 60-day period. All eggs were counted and eggs collected from eight Wednesdays were used for hatching success, deformities and F_1 larval and juvenile growth and survival in the 58-day Phase 3 exposure. Larvae were examined for developmental endpoints including edema, delayed development, and skeletal, eye, craniofacial, and fin deformities.

Effects Data: A summary of the endpoints by each treatment level is shown below.

Table 1. Summary of pupfish toxicity endpoints by exposure treatment (average across all replicates). There were no statistically significant differences across controls and selenium amendment treatments for any of the endpoints shown here (1-way ANOVA, α=0.05).

Endpoint[a]	Control	Se-1	Se-2	Se-3	Se-4	Se-5
F0 survival, day 28	100	100	100	100	100	98
F0 survival, day 56	100	100	100	100	100	100
F0 survival, day 85	100	100	100	100	100	100
F0 survival, day 150	91	94	94	94	91	97
F0 growth, day 28	213	206	204	198	213	203
F0 growth, day 56	535	526	486	469	509	447
F0 growth, day 85	935	998	941	934	914	1053
F0 growth, day 150	1718	1763	1776	1755	1673	1606
F1 survival, day 30	100	100	100	100	98	98
F1 survival, day 58	100	100	93	90	95	88
F1 growth, day 30	73	73	76	78	77	58
F1 growth, day 58	260	264	286	286	288	255
total number eggs	6845	6331	4143	4386	3337	5225
% reduction eggs	NA	8	39	36	51	24
avg % deformities, main	5.3	2.7	4.9	2.4	11.4	8.1
avg % deformities, preliminary	4.4	8.8	11.6	14.3	10.7	21

[a] Endpoint units: survival, %; growth, mg wet weight; % reduction eggs is relative to the control.

The authors observed no significant differences in pupfish survival or growth among treatments. The authors hypothesized the lack of statistically significant acute effects was because the pupfish in this study were near their chronic toxicity threshold, as suggested by the (non-significant) mean reductions in growth (7% in F_0 day 150) and survival (12% in F_1 day 58) in the highest selenium treatment (Se-5), relative to controls (Table 1).

Egg hatching and larval survival in all selenium treatments (not listed in Table 2) were within 10 percent of control means, and differences among treatments were

not related to selenium exposure. The authors noted that the highest selenium treatment, Se-5, did have the lowest larval survival (84%) and lowest combined egg hatching and larval survival (76 percent). The means frequencies of deformities were higher in the two highest Se treatments (Se-4 and Se-5, Table 1); however % deformities across treatment levels were not statistically significant (1-way ANOVA, p=0.13; Beckon et al. (2012). However, overall deformity rates were statistically significantly higher in a preliminary reproduction than in the main reproduction test. Beckon et al. (2012) hypothesized that the reason for the difference in deformity rates between the two tests was related to the time the eggs were collected relative to the time the respective spawning groups were isolated. Eggs were collected in the preliminary reproductive study 1 - 9 days after the spawning groups were isolated, whereas spawns used to characterize deformities in the main reproduction test were collected at least 14 days after the onset of spawning. The larvae produced from the earlier collected eggs may have been exposed to higher selenium concentrations in the egg. The pattern of a gradual decrease in egg selenium concentration over time was observed in the life cycle study.

Egg production varied considerably over the 23 collection dates (Table 2 and Figure 1). Although each of the selenium treatments had a lower total number of eggs relative to the control, one-way ANOVAs of cumulative egg production did not indicate significant differences among treatments on either a per-replicate basis (p=0.34) or on a per-female basis (p=0.20). Similarly, repeated measures ANOVA indicated no differences between treatments, but the authors indicated significant differences among sampling dates and significant interactions of treatment and date. Because of the lower number of eggs in the selenium treatments and the significance of the interaction of treatment and time, the authors concluded that pupfish egg production was adversely affected by elevated selenium exposure and reported significant reductions in egg production at treatment levels Se-2 through Se-5 (4.4 to 27 mg/kg dw Se in eggs). The authors recognized that typically larval survival and deformities are the most sensitive reproductive endpoint for selenium toxicity and not egg production and suggested more study is needed to confirm the unusual sensitivity of pupfish egg production to selenium.

Table 2. Number of pupfish collected on each sampling date throughout the study, by treatment level. Values represent the sum of all eggs collected on a given date for a given Se treatment.

Day	Control	Se-1	Se-2	Se-3	Se-4	Se-5
2	136	112	90	67	122	94
4	275	173	123	142	188	162
7	307	273	301	283	160	432
9	265	252	226	169	271	283
11	401	136	424	319	265	380
14	417	359	333	246	198	401
17	448	456	206	163	145	232
21	303	664	404	204	163	400
23	287	205	141	143	177	175
25	340	308	94	143	150	228
28	366	273	103	101	95	181
30	130	164	104	52	82	132
32	323	304	271	78	75	151
35	320	427	81	150	74	223
37	236	176	41	113	38	38
39	326	151	159	184	113	140
42	507	140	55	193	101	140
44	251	133	66	152	69	137
51	380	359	227	338	305	370
53	278	63	38	197	56	188
56	199	478	138	195	238	222
58	202	329	331	410	143	320
60	148	396	187	344	109	196

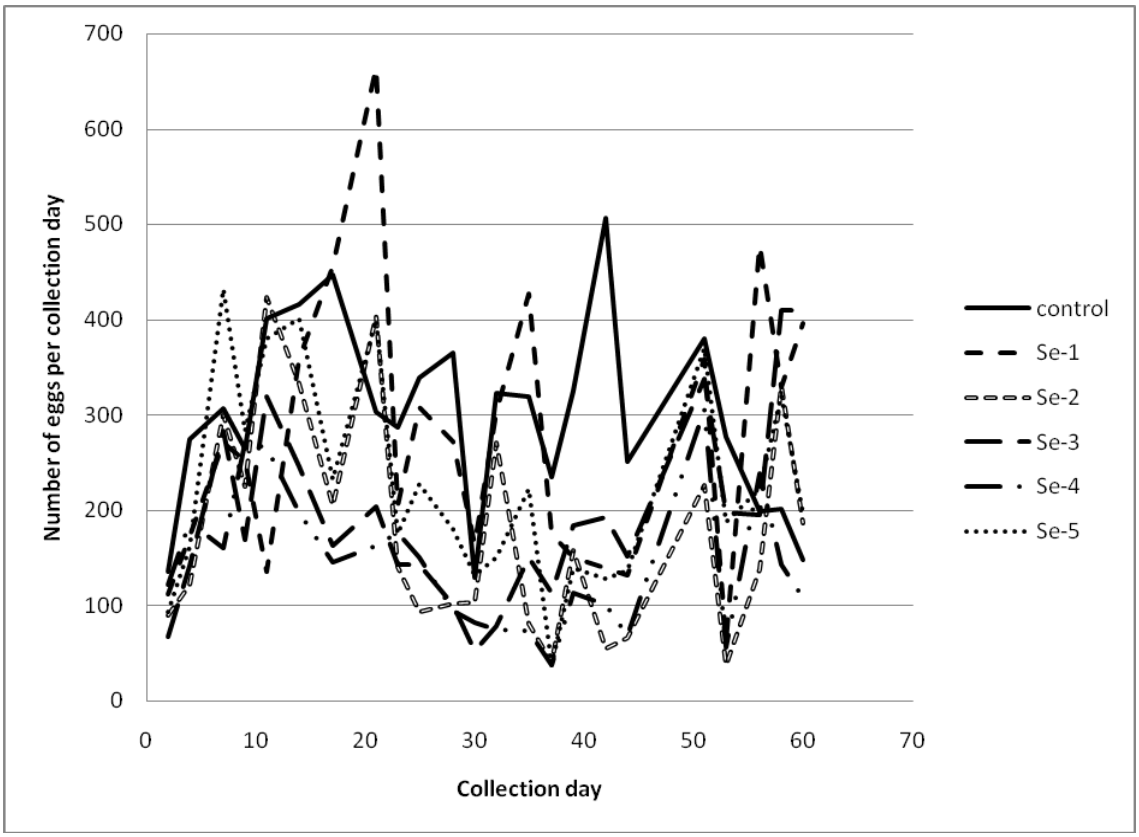


Figure 1. Pupfish egg production by sampling date

Several findings from the pupfish study put a clear demonstration of effect due to selenium in question. The fact that the typical sensitive endpoints for selenium, larval survival and deformities, were not demonstratively responsive to selenium through the highest treatment level, the fact that the egg production data did not show significance among treatments alone, and the fact that egg production increased at the highest selenium treatment level provide sufficient doubt of a clear effect due to selenium. These issues are discussed below.

Examination of the Repeated Measures Analysis:

Analysis Using the Full Dataset: The effects of selenium treatment and sampling date on pupfish egg production (eggs per female per day) were reanalyzed. First, the data were reanalyzed using repeated measures ANOVA. Results of the repeated measures ANOVA analysis were qualitatively similar to those reported in Besser et al. (2012) and are shown in the following table.

Between Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Se treatment	2,202.6	5	440,5	1.755	0.143
Error	10,543.5	42	251.0		

Within Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Sampling Date	1,867.5	22	84.89	4.973	<0.001
Se Treatment x Sampling Date	2,566.3	110	23.33	1.367	0.010
Error	15,771.8	924	17.07		

As with the results reported in Table 7 of Besser et al. (2012), there was no main effect of Se treatment (note – for purposes of these analyses and associated text, "Se treatment" is defined as the control plus the 5 treatments that received Se amendments), but there was a statistically significant ($p \leq 0.05$) effect of sampling date and a significant date by Se treatment interaction. Results were qualitatively similar because the p-values for Se treatment and sampling day were identical in both analyses, yet the p-values for the day by Se treatment interaction term were nearly identical.

A statistically significant sampling date effect means that there were significant differences in overall egg production on different sampling dates. Daily egg production per female ranged from 2.176 on day 2 to a high of 7.294 on day 11, and was variable throughout the study. Of greater interest is the statistically significant day x Se treatment interaction. What this means is, although there was not an overall significant effect of Se treatment on egg production per female, there was a significant Se treatment effect ($p<0.05$) on egg production per female on at least one of the 23 sampling dates.

Analysis after Removal of Control Replicate Outlier: Repeated measures ANOVA analysis confirmed the results reported in Besser et al. (2012). However, as shown on Figure 8b of Besser et al. (2012), one replicate chamber (replicate g) within the control treatment had only one surviving female pupfish from day 7 through the end of the test (day 60), and that replicate also had the highest overall egg production per female of any test chamber. All replicate chambers in all treatments began with three female pupfish, and the replicate described above was the only one with only one surviving female. All three females survived the 60 day test in the majority of the replicate chambers. In order to determine whether the significant date by Se treatment interaction was an artifact of this one test chamber, data were reanalyzed after removing this replicate.

One requirement of repeated measures ANOVA is that the model cannot contain any missing values. An alternative to repeated measures ANOVA when data are missing, and the most commonly followed procedure under these circumstances, is to analyze the data using a mixed model. This was the procedure followed here.

The results of a fully balanced mixed model (no missing data) should be identical to repeated measures ANOVA. As an initial check, the full dataset was reanalyzed as a mixed model. Sample chamber was the random effect parameter, and Se treatment, sampling date, and Se treatment by sampling date were the fixed effect parameters. As expected, the F-ratios for the effects of selenium treatment, sampling date, and the sampling date by Se treatment interaction were identical. Next, the data were reanalyzed after removing data from control replicate g from all sampling dates. Results of this analysis are reported in the table below.

Mixed Model – Fixed Effect

Effect	Numerator df	Denominator df	F-ratio	p-Value
Se Treatment	5	902	1.087	0.366
Sampling Date	22	902	6.042	<0.001
Se Treatment x Sampling Date	110	902	1.310	0.023

T

The statistically significant interaction between Se Treatment and Sampling Date persisted after removal of the potentially anomalous control treatment chamber with one female pupfish. In other words, even after removing the one potentially anomalous control replicate, there were still some individual sampling dates where the effects of Se treatment were statistically significant ($p<0.05$).

Se Treatment x Sampling Date Interaction: When a significant interaction is observed in a repeated measures ANOVA, the next recommended step in the process is to examine each of the repeated measures (sampling dates) separately to identify those dates where the significant difference in Se treatment level occurred. When individual dates for the full dataset (including the replicate with one surviving female) were analyzed separately, there were significant ($p<0.05$) effects of Se treatment level on egg production on days 28, 35, 37, 42, and 53 (1-way ANOVA, $df_{5,42}$). There were no significant Se treatment effects on the remaining 18 sampling dates. ANOVA results are summarized in the table below.

Sampling Date	F-ratio	p-value
28	2.501	0.045
35	2.704	0.033
37	3.351	0.012
42	4.294	0.003
53	3.352	0.012

Because of the large number of comparisons (23 individual ANOVA models for each sampling date), an alpha of 0.05 is inappropriate for this particular analysis. This is because an alpha of $p<0.05$ means that a statistically significant result will be observed 5% of the time due to chance alone (Type I error). In order to control for the increased likelihood of a Type I error when making multiple comparisons, the alpha level of 0.05 was adjusted using Sidak's correction (Abdi 2007). For 23 comparisons and an alpha of 0.05 for one comparison, the adjusted alpha using Sidak's correction is as follows:

$$1 - (1 - 0.05)^{\frac{1}{23}} = 0.0027$$

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As a result, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Each of the 23 sampling dates for the dataset where the replicate chamber from the control treatment with one surviving female pupfish was excluded were also analyzed using one-way ANOVA to determine which sampling dates had significant Se treatment effects. Significant differences among Se treatment levels at alpha 0.05 are shown in the table below.

Sampling Date	F-ratio	p-value
35	2.839	0.027
42	3.164	0.017
53	2.549	0.042

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As with the full dataset, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Summary of Repeated Measures Analysis: This analysis demonstrated that although there was a significant Se treatment by sampling date interaction, regardless of whether or not the control treatment chamber with one female pupfish was excluded, differences among Se treatment levels were only observed for a small subset of the 23 sampling dates. Furthermore, after adjusting alpha to account for multiple comparisons, one-way ANOVA analyses conducted separately for each sampling date to locate the source of the Se Treatment x Sampling Date interaction determined that there were no statistically significant differences among Se treatment levels on any sampling date, precluding the need to perform *post hoc* comparison of means tests to identify significant differences among individual Se treatments.

Combining Effect Metrics Using a Population Model: To improve the certainty of any conclusions to be made about the sensitivity of pupfish to selenium, it is also worthwhile to consider the biological (as opposed to statistical) significance of the observations. But for total egg production, survival, and deformities, the concentration-response curves did not show a sufficient concentration-related effect to calculate an EC10. Nevertheless, because Besser et al. (2012) raised the issue of an interaction of egg production with time, there is a particular concern that there could be a delay in egg production that would reduce population growth rate, even while total numbers of eggs were not significantly affected. This question was evaluated by constructing a population model corresponding to data available from the test.

This modeling approach allows for combining and properly weighting effects on egg production, timing of egg production, and survival. Percent hatch and percent deformities were also considered in alternate calculations. Because the model is only intended for combining the lab data into a unified concentration-response curve, it cannot be interpreted as making real-world population predictions. The relevant data were taken from spreadsheets Besser et al. (2012b and 2012c), which were provided by Besser.

The reproduction and larval endpoints spreadsheet, Besser et al. (2012b), presents egg production at 23 time points. This information thus allows for 23 adult life stages, each assigned its own fecundity. Another page of this spreadsheet provides larval survival data, thus defining survival of the early life stage. The juvenile and adult survival spreadsheet, Besser et al. (2012b), defines a survival rate shared by these life stages.

For each treatment, the data from the test thus provide *all* the needed input for 25 life stages: (1) an embryo-larval stage with its own daily survival probability (along with hatching and deformity percentages, when considered in alternative calculations), (2) a non-reproducing juvenile stage sharing its treatment's daily survival probability with the adult stages, and (3 – 25) 23 short-duration adult stages each with its own egg production, but sharing its treatment's daily survival probability with the treatment's other adult stages. Use of the data is detailed below.

Egg Production: Egg production at the test's 23 observation time points is from the spreadsheet Besser et al. (2012b), expressed as eggs per female per day. The intent of Besser et al. (2012) was for each treatment to have eight replicates, and each replicate was to have one male and three females. Only replicates matching that design were used. Early in the test Control Replicate "g" ended up with only one female, and was therefore not used here. Se-1 Replicate "h" and Se-3 Replicates "d" and "h" had been inadvertently stocked with two males and two females, and were likewise not used here. Table 3 shows the time course of egg production incorporated into the population model. For each treatment, model fecundity, m_i, for life stages i = 3 – 25, is the observed egg production *divided by 2*, in order to provide *female eggs* per female per day.

Percent Hatch: The spreadsheet Besser et al. (2012b) presents percent hatch for eggs collected at selected time points. Within each treatment these were averaged. In selenium reproductive studies percent hatch is often treated as a noise variable unrelated to selenium exposure. Consequently, the population growth calculations were run with and without including percent hatch. When hatch was incorporated into the calculation, daily fecundity was reduced by multiplying by percent hatch.

Deformities: The Besser et al. (2012b) spreadsheet also provides deformity counts for the study's preliminary test and for its main test. Only the main test results were used here. Counts were totaled for each treatment, and a percentage calculated. Population growth calculations were performed both with and without consideration of deformity percentage. For simplicity when considered, a worst case assumption was made that deformed individuals do not contribute to the

population. Percent deformity was thereby handled in manner parallel to percent hatch, by multiplying daily fecundity by percent free of deformity.

Table 3. Life stage durations, and observed eggs per female per day at observation time points for control and selenium treatments, *only with replicates having the design three females and one male*. Model fecundity, m, is set at one-half the observed, to yield female eggs per female.

Repro Study Observation Day	Assigned Life Stage Number	Life Stage Duration	Observed Eggs/Female/Day					
			Control	Se-1	Se-2	Se-3	Se-4	Se-5
-	1	35	-	-	-	-	-	-
-	2	85	-	-	-	-	-	-
2	3	2	2.690	2.571	1.875	1.319	2.542	1.958
4	4	2	5.548	4.048	2.563	2.153	3.917	3.375
7	5	3	4.333	4.302	4.181	3.185	2.222	6.000
9	6	2	5.762	5.524	4.708	3.639	5.646	5.896
11	7	2	8.024	3.238	8.833	4.528	5.521	7.917
14	8	3	6.540	4.905	4.625	2.296	2.750	5.569
17	9	3	6.429	7.143	2.861	1.481	2.014	3.222
21	10	4	3.345	7.881	4.208	1.764	1.698	4.167
23	11	2	5.786	4.643	2.938	3.806	3.688	3.646
25	12	2	6.905	7.286	1.958	2.792	3.125	4.750
28	13	3	4.794	4.317	1.431	1.306	1.319	2.514
30	14	2	1.881	3.881	2.167	1.403	1.708	2.750
32	15	2	5.464	7.286	5.646	1.444	1.563	3.146
35	16	3	4.373	7.310	1.132	2.880	1.028	3.097
37	17	2	5.631	4.417	0.927	1.556	0.792	0.792
39	18	2	6.119	3.917	4.240	3.556	2.354	2.917
42	19	3	7.349	2.222	1.056	2.500	1.403	1.944
44	20	2	4.798	3.274	1.719	3.194	1.438	2.854
51	21	7	1.847	2.139	1.571	2.532	2.060	2.202
53	22	2	6.310	1.512	0.823	5.403	1.333	3.917
56	23	3	3.183	7.317	2.076	2.491	3.528	3.083
58	24	2	3.405	7.810	8.469	9.597	3.104	7.656
60	25	2	3.810	8.226	4.115	6.347	2.271	4.271
Total as $\sum$ (duration · eggs/f/d) =			281.6	294.3	181.9	174.7	142.0	220.1

Larval Survival: The Besser et al. (2012b) spreadsheet also has data for larval survival after 14 and 21 days for eggs collected at three time points. The fraction surviving 21 days was used here. For each treatment, the probability of the early life stage (i=1) surviving each day equals the fraction surviving for 21 days, raised to the 1/21 power: $\sigma_1 = \sigma_L = (21\text{-d Surv})^{1/21}$, shown in Table 4.

Juvenile and Adult Survival: A second spreadsheet, Besser et al. (2012c), has data on juvenile and adult survival after 30 and 58 days. The fraction surviving 58 days was used (Table 4). Parallel to the handling of larval survival, for each treatment the juvenile-adult daily survival probability, σ_{JA} = (58-d Surv)$^{1/58}$, as shown in the table. This value applies to life stages i=2-25 (σ_2 through σ_{25}).

Table 4. Pupfish observed survival and modeled daily survival; fraction hatching and fraction free of deformity.

Treat-ment	Conc	21-d Larval Surv	Larval Daily Surv (σ_L)	58-d Juv+Adlt Surv	Juv+Adlt Daily Surv (σ_{JA})	Fraction Hatch	Fraction Free of Deformity
Control	1	0.9038	0.9952	1.0000	1.0000	0.9023	0.9489
Se-1	3	0.9770	0.9989	1.0000	1.0000	0.9026	0.9727
Se-2	4.4	0.9109	0.9956	0.9250	0.9987	0.8197	0.9563
Se-3	8	0.9600	0.9981	0.9000	0.9982	0.8922	0.9750
Se-4	13	0.9586	0.9980	0.9500	0.9991	0.8988	0.9048
Se-5	27	0.8396	0.9917	0.8750	0.9977	0.9104	0.9174

Formulation of the Population Model: The population growth equation is shown below, in abbreviated form.

$$\begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_t = \begin{bmatrix} \sigma_1(1-\gamma_1) & 0 & \sigma_3 m_3 & \cdots & \sigma_{25} m_{25} \\ \sigma_1\gamma_1 & \sigma_2(1-\gamma_2) & 0 & \cdots & 0 \\ 0 & \sigma_2\gamma_2 & \sigma_3(1-\gamma_3) & \cdots & 0 \\ \vdots & \vdots & \vdots & \cdots & 0 \\ 0 & 0 & 0 & \cdots & \sigma_{25}(1-\gamma_{25}) \end{bmatrix} \begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_{t-1}$$

The diagonal of the 25x25 projection matrix has $\sigma_i(1-\gamma_i)$, the sub-diagonal has $\sigma_i\gamma_i$, and the top row has $\sigma_i m_i$. All other elements are 0. For life stage i, σ_i is the daily survival probability, γ_i is the daily probability of graduating to the next life stage, and m_i is the fecundity expressed as number of female eggs produced per female per day, set at one-half the observed eggs/female/day.

The graduation probability, γ_i, for individuals in each life stage was calculated as follows:

$$\gamma_i = \frac{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - \left(\frac{\sigma_i}{\lambda}\right)^{(Dur_i - 1)}}{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - 1}$$

where λ is the population growth rate and Dur_i is the duration of the life stage. In a 2-day duration life stage, were survival 100% (σ=1) and were the population not growing (λ=1), exactly one half (1/*Dur*) would graduate each day from the 2-day life stage. However, with σ<1 and λ>1, there would be a slight youthful bias

within the life stage, such that slightly more than half would be only 1 day into the life stage and not ready to graduate, and slightly less than half would be in their second day and ready to graduate. The above function adjusts for that.[1] The projected population growth rate for each treatment was calculated as follows. The 25x25 projection matrix was placed on an Excel spreadsheet. Each cell in the diagonal was then modified to subtract the eigenvalue, λ, which represents the population growth rate. That is, each cell in the diagonal was rewritten as $\sigma_i(1-\gamma_i) - \lambda$. The determinant of the 25x25 matrix was then calculated by function MDETERM. To obtain the population growth rate, Excel's Solver was then tasked with finding a value for λ that yielded a value of zero for the matrix determinant. In this case,-10^{-18} < MDETERM < $+10^{-18}$ was deemed sufficiently close to zero. Introducing the constraint to look for λ values between 1.01 and 1.04 was found helpful for Solver to find the dominant eigenvalue. When Solver occasionally could not get the determinant within 10^{-18} of zero, probably due to a solution oscillation that can occur because the input values γ_i are expressed as a function of the solution output λ, digits were removed from Solver's best estimate for λ, to provide a new starting value with which Solver could complete the solution.

Effects on Projected Population Growth Rates: Table 5 and Figure 2 show the model results. Figures 2-B, -C, and -D are almost indistinguishable from Figure 2-A, because hatch and deformity rates varied so little across treatments. Although population growth rates at 4.4 – 27 mg Se/kg are less than at 1 – 3 mg Se/kg, the 6-fold increase in concentration from 4.4 – 27 mg Se/kg yields no change in response. Consequently, the results do not suggest a selenium-related effect, and no EC_{10} can be calculated. Based on the combined influences of egg production and timing, and survival (with or without percentage hatch and deformities), pupfish does not appear to be among the most sensitive species.

[1] The formula for γ is undefined (0/0) under the condition σ=1 and λ=1, so it is not obvious from inspection how it behaves. This function addresses a model artifact that is called numerical dispersion when it occurs in pollutant transport models. It prevents overoptimistic rates of moving through the life stages, particularly in the 35-day and 85-day larval and juvenile stages, and allows a 25-stage model of life duration 180 days to yield precisely the same growth rate as a 180-stage (one day per stage) model, which was also constructed and checked for comparison. However, in this application where absolute growth rates have no particular meaning and only relative differences between treatments are of interest, the function does not change the overall perspective.

Table 5. Model output: daily population growth rates as λ (factor increase) and r (=ln λ), for models that account for survival, fecundity and its timing, and optionally also hatch and/or deformities. Because λ is responding to all the treatment parameters included in the model, its treatment-to-treatment variations do not exactly track the variations in any single input.

Treat-ment	Conc	Factors included in model:							
		All account for survival (σ_L , σ_{JA}) and fecundity (m) and its timing							
		-		Hatch		deformity		hatch & deform.	
		λ	r	λ	R	λ	r	λ	r
Control	1	1.0337	0.0332	1.0330	0.0324	1.0334	0.0328	1.0326	0.0321
Se-1	3	1.0346	0.0340	1.0338	0.0333	1.0344	0.0338	1.0336	0.0331
Se-2	4.4	1.0299	0.0294	1.0284	0.0280	1.0295	0.0291	1.0281	0.0277
Se-3	8	1.0285	0.0281	1.0277	0.0273	1.0283	0.0279	1.0275	0.0271
Se-4	13	1.0291	0.0287	1.0283	0.0279	1.0283	0.0279	1.0276	0.0272
Se-5	27	1.0294	0.0290	1.0288	0.0283	1.0288	0.0284	1.0281	0.0277

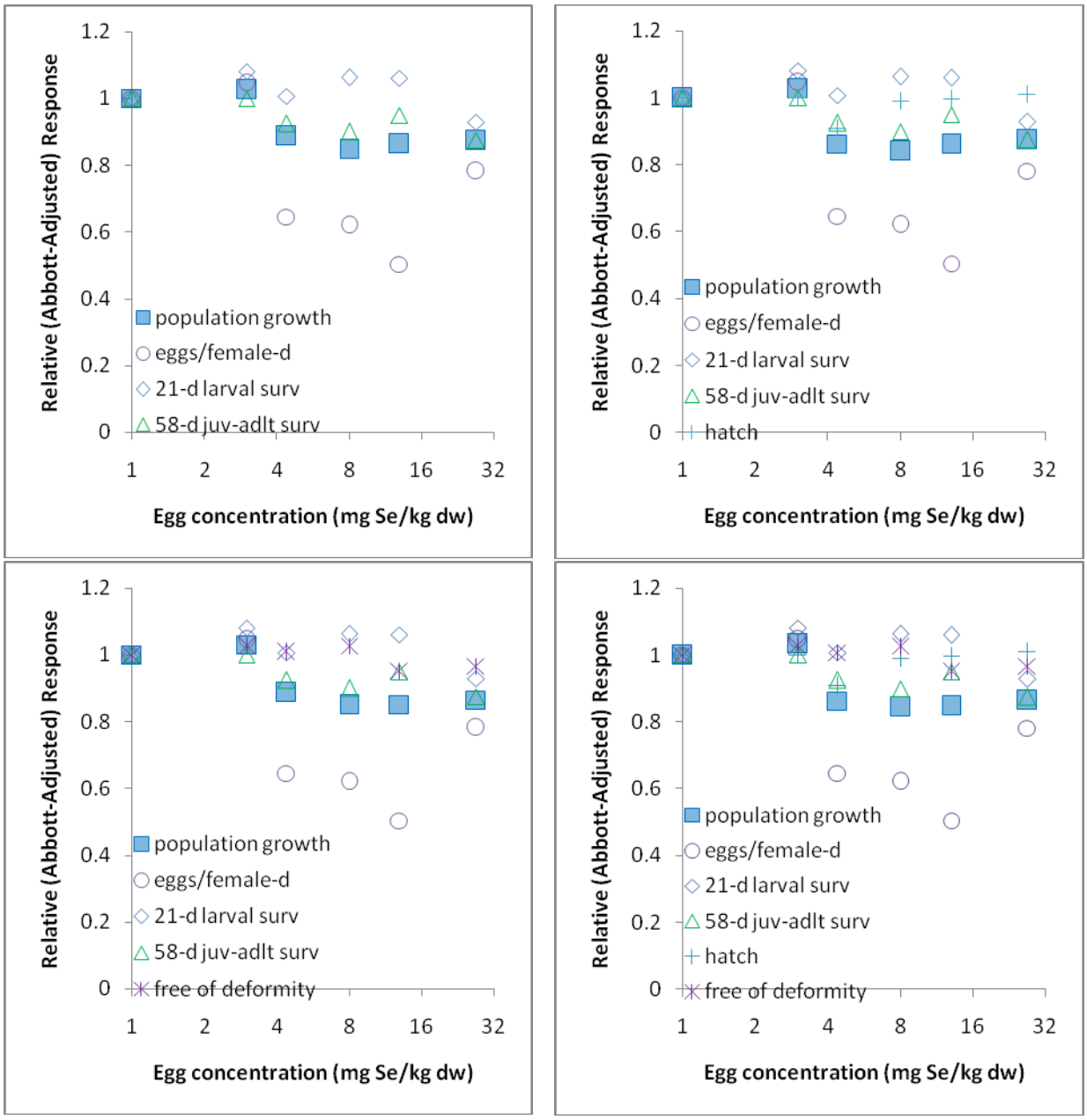


Figure 2. Abbott-adjusted pupfish response as modeled population growth rate (solid-filled symbols) and observed eggs per female per day, larval survival, and juvenile and adult survival (open symbols). Where used in the population model (to modify fecundity), hatch and deformity are shown as open symbols. Some open-symbol points are obscured beneath solid-symbol points. (A) Upper left, egg production and survival only, (B) upper right, adds in influence of percent hatch, (C) lower left, adds in influence of deformities, and (D) lower right, adds in influence of percent hatch and deformities.

Isolating the Influence of Timing of Egg Production: By combining survival with egg production and its timing in the above analysis, the assessment obscures the influence of timing: the issue that was the main reason for undertaking population modeling in the first place. The concern is whether selenium exposure could delay reproduction, thereby yielding reduced population growth. To help isolate the influence on the timing of egg production, two population model runs were performed where all treatments were assigned one of two daily survival rates (0.99 or 0.999) spanning the full range of daily survival rates observed in the 21 and 58 day survival calculations. That is, with survival held constant, the only factors varying across treatments were egg production and timing.

The results are shown in the table below. The Abbott-adjusted results are plotted in Figure 3. Although the relative differences in Figure 3 population growth rates are subdued compared to the wider variation in egg production, this is merely a consequence of the predicted population growth rate being more responsive to survival than to reproduction. It is still apparent that the variations in total egg production are affecting growth rate. The question to be addressed here is whether increasing selenium concentration yields a decline in growth rate beyond the pattern reflecting total egg production.

Population growth rates, as influenced only by differences in egg production and timing					
		With only egg production (*m*) and its timing variable across treatments			
		σ=0.999		**σ=0.99**	
Treat-ment	**Conc**	**λ**	**r**	**λ**	**r**
Control	1	1.0339	0.0334	1.0246	0.0243
Se-1	3	1.0338	0.0333	1.0245	0.0242
Se-2	4.4	1.0310	0.0306	1.0217	0.0215
Se-3	8	1.0293	0.0289	1.0201	0.0199
Se-4	13	1.0293	0.0288	1.0200	0.0198
Se-5	27	1.0324	0.0318	1.0231	0.0228

Inspection of Figure 3 indicates that when survival is assigned a constant value across treatments, the pattern of population growth differences across treatments does not suggest an additional selenium-accentuated factor depressing population growth rate. Population growth at 13 and 27 mg Se/kg is slightly higher than might be expected from total egg production, when compared to lower concentrations. The lack of influence of selenium exposure on timing of egg production is also illustrated by comparing each treatment's cumulative proportion of egg production over the course of the test, as shown in Figure 4. Although the treatments differ somewhat in the temporal pattern of their egg production, there is no consistent relationship with selenium exposure.

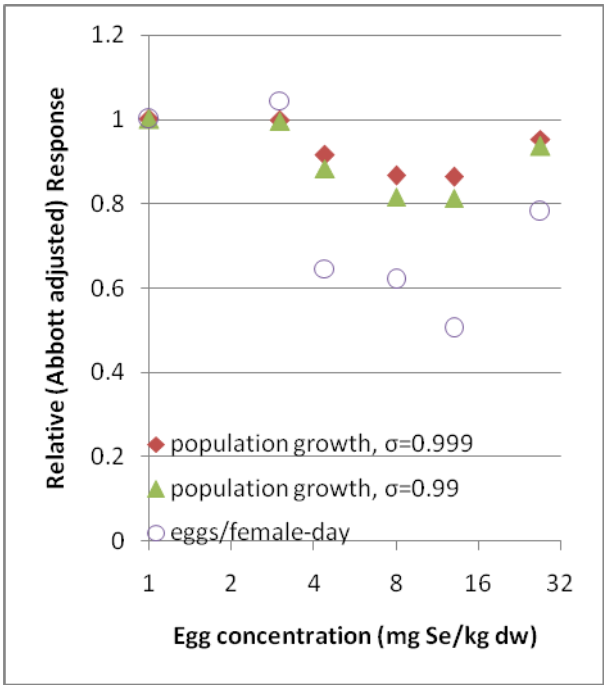


Figure 3. Predicted population growth rate calculated considering differences only in egg production and timing (having assigned uniform survival rates across treatments).

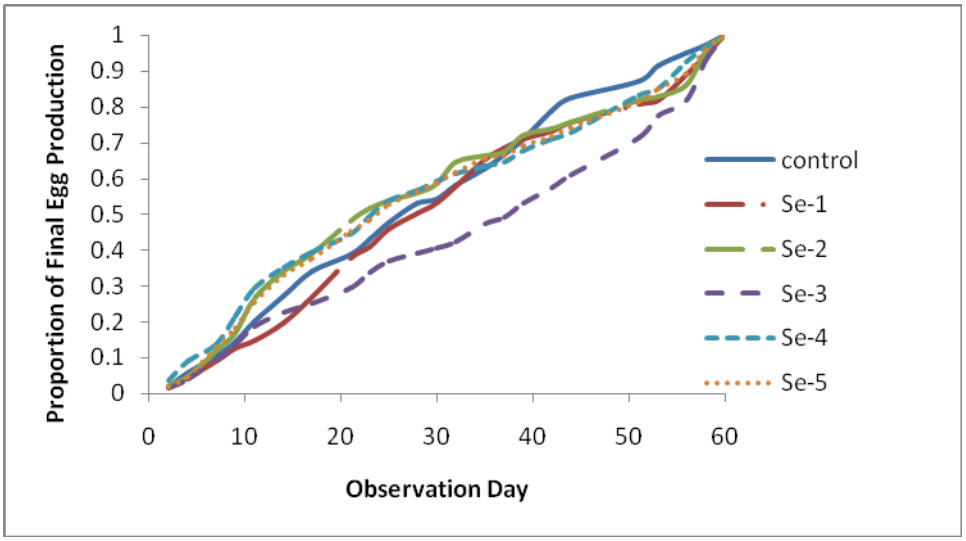


Figure 4. Cumulative pattern of egg production over time. (Control: continuous line. Se-1: dot, dot, long dash. Se-2: long dashes. Se-3: medium dashes. Se-4: short dashes. Se-5: dots.)

Chronic Value: In other selenium studies, egg production and percent hatch have not generally been thought to be related to selenium exposure. Although Besser et al. (2012) noted that repeated measures ANOVA indicated a potential interaction between selenium treatment and egg production on particular sampling dates, a thorough examination of the study data from multiple perspectives indicates no statistically significant or biologically apparent effect of selenium on egg production, timing of egg production, or percent hatch at or below the highest tested concentration of 27 mg Se/kg (dw). Likewise there was no discernible effect on deformity rates.

In the separate tests of F1 larval survival at 21 days and of F1 juvenile-adult survival at 58 days, the highest treatment, 27 mg Se/kg (dw), displayed lower survival than any other treatment. Although the reduction was not sufficient to be statistically significant, Besser et al. (2012) suggest that this is indicative of a threshold. Note that among toxicity tests in general, the 10% effect level of the EC_{10} might or might not be statistically significant from the perspective of hypothesis testing.

Shown below are the survival rates for the 27 mg Se/kg treatment adjusted to the control (Abbott-adjusted), or similarly adjusted to the average survival at all lower treatments (some of which had better survival than the controls). Either way the adjustment is done, results are similar. (These survival data, Abbott-adjusted, are included in Figure 2.)

27 mg Se/kg treatment:	**Larval Surv at 21 days**	**Juv-Adlt Surv at 58 days**
adjusted to control	92.9%	87.5%
adjusted to all lower treatments	89.1%	91.6%

The effect level at 27 mg Se/kg was thus 7% – 13% in the above comparisons. While the concentration response curve is not sufficiently defined to allow confident assignment of an EC_{10}, the data suggest a chronic value in the general neighborhood of 27 mg Se/kg.

An effect level of 27 mg Se/kg egg for the pupfish in this study is consistent with the findings of Saiki et al. (2012a) who evaluated selenium in two related species in the Salton Sea, California. These authors measured 3.09 to 30.4 mg/kg whole body Se levels in mosquitofish and sailfin mollies and based on a lack of a negative relationship with the catch-per-unit-effort deduced these species were not adversely affected by selenium. They extrapolated the finding of selenium tolerance to the pupfish based on the results of another study (Saiki et al 2012b) in which mosquitofish and sailfin mollies accumulated similar levels of selenium to the pupfish. Note: the ratio of selenium in whole body to egg tissues in the pupfish was approximately 1:1 in the Besser study (see first table in the pupfish study summary above).

Staub, B.P. W.A. Hopkins, J. Novak, J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Arch. Environ. Contamin. Toxicol. 46:96-101.

Test Organism: Eastern mosquitofish (*Gambusia holbrooki*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from a contaminated ash basin (ASH) and a reference pond (REF)

Study Design: In July 1999, male eastern mosquitofish were collected from ASH and REF (n=26, n=20, respectively) for measurement of standard metabolic rate (SMR). In July 1999, gravid female eastern mosquitofish were collected from ASH and REF and transported to a laboratory for testing. To ensure all females were fertilized in the field, all offspring used in testing were limited to three weeks after collection. (Eastern mosquitofish are live-bearers with a four week gestation period.) Response variables compared between ASH and REF were (1) SMR of males, (2) brood size of females, (3) percent of live offspring at parturition, and (4) trace element concentration in females and offspring.

Effects Data: SMRs of males, brood size of females, and offspring viability were not significantly different between sites. Average (n=5) concentrations of selenium in females were 11.85 and 0.61 mg/kg dw in ASH and REF sites respectively. The average concentrations of selenium in offspring were 15.87 mg/kg dw and below detection in ASH and REF sites, respectively. The authors point out that the selenium concentrations are an under-estimate of the field levels since the females were allowed to depurate during their time in the laboratory prior to parturition.

Chronic Value: >11.85 mg Se/kg dw whole body

Saiki, M.K., B.A. Martin, and T.M. May. 2004. Reproductive status of western mosquitofish inhabiting selenium-contaminated waters in the grassland water district, Merced County, California. Arch. Environ. Contamin. Toxicol. 47:363-369.

Test Organism: Western mosquitofish (*Gambusia affinis*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from selenium-contaminated sites and reference sites in the San Joaquin River watershed.

Study Design: Western mosquitofish were collected in June and July 2001 from San Luis Drain (SLD) at Gun Club Road (Se-contaminated site), North Mud Slough at Gun Club Road (MSN1; reference site); North Mud Slough at State Highway 140 (MSNs; Se-contaminated site); San Joaquin River at Lander Avenue (SJR; reference site). 20 gravid females from each site were held in the laboratory for two weeks to quantify live and dead births and to make other measurements. Only 17 females from SLD were collected. Live and dead fry were visually examined under low magnification with a binocular microscope for evidence of external abnormalities (teratogenic symptoms such as spinal curvature, missing or deformed fins, eyes and mouths and edema).

Effects Data: The percentage of live births was high at both Se-contaminated sites (96.6 to 99.9%) and reference sites (98.8 to 99.2%). There were no obvious anomalies (e.g., deformities, edema) observed during the study. The concentration of selenium in 4 postpartum females from the site with the highest selenium concentration, SLD, ranged from 13.0 to 17.5 mg Se/kg dw (geometric mean of the high and low is 15.1 mg Se/kg dw. The concentration of selenium of western mosquitofish collected at each site is in Table D-8.

Chronic Value: >15.1 mg Se/kg dw whole body

Table D-8. Selenium in whole body samples of western mosquitofish from study sites

Site	N	[Se], mg/kg dw
SLD	8	18.1
MSN2	24	9.31
MSN1	20	2.72
SJR	22	0.907

Coughlan, D.J. and J.S. Velte. 1989. Dietary toxicity of selenium-contaminated red shiners to striped bass. Trans. Am. Fish Soc. 118:400-408.

Test Organism: Striped bass (*Morone saxitilis*; adults from Lake Norman, NC, approximately 250 g each)

Exposure Route: dietary only
Treated fish were fed selenium contaminated red shiners (1 g) from Belews Lake, NC (9.6 mg Se/kg ww or 38.6 mg Se/kg dw based on a mean reported moisture content of 75.1 percent). Control fish were fed golden shiners from a local bait dealer (0.3 mg Se/kg ww or 1.3 mg Se/kg dw based on a mean reported moisture content of 76.3 percent).

Test Treatments: Test treatments were as described above. Two tanks contained treated fish (n = 20 fish total), and one tank of fish served as the control (n = 10 fish). Each tank received a continuous flow of soft well water (hardness and alkalinity approx. 30 mg/L as $CaCO_3$) throughout the exposure.

Test Duration: 80 days

Study Design: During the experiment, all striped bass (n = 10 per tank) were fed to satiation three times per day. Pre-weighed rations of live red shiners (treated fish) and golden shiners (controls) were added to the tanks and allowed 5 hours to feed. Uneaten prey was removed and weighed. Composite whole-body samples of each prey fish were collected at regular intervals throughout the study for whole-body tissue selenium analysis. The final selenium concentration in epaxial white muscle was determined for surviving striped bass at the end of the test. Moribund striped bass were sacrificed so as to obtain muscle tissue samples for selenium analysis. Samples of liver and trunk kidney of these and the surviving striped bass were dissected for observations of histopathology.

Effects Data: Striped bass fed selenium-laden red shiners exhibited changes in behavior (lethargy, reduced appetite), negligible weight gain, elevated selenium concentrations in muscle, histological damage, and death. Control fish ate and grew well, and behaved normally. Average selenium ingestion was between 60 and 140 Φg Se/fish per day until day 30. Appetite of the treated fish appeared to be significantly reduced beyond this point compared to the appetite of the control group. By day 78, all striped bass fed the Se-laden red shiners either had died or were moribund and sacrificed for analysis. The final selenium concentration in muscle of treated striped bass averaged from 3.5 (tank 1) and 4.0 (tank 2) mg/kg ww, or 16.2 and 18.5 mg/kg dw, respectively, assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species. The final selenium concentration in muscle of control striped bass fed uncontaminated golden shiners averaged 1.1 mg/kg ww, or 5.09 mg/kg dw (assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species).

Chronic Value: The chronic value for percent survival of striped bass relative to final selenium in muscle tissue after being fed Se-laden red shiners is <16.2 mg/kg dw.
An EC_{20} value could not be calculated for this data set because the data did not meet the assumptions required for analysis.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1984. Roxboro Steam Electric Plant 1982 Environmental Monitoring Studies, Volume II, Hyco Reservoir Bioassay Studies. Environmental Technology Section. Carolina Power & Light Company.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* embryos and larvae)

Exposure Route: dietary and waterborne - field exposure
Native adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. No selenium values were given for Hyco Reservoir, total selenium was not detected in the control lake (<1 µg/L). A mean selenium for the ash pond effluent from a previous study was 53 µg/L (N=59; range 35-80 µg/L).

Study Design: All combinations of crosses between the Hyco and control fish were made using gametes from the collected fish. Fertilized eggs were exposed in egg cups to 0, 20 and 50 percent ash pond effluent under flow-through conditions. Percent hatch and swim-up successes were measured. Swim-up larvae were released to exposure tanks where there were fed zooplankton collected from Hyco and the control lake. Larvae were observed for 28 days at which time survival and weight were measured.

Effects Data: Survival to the swim-up stage was different between larvae from Hyco females fertilized with either male type and those larvae from control females fertilized with either male type. All crosses involving a Hyco female resulted in larvae exhibiting 100 percent mortality prior to reaching swim-up. Percent survival from hatch to 28 days for larvae from control females exposed to control water and fed control lake zooplankton was only 5 and 12 percent for the two replicates so no meaningful comparisons can be made to the different dilution exposures or diet exposure. The mean concentrations of selenium in the ovaries, female liver and female muscle were 49, 130, and 84 mg/kg dw, respectively.

Effect level: <49, <130 and <84 mg Se/kg dw in adult ovaries, liver and muscle, respectively

Chronic Value: <49.65 mg Se/kg dw in whole body using the muscle to whole body equation
<84 mg Se/kg dw maternal muscle
<49 mg Se/kg dw ovary

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; 30-day old larvae)

Exposure Route: Dietary and waterborne - field exposed adults
Juvenile bluegill from crosses with females in 0, 20 and 50 percent ash pond effluent were transferred to control water and fed zooplankton from either Hyco or the control lake. Selenium in Hyco and control zooplankton was 45 and 1.9 mg/kg dw, respectively. Duration was not given.

Study Design: Survival and observations on pathology and morphology were made in the two diet treatments.

Effects Data: Mortality in larvae fed control zooplankton was 23.7 percent, whereas mortality in larvae fed Hyco zooplankton was 97.3 percent. There were no differences in survival (for two diet treatments) in larvae that were raised for the 30 days prior to the test in different effluent concentrations (0, 20 50 percent). The average selenium concentrations in the larvae fed control and Hyco zooplankton were 1.9 and 24.7 mg/kg dw, respectively.

Effect level for larval survival: <24.7 mg Se/kg dw in larvae

Chronic Value: None recommended for larval tissue.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1985a. Roxboro Steam Electric Plant Hyco Reservoir 1983 Bioassay Report. Environmental Services Section. Carolina Power & Light Company. September 1985.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; embryos and larvae)

Exposure Route: dietary and waterborne - field exposed
Resident adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. For embryo/larval study up to swim-up stage, control fish were collected from the unaffected portion of Hyco.

Study Design: Repeat of 1982 28-day Embryo/Larval Study. Three crosses between: Hyco female and Hyco male; control female with Hyco male; and control female with control male. Gametes were fertilized and maintained for the 28-day test in ash pond effluent dilutions of 0, 20 and 50 percent. Percent hatch, percent swim-up success and survival were measured to 28 days post hatch. Two treatments were replicated and fed zooplankton collected from Hyco-affected and Hyco-unaffected (control). Larvae were observed for 28 days at which time survival and weight were measured.

Embryo/Larval Study up to Swim-up Stage. Five crosses were made between fish collected from the affected and unaffected areas. Percent hatch, percent swim-up and survival were measured until swim-up (approximately 3-4 days after hatch).

Effects Data: 28-day Embryo/Larval Study. All larvae that hatched from eggs obtained from Hyco females died prior to completing swim-up (see table below).

Effect level (larval survival): <30, <33 and <59 mg Se/kg dw for adult female bluegill in ovaries, liver and muscle, respectively

Summary of 28-day embryo larval study										
% effluent	Parent source in cross M X F	% hatch	% swim-up	% survival, 28-days	Adult tissue, mg Se/kg dw					
					Gonad		Liver		Muscle	
					M	F	M	F	M	F
0	H X H	92	0	0	33	30	43	33	62	59
20	H X H	98	0	0	33	30	43	33	62	59
20	H X H	92	0	0	33	30	43	33	62	59
50	H X H	97	0	0	33	30	43	33	62	59
0	H X C	89	87	18	33	2.2	43	4.4	62	2.7
20	H X C	96	96	34	33	2.2	43	4.4	62	2.7
50	H X C	60	84	58	33	2.2	43	4.4	62	2.7
0	C X C	79	95	40	nd	2.2	37	4.4	27	2.7
20	C X C	90	96	36	nd	2.2	37	4.4	27	2.7
20	C X C	88	97	25	nd	2.2	37	4.4	27	2.7
50	C X C	72	92	42	nd	2.2	37	4.4	27	2.7

Chronic Value: <36.49 mg Se/kg dw in whole-body using the muscle to whole body equation.
<59 mg Se/kg dw muscle
<30 mg Se/kg dw ovary

Embryo/larval study to swim-up. Percent swim-up of larvae from parents collected in non-affected Hyco averaged 93 percent, whereas percent swim-up from larvae collected from affected Hyco was 12 percent. Effect levels were determined for adult female and larval tissues. Larval tissues were averaged across effluent concentrations (geometric mean).

Effect level (percent swim-up):
Adult female ovaries: >9.1 mg/kg dw; <30 mg/kg dw
Adult female liver: >26 mg/kg dw, <33 mg/kg dw
Adult female muscle: >25 mg/kg dw, <59 mg/kg dw
Larvae: >12.8 mg/kg dw; < 165 mg/kg dw

Summary of Embryo/Larval Study up to Swim-up - Affected vs Unaffected Hyco											
date of fert.	Parents' capture location in Hyco	Percent hatch			Percent swim-up			Selenium in tissue, mg/kg dw			
		at % effluent			at % effluent			Adult female			
		0	20	50	0	20	50	Ovary	Liver	Musc	Larvae
6-24	affected	93	98	94	0	0	0	30	33	59	0: 130 20: 120
6-27	affected	99	88	77	0	0	0	30	33	59	0: 130 20: 120
6-28	affected	29	34	35	25	14	3	30	33	59	0: 130 20: 120
6-28	affected	98	86	91	5	0	0	30	33	59	0: 130 20: 120
6-29	affected	88	93	85	59	42	25	30	33	59	0: 130 20: 120
7-14	unaffected	92	80	84	79	92	89	9.1	26	25	0: 19 20: 11 50: 10
7-26	unaffected	99	94	93	100	98	98	9.1	26	25	0: 19 20: 11 50: 10
7-27	unaffected	76	84	86	100	89	91	9.1	26	25	0: 19 20: 11 50: 10

Chronic Value: The chronic value estimated for the percentage larvae reaching the swim-up stage is presented as a range:
>25 mg Se/kg dw (unaffected area) and <59 mg Se/kg dw muscle (affected area)
>30 mg Se/kg dw (unaffected area) and <9.1 mg Se/kg dw ovary (affected area)

Bryson, W.T., K.A. MacPherson, M.A. Mallin, W.E. Partin, and S.E. Woock. 1985b. Roxboro Steam Electric Plant Hyco Reservoir 1984 Bioassay Report. Environmental Services Section. Carolina Power & Light Company

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile- hatchery raised)

Exposure Route: Dietary only

Test Treatments: 5 diets: Se form (nominal selenium concentration in base diet)
seleno-DL-cystine (5 mg/kg)
seleno-DL-cystine (10 mg/kg)
seleno-DL-methionine (5 mg/kg)
sodium selenite (5 mg/kg)
Hyco zooplankton (5 mg/kg)

Test Duration: 60 days

Study Design: Each treatment contained 40 fish which were maintained in a flow-through system. Fish were fed at 3 percent of their body weight. Length and weight were measured on days 30 and 60. Total selenium was measured in liver and whole-body.

Effects Data: No decreased length or weight in any of the Se-diets relative to the control.

Chronic Value: all values are whole-body
seleno-DL-cysteine: >2.16 mg Se/kg dw
seleno-DL-cysteine-2X: >3.74 mg Se/kg dw
seleno-DL-methionine: >2.46 mg Se/kg dw
sodium selenite : >1.21 mg Se/kg dw
Hyco zooplankton: >2.35 mg Se/kg dw

Because none of the selenium-spiked diet formulations affected growth of juvenile fish at the concentrations tested, the chronic value selected for this study is >3.74 mg Se/kg dw for the seleno-DL-cysteine-2X formulation.

Source and Exposure Embryo-Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* Adults from Hyco and a control lake)

Exposure Route: Dietary and waterborne - field exposure

Test Treatments: Four treatments:
Hyco–collected fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.
Control fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.

Test Duration: Adult fish were in spawning tanks 4-7 months

Study Design: Eggs from each treatment were observed for percent hatch and percent swim-up.

Effects Data: Fish collected from the control lake did not spawn. Percent hatch and percent swim-up from Hyco fish in Hyco and control water are given in the table below. The percent hatch and percent swim-up were >83 and >83 for all the Hyco fish suggesting no effect for these endpoints.

Source of parents	Se in parental liver tissue, mg/kg dw	Water type for eggs and larvae	N	Percent hatch	Percent swim-up
Hyco	18.6	Hyco	16	86.6	91.1
Hyco	18.6	well water	10	83.8	95.5
Control	13.8	Hyco	a	a	83.3
Control	13.8	well water	12	86.0	97.4

[a] percent hatch unknown.

Chronic Value: The chronic value for this study is >18.6 mg Se/kg dw liver tissue.

Gillespie, R.B. and P.C. Baumann. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans. Am. Fish. Soc. 115:208-213.

Test Organism: Bluegill sunfish, wild-caught (*Lepomis macrochirus*; adults; embryos and larvae)

Exposure Route: dietary and waterborne - field exposure

Test Treatments: High selenium adult fish were collected (electrofishing and with Fyke nets) from Hyco Reservoir. Low selenium adult fish were collected from Roxboro City Lake, Roxboro, NC.

Study Design: All possible combinations of bluegill parents from Hyco Reservoir and Roxboro City Lake were artificially crossed in June and July, 1982 and 1983, respectively. Fertilization success was assessed by stripping subsamples of 100 to 500 eggs per female and combining them with 2 ml of sperm. All zygotes were reared in Roxboro City Lake water and percent fertilization was estimated 2-3 hours later as the proportion of mitotically active zygotes. To estimate hatching success, gametes were combined as before and subsamples of 100 to 300 embryos per cross were transferred to egg cups and maintained in closed aquaria receiving re-circulated Roxboro City Lake water. Percent hatch (approx. 2d at 22 to 25^{o}C) was based on the number of yolk-sac larvae. In 1982, about 200 embryos from 8 crosses were observed and preserved at intervals up to 40 h after fertilization, and about 450 larvae were preserved at intervals of 40 to 180 h after fertilization. In 1983, about 1,800 larvae were observed and preserved from 40 to 150 hr from crosses involving females from Hyco Reservoir, and about 40-300 hr for crosses involving females from Roxboro City Lake (10 crosses total).

Effects Data: No significant differences were found in percent fertilization or in percent hatch among parent combinations from the 18 crosses made in June 1982 and July 1983. In contrast, larvae from all crosses involving a Hyco female were edematous; 100 percent of the larvae were abnormal in 7 of 8 crosses. Note: This outcome was observed when the same female from Hyco Reservoir was crossed with males from either Hyco Reservoir or Roxboro City Lake. The range of selenium concentrations in the ovaries of Hyco Reservoir females used for the cross experiments was from 5.79 to 8.00 (GM = 6.945 mg/kg ww; n=7). The reported concentrations of selenium in ovaries and carcasses of females collected from Hyco Reservoir in 1982 and 1983 were 6.96 and 5.91 mg/kg ww (n=22 and 28, respectively). The reported concentrations of selenium in ovaries and carcasses of females collected from Roxboro City Lake in 1982 and 1983 were 0.66 and 0.37 mg/kg ww (n=14 and 19, respectively). The mean selenium concentration in bluegill larvae (n=222) from artificial crosses of parents from Hyco Reservoir was 28.20 mg Se/kg dw.

Chronic Value: <46.30 mg Se/kg dw ovary using 85 percent moisture for ovaries measured in study.

Doroshov, S., J. Van Eenennaam, C. Alexander, E. Hallen, H. Bailey, K. Kroll, and C. Restrepo. 1992. Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River; Part II, Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (*Lepomis macrochirus*). Final Report to State Water Resources Control Board, State of California. Contract Number 7-197-250-0.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); Population A: selenium bioaccumulation observations used 113 g (range 30-220 g) obtained from Rainbow Ranch Fish Farm, California. Population B: spawning performance observations used 106 g (range 65-220 g) females and 164 g (range 80-289 g) males obtained from Chico Game Fish Farm.

Exposure Route: Dietary only
Dietary
Seleno-L-methionine added to trout chow; the three nominal dietary concentrations of 8, 18 and 28 mg/kg seleno-L-methionine were measured at 5.5, 13.9, and 21.4 mg/kg Se (moisture content 13 to 16%).

Test Duration: 140 days

Study Design: Population A fish and Population B females were fed nominal dietary treatments 8, 18 and 28 mg/kg seleno-L-methionine; Population B males were fed untreated diets until the start of spawning. Population A fish were sampled on days 0, 30, 58, 86 and 114 for Se measurement. At least 3 females were sampled each event. Fish remaining after day 114 were transferred to an outdoor pond fed untreated diet and sampled on day 144 for depuration analysis.

On day 120 Population B males and females were paired for natural spawning which had limited success. Fish were maintained in treatment tanks and females were monitored for egg ripeness. When ripe, females were induced to ovulate and ova were fertilized *in vitro* with semen stripped from males. Fertilized eggs were sampled for fertilization success, Se content, and two live sub-samples for bioassay, one a 30-day embryo-larval test and another for larval development during first 5 days after hatching.

Larval development: after hatching, 100 larvae were transferred to beakers and samples were examined daily for normal, abnormal and dead were recorded.

Larval bioassay: 90 fertilized eggs from each female were placed in groups of approximately 30 eggs. Larvae and fry were fed rotifers and brine shrimp nauplii through the 30 day observation.

Effects Data: Selenium concentrations in parental tissues for Populations A and B are given in Tables 1 and 2, respectively. Treatment effects were only observed on early development bioassays. In the 5-day larval bioassay, systemic edema and underdeveloped lower jaw were apparent in all larvae in the 28 mg/kg dietary treatment by day 3 and complete mortality by day 5, except for two progenies where 10% of the larvae appeared normal. No abnormalities were observed in control and 8 mg/kg treatment. 3 of the 6 progenies in the 18 mg/kg treatment exhibited 10 to 20% larvae with similar abnormalities (Table 3). The average proportion of larvae with edema were 5% in 18 and 95% in 28 mg/kg, both of

these were statistically different from the control (0% edema).

For analysis of the effect level determination, 4-day edema observations were used (Table 4) rather the 5-day data because the latter were difficult to interpret relative to edema because of almost complete mortality at the highest concentration (although the 4-day and 5-day edema observations were almost identical). Of the 33 edema measurements, only 15 could be used because not all the individual-replicate egg concentrations were reported. Table 4 also shows the treatment averages, which are only slightly different than the 5-day edema data. These averages do not match the average of the individual replicates in this table because they are for all the replicates, not just those with which concentrations could be paired.

The Se egg and edema data from Table 4 are plotted on Figure 1. The individual replicates are analyzed using TRAP. TRAP warns about inadequate partial responses because the partial responses are less than 10% or greater than 90%, and there are no data between 10 and 90%. However, for this dataset, these partial responses at both ends, albeit small, are sufficiently informative based on multiple lines of evidence (e.g., same response on both days 4 and 5, other endpoints that show effects at treatment 18, and several instances of edema at treatment 18 in contrast to absolutely none for many observations at any lower concentration). And because treatment 18 does have an effect of several percent or so, estimating the EC_{10} near these points is defensible; the EC_{10} is 22.6 mg/kg egg. The EC_{10} of 22.6 mg Se/kg egg dw was selected for the chronic value because it was determined using the individual replicates rather than treatment averages as was done in the previous draft document. The EC_{10} of 22.6 mg Se/kg egg is slightly higher than that in the previous draft which used means rather than replicate data (Figure 3).

In the 30-day larval survival bioassay, statistical difference was only in the highest test treatment for survival and growth measurements, length and weight (Table 5). The proportion of abnormal larvae was higher in the selenium-treated diets but was not significantly different from the control. The percent of abnormal larvae in the 18 mg/kg treatment (7.2%) was only slightly higher than the control (6.3%).

Authors present the effect level for bluegill at the 18 mg/kg dietary treatment (NOEC 8 mg/kg) based on proportions of edema and delayed resorption of the yolk sac. The latter endpoint is based on significantly greater yolk area and oil globule area in the 18 and 28 mg/kg treatments.

The most sensitive endpoint, percent edema, as a function of selenium in maternal muscle dw, was fitted to a TRAP tolerance distribution analysis using the individual replicates (Figure 2). The response is steep and the EC_{10} estimate is 15.7 mg/kg. This basically is setting the EC_{10} to the average of the two replicates with nominally 10% edema (15.4 and 16.6 mg/kg), with 90% edema occurring at only a slightly higher concentration (17.3 mg/kg).

Chronic Value: EC_{10} value (edema) at 22.6 mg Se/kg egg dw or 15.7 mg Se/kg muscle dw Chronic Value is 22.6 mg Se/kg eggs dw.

Table 1. Selenium Concentrations (mg/kg dw) in Bluegills from Population A Day 113 of				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Ovary	2.17 (0.05)	10.89 (1.83)	26.17 (0.07)	40.32 (2.44)
Female liver	2.51 (0.32)	NA	22.75 (2.96)	40.68 (2.14)
Testis	2.65 (0.21)	9.87	16.38 (0.71)	29.70 (5.02)
Male liver	4.10 (0.37)	14.32	24.28 (4.54)	52.47 (5.23)

Table 2. Selenium Concentrations (mg/kg dw) in Bluegill Parents (Population B) Used in Larval Toxicity Tests				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Male liver	4.07 (0.23)	6.94 (1.58)	20.46 (3.46)	31.63 (1.75)
Testis	1.87 (0.11)	3.64 (0.47)	9.96 (0.45)	15.25 (0.45)
Female liver	4.00 (0.26)	12.33 (1.09)	25.98 (4.28)	47.60 (4.11)
Female muscle	**1.47 (0.14)**	**5.80 (0.79)**	**10.41 (2.02)**	**23.64 (2.04)**
Ovary	2.23 (0.11)	6.34 (0.47)	14.10 (2.62)	30.63 (3.23)
Eggs	**2.81 (0.14)**	**8.33 (0.63)**	**19.46 (3.83)**	**38.39 (3.14)**
Larvae	NA	NA	NA	35.30 (4.16)
Fry	1.48 (0.11)	1.25 (0.02)	1.37 (0.06)	1.46 (0.03)

Table 3. 5-day Larval Development Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Free of Edema, %	100	100	95 (2)*	4.3(2.7)*

Table 4. 4-day Edema Observations by Replicate from 5-day Larval Toxicity Test			
Treatment/Replicate ID	**Se egg, mg/kg dw**	**Se muscle, mg/kg dw**	**Percent edema (n=10)**
08-2C	3.54	2.25	0
18-4C	3.25	0.95	0
8-1S	11.49	7.07	0
8-2S	8.31	5.80	0
8-6S	6.18	1.41	0
18-1S	8.55	2.75	0
18-3S	22.06	15.44	10
18-6S	30.20	16.58	10
28-1S	44.02	NA	100
28-2S	36.31	31.10	100
28-3S	25.21	17.28	90
28-4S	52.18	27.40	100
28-5S	42.40	24.00	100
28-6S	38.47	24.66	100
28-7S	30.12	17.42	90
Treatment	**Se egg, mg/kg dw treatment avg**		**Percent edema treatment avg**
C	2.81	--	0 (n=140)
8	8.33	--	0 (n=50)
18	19.5	--	6.67 (n=60)
28	38.4	--	97.1 (n=70)

Table 5. Results from 30-day Embryo-larval Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Larval survival, %	71 (8.5)	51.9 (26.5)	64.4 (3.4)	2.5 (3.5)*
Larval length, mm	19.1 (1.2)	19.9 (1.2)	19.3 (0.8)	16.6 (2.5)*
Larval weight, mg	114 (24)	133 (27)	119 (16)	81 (37)*
Abnormalities in larvae, %	6.3 (7.9)	15.0 (5.8)	7.2 (3.1)	25.0 (43.3)

* Statistically significantly different from control

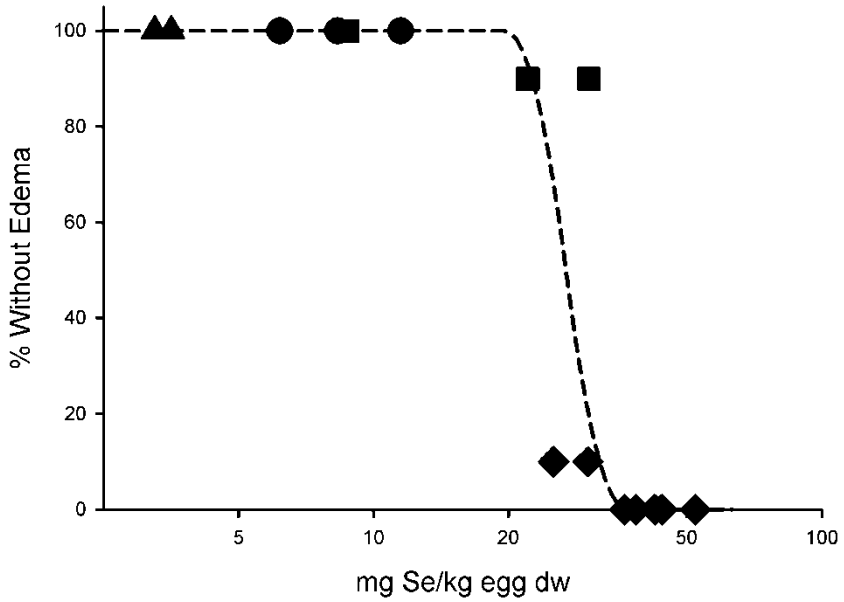


Figure 1. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

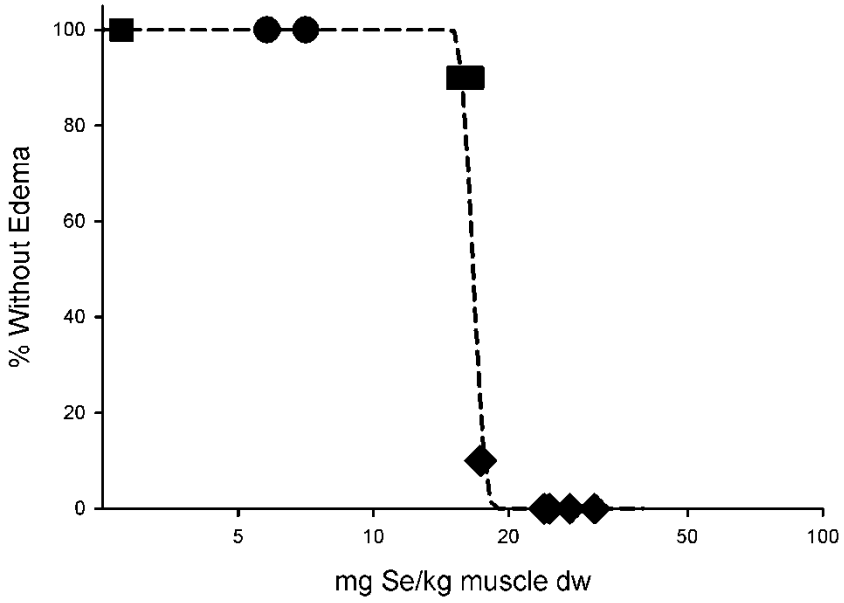
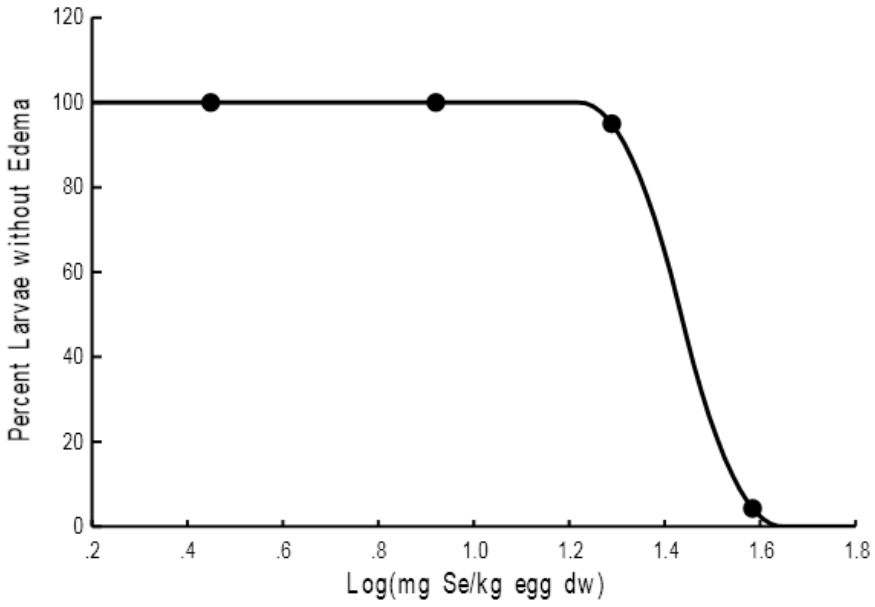


Figure 2. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in maternal muscle. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

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Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4428	1.4342	0.0000	1.4342	1.4342
S	5.452	4.712	0.000	4.712	4.712
Y0	98.33	100.00	0.00	100.00	100.00

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	27.18		
20.0	22.71		
10.0	20.75		
5.0	19.460		

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Figure 3. (From previous draft document) TRAP analysis of bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs.

Hermanutz et al. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ. Tox. & Chem. 11: 217-224

Hermanutz et al. 1996. Exposure of bluegill (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. EPA Report. Mid-Continent Ecology Division. Duluth, MN.

Tao, J., P. Kellar and W. Warren-Hicks. 1999. Statistical Analysis of Selenium Toxicity Data. Report submitted for U.S. EPA, Health and Ecological Criteria Div. The Cadmus Group.

Test Organism: Bluegill (*Lepomis macrochirus*; 3 to 4-year old adults)

Exposure Route: Dietary and waterborne followed by dietary only

Dietary and waterborne

Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Dietary only

Recovering streams exposed bluegill to selenium in prey organisms. Selenite addition to water was ceased (selenium in water was below detection level).

Study Design: Eight Monticello artificial streams were used for three separate studies between 1987 and 1990.

Table 1. Study Design.

Stream	**Study I**	**Study II**	**Study III**
Dates **BG[a] put in station 0-2** **BG transferred to sta. 6 End of study**	**9-1-87** **5-16-88** **8-22-88**	**10-88** **5-89** **8-89**	**11-89** **5-90** **7-90**
1	Unused	Control	Control
2	Unused	2.5 µg/L	Recovering
3	10 µg/L	10 µg/L	Recovering
4	30 µg/L	Recovering	Recovering
5	Control	Control	Control
6	30 µg/L	Recovering	Recovering
7	Control	2.5 µg/L	Recovering
8	10 µg/L	10 µg/L	Recovering

[a] BG = Bluegill

The design of the three Hermanutz et al. studies is included in Table 1 and a schematic diagram of an artificial stream is provided below (Figure 1). For each study, a random sample of 22-50 adult bluegill were transferred from stations 0-2 (provided temperatures above 4°C during winter) to station 6 (most suitable for nests) during mid-May for spawning. Spawning activity was monitored in the streams. Embryo and larval observations were made *in situ* and in the laboratory from fertilized eggs taken from the streams and incubated in the lab.

Figure 1. Schematic Design of One of the Artificial Streams in the Monticello Study

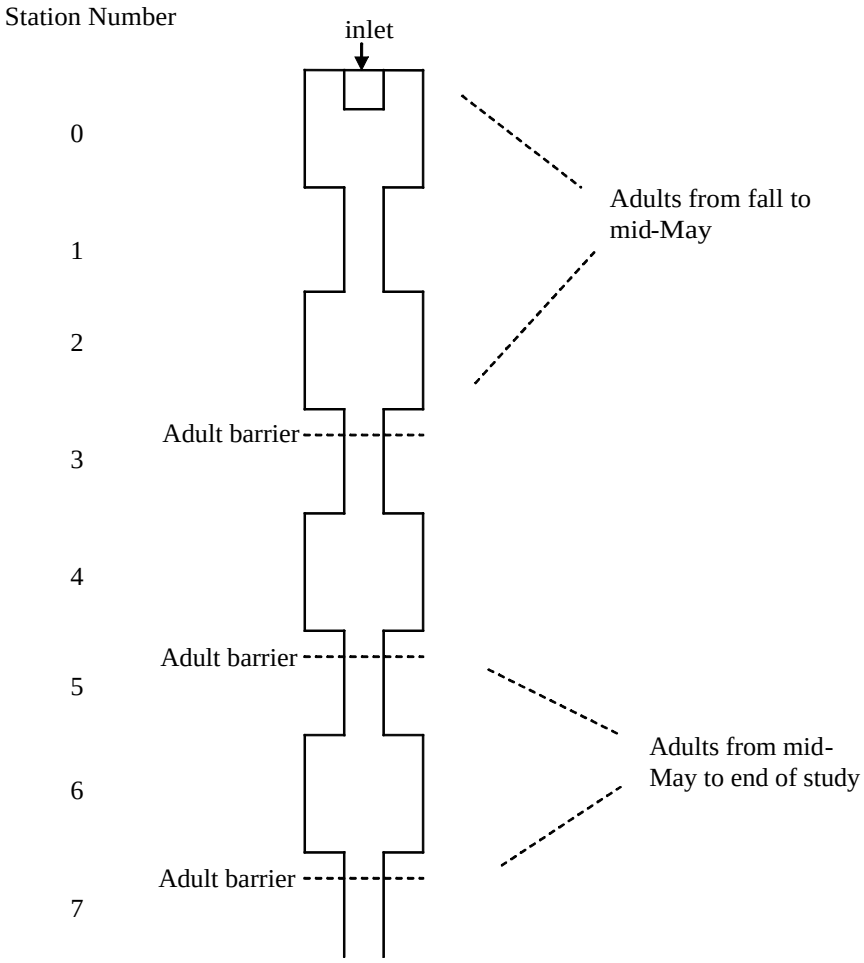


Table 2. Effects on Progeny - Study I[a]

Egg cup observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	% hatch mean ± SD	% survival to 4th day mean ± SD	% edema mean ± SD	% lordosis mean ± SD	% hemorr mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	93.3 ± 9.1	69.7 ± 13.9	0.1 ± 0.2	1.8 ± 2.6	0.1 ± 0.3
control	7	0.47	0.01	0.07	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	71.5 ± 22.5	28.8 ± 23.1	80 ± 1.0	11.6 ± 15.9	28.5 ± 40.6
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L	4	3.71	NA	3.71	15.46	15.46	60.3 ± 25.8	9.1 ± 12.9	50.3 ± 64.1	6.3 ± 1.8	26.8 ± 20.2

Nest observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	# active nests mean ± SD	# embryos Collected mean ± SD	% dead Embryos mean ± SD	# larvae Collected mean ± SD	% dead Larvae mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	6.5 ± 2.1	1441 ± 205	0.9 ± 0.03	3947 ± 1888	3.0 ± 1.1
control	7	0.47	**NA**	**0.47**	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	5.0 ± 4.2	1282 ± 457	3.2 ± 2.9	1169 ± 1093	17.0 ± 21.3
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L [c]	4	3.71	NA	3.71	15.46	15.46	1.0 ± 1.4	361 ± 510	0.4	157 ± 222	12.1

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Hermanutz et al (1992).

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

[c] No active nests, embryos, or larvae found in one of the 30 µg/L streams. Therefore, N = 1 for % dead embryos and dead larvae in the 30 µg/L treatment

Table 3. Effects on Progeny - Study II[a]

Egg cup observations												
treatment	stream	No. of trials	% hatch	% survival to 3rd day	% edema	% lordosis	% hemorr	% healthy[b]	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
									Early	Final	Geometric Mean	
control	1	6	93.0	75.2	0	0	0	97.8	1.02	0.78	0.89	3.72
control	5	5	96.4	71.5	0	0	0	97.9	1.09	0.76	0.91	3.79
2.5 µg/L	2	0	NA	NA	NA	NA	NA	NA		1.82	1.82	7.58
2.5 µg/L	7	4	81.4	71.6	0	0	3.6	92.2	2.02	3.36	2.61	10.86
10 µg/L	3	3	83.3	57.7	100	11.1	49.3	0		8.1	8.10	33.75
10 µg/L	8	2	91.1	57.1	100	18.2	41.1	0	6.96	12.6	9.36	39.02
rec 30 µg/L	4	0	NA	NA	NA	NA	NA	NA				
rec 30 µg/L	6	6	92.9	73.0	17.4	0	11.5	70.7	5.87	13.2	8.80	36.68

Nest Observations														
Treatment	Stream	# active Nests	# embryos Collected	% dead embryos	# larvae collected	% dead larvae	#samples w larvae	% edema	% lordosis	% hemorr	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
											Early	Final	Geometric Mean	
control	1	6	2458	0.94	3252	0.03	7	0	0	0	1.02	0.78	0.89	3.72
control	5	9	1329	0	3435	1.05	13	0	0	0	1.09	0.76	0.91	3.79
2.5 µg/L	2	1	0		2497	0.20	3	4.1	25	77.6		1.82	1.82	7.58
2.5 µg/L	7	5	1462	0	4717	0.08	8	0	0	52	2.02	3.36	2.61	10.86
10 µg/L	3	2	672	0	5376	0.50	9	81.4	5.0	55.5		8.1	8.10	33.75
10 µg/L	8	3	931	0.32	750	0.40	4	50	14.7	26.7	6.96	12.6	9.36	39.02
R 30 µg/L	4	0	NA	NA	NA	NA	NA	NA	NA	NA				
R 30 µg/L	6	8	646	0	6782	7.8	16	27.3	0	17.1	5.87	13.2	8.80	36.68

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Tao et al. (1999).

[b] Among live larvae that survived up to third day after first larvae hatched; assumes the observations of multiple abnormality types always co-occurred in the same organism. This may overestimate the actual % healthy when this assumption is violated.

[c] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Table 4. Effects on Progeny - Study III[a]

Egg cup observations									
treatment	**Stream**	**number of trials**	**% hatch**	**% survival to 3rd day**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	92	58.6	0	0	0	1.2	5.0
control	5	3	76.7	69.2	0	0.9	0.8	0.93	3.88
R 2.5 µg/L	2	3	87.3	66	0	0	0	1.84	7.67
R 2.5 µg/L	7	6	87.2	76.5	0	0	0	1.97	8.21
R 10 µg/L	3							6.25	26.04
R 10 µg/L	8	3	75.3	74.5	0	0	0	2.44	10.17
R 30 µg/L	4	5	92	78				3.82	15.92
R 30 µg/L	6								

Nest observations								
treatment	**stream**	**# active nests**	**# samples with larvae**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	5	0	0	0	1.2	5.0
control	5	2	3	0	0	0	0.93	3.88
R 2.5 µg/L	2	5	5	0	0	0	1.84	7.67
R 2.5 µg/L	7	5	2	0	0	0	1.97	8.21
R 10 µg/L	3	2	4	0	0	0	6.25	26.04
R 10 µg/L	8	4	4	0	0	0	2.44	10.17
R 30 µg/L	4	9	13	0	0	0	3.82	15.92
R 30 µg/L	6							

[a] The NOAEC for the study are from recovering 30 µg Se/L treatment.

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Effects Data: Tables 2 through 4 include exposure and effects data for Study I, II, and III, respectively. Study I & II deformity and survival data reported in the tables above from the nest and egg in response to Se concentrations in parental ovaries (mg/kg dw) were compiled in Table 5 for TRAP analysis. Study I effects data were obtained from Hermanutz et al. (1992), and corresponding Study I ovary Se concentrations were obtained from Hermanutz et al. (1996). Study II and III exposure data were obtained from Hermanutz et al. (1996) and effects data from Tao et al. (1999).

In this study ovary concentrations were measured in an aliquot of females taken from each treatment. The exposure and effects data are thus not as directly linked as they would be in field studies of more recent design – where offspring health can be directly linked to measured tissue concentrations of their female parent.

In a change from the analyses published in drafts of this criterion document, ovary, muscle, and whole-body concentrations measured too early in the exposure period (that is, during the month of May, and labeled "early" in Tables 2 and 3) have not been used because they were not sufficiently co-occurrent with the effects measurements. On the other hand, the data for the Study II recovering stream and all Study III recovering streams *are* included in the analyses. For this analysis, the nest data continue not to be used, because they were less consistent than the egg-cup data.

EC_{10}s are based on the combined effects on survival and deformities: that is, reduction in the percentage of individuals surviving and normal. Table 5 shows the exposure and effects data used. Figures 2 and 3 show the ovary, and whole-body concentration-response curves and an explanation of how the EC_{10} values were derived. The same approach was used for the muscle data, that is, an interpolation using a nonlinear regression threshold sigmoid equation. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 11.2 mg/kg muscle and the average background survival/normal of 69.1% and the second point set to the LOEC of 21.0 mg/kg and a survival/normal of 5.8%. The resulting EC_{10} is 13.4 mg/kg muscle dw. The EC_{10} estimates for the three tissues (below) are slightly different than the EC_{10} values in the previous draft document. The reason for the difference is the use of the interpolation method in the current version rather than an inappropriate usage of a TRAP model in the previous document.

Chronic Value: This study's chronic values for bluegill based on percentage surviving and free of deformities are the following EC_{10} values:
Ovary: 14.7 mg Se/kg ovary dw
Muscle: 13.4 mg Se/kg muscle dw
Whole body: 10.6 mg Se/kg WB dw.

Table 5. Final Exposure Concentrations and Egg Cup Survival and Deformity Rates Used for TRAP Analysis (Studies I, II, & III). The percent deformity is the maximum percentage of the individual deformity types for each treatment.

		Tissue concentration at end of exposure (dw)			Effects data from Hermanutz et al. (1996) and Tao et al. (1999)		
Study	**Treatment (µg/L)**	**Se ovary (mg/kg)**	**Se muscle (mg/kg)**	**Se WB (mg/kg)**	**% Survival**	**% Deformity**	**%Normal +Surviving**
I	Control	2.21	2.05	1.546	69.7	1.8	68.4
I	10	16.73	21.03	18.131	28.8	80	5.76
I	30	>25[1]	No data	No data	9.1	50.3	4.52
II	Control	3.25	1.96	1.63	75.2	0	75.2
II	Control	3.17	2.61	1.47	71.5	0	71.5
II	2.5	7.58	6.73	5.40	No data	No data	No data
II	2.5	14	7.13	4.40	71.6	3.6	69
II	10	33.75	36.51	16.47	57.7	100	0
II	10	52.5	55.25	26.79	57.1	100	0
II	R-30	55	39.78	24.29	79	17.4	65.3
III	Control	5.0	3.37	1.27	62.9	0	62.9
III	Control	3.88	3.11	2.66	68	0	68
III	R-2.5	7.67	5.78	4.17	71.3	0	71.3
III	R-2.5	8.21	6.48	4.25	72.2	0	72.2
III	R-10	10.17	11.20	9.29	63.4	0	63.4
III	R-30	15.92	15.12	13.77	81.1	No data	No data

[1] No data were recorded for this treatment, but a value 50% higher than the 10 µg/L treatment was added for inclusion in the analysis.

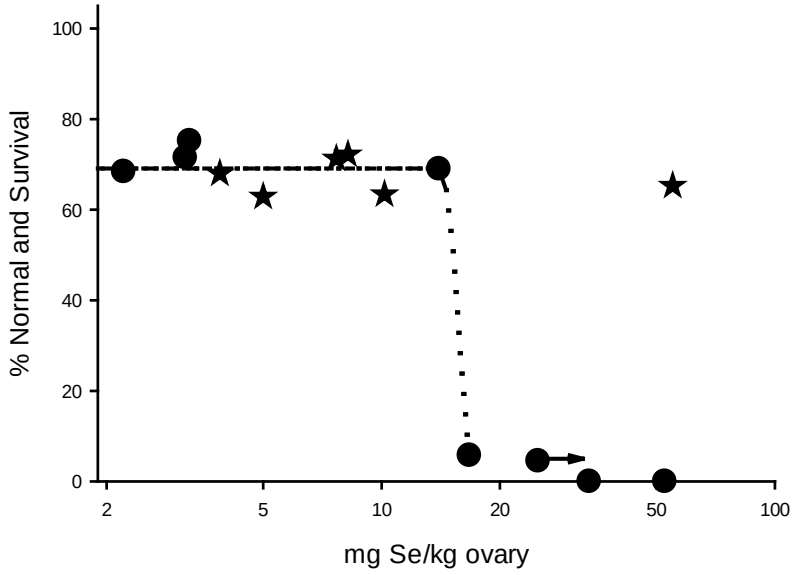


Figure 2. TRAP interpolation curve for the Table 5 ovary data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 14.0 mg/kg and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.7 mg/kg and a survival/normal of 5.76%. The resulting EC_{10} is 14.7 mg/kg ovary dw.

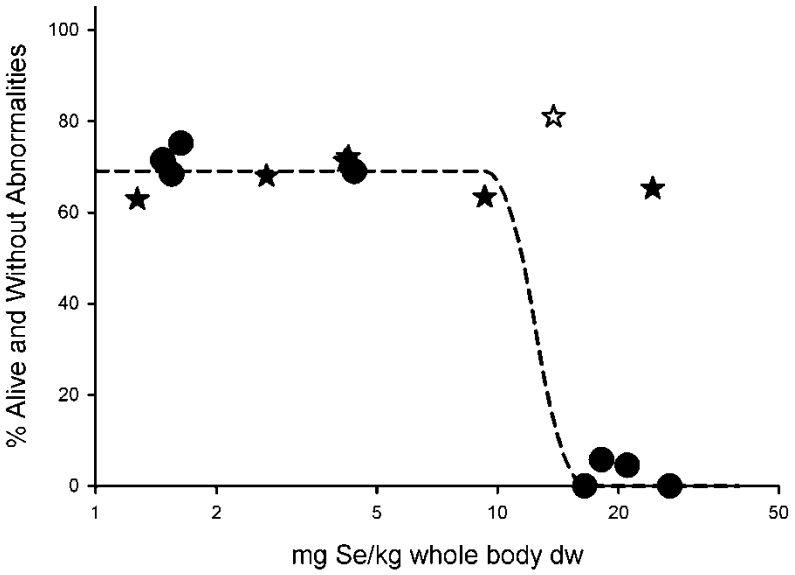


Figure 3. TRAP interpolation curve for the Table 5 whole body data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 9.3 mg/kg whole body and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.5 mg/kg and a survival/normal of 0%. The resulting EC_{10} is 10.6 mg Se/kg whole body dw.

Coyle, J.J., D.R. Buckler and C.G. Ingersoll. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). Environ. Toxicol. Chem. 12:551-565.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; two-year old pond-reared adult fish and resultant fry)

Exposure Route: Dietary and waterborne
Dietary
Seleno-L-methionine added in an aqueous solution to Oregon moist pellets; moisture content of diet was 25 percent.
Waterborne
Flow through 10 µg Se/L nominal, 6:1 ratio of selenate:selenite, 98 percent purity, adjusted to pH 2 with HCl to prevent bacterial growth and change in oxidation states of Se(IV) and Se(VI).

Test Duration: 140 days

Study Design: The experiment consisted of a test control and food control (see Test Treatment table below) with fish (n=28 initially) in the four remaining treatments fed one of the four seleno-methionine diets in combination with 10 µg Se/L in water. Spawning frequency, fecundity, and percentage hatch were monitored during the last 80 days of the exposure period. Survival of resulting fry (n=20) was monitored for 30 days after hatch. Adults and fry were exposed in separate, modified proportional flow-through diluters. Fry were exposed to the same waterborne selenium concentrations as their parents. Adults were fed twice daily *ad libitum*. Whole-body selenium concentrations in adult fish were measured at days 0, 60, and were calculated from individually analyzed carcass and gonadal tissue (ovaries and testes) at day 140. Eggs not used in percentage of hatch determinations were frozen and analyzed for total selenium.

Measured Se in:	**Test Treatments**					
	1 (test control)	2 (food control)	3	4	5	6
water (µg Se/L)	0.56	8.4	10.5	10.5	10.1	11.0
diet (mg Se/kg dw)	0.76	0.76	4.63	8.45	16.8	33.3

Effects Data: There was no effect of the combination of highest dietary selenium concentration (33.3 mg/kg dw) in conjunction with exposure to a waterborne selenium concentration of 11.0 µg/L on adult growth (length and weight), condition factor, gonad weight, gonadal somatic index, or reproductive endpoints (i.e., spawning frequency, number of eggs per spawn, percentage hatch) during the 140-day exposure (Table 1). The mean corresponding whole-body selenium concentration in adults exposed to this waterborne and dietary selenium combination was 19 mg/kg dw. Survival of fry from the exposed adults was affected by 5 days post-hatch. Concentrations of whole-body selenium in adult tissue at day 60 were used to determine effects in the fry because eggs were taken for the larval tests beginning at day 60 of the adult exposure.

Table 1. Effects on Adults

Se in diet, mg/kg dw	Se in water, µg/L	whole-body Se (140 d), mg/kg dw	replicate	total no. spawns	eggs/spawn	hatchability, %
0.8	0.5	0.8	A	15	14,099	94.5
			B	10	5,961	90.5
0.8	7.9	1.0	A	12	9,267	89.5
			B	11	9,255	84.5
4.6	10.5	3.4	A	20	9,782	86.5
			B	12	13,032	96.5
8.4	10.5	6.0	A	2	10,614	96.5
			B	9	7,995	90
16.8	10.1	10	A	13	10,797	83
			B	13	9,147	91.5
33.3	10.1	19	A	14	8,850	80
			B	4	8,850	80

In the 30-d survival after hatch test, there was complete mortality after one week at the highest exposure and no significant differences in survival at lower concentrations. Table 2 provides the survival data at 5 days post hatch used in the analysis of the effect concentration. The day 5 data are given in Table 2 because this was the only day in which control survival was over 90%, with the control and all the treatments showing substantial and increasing toxicity over the next 4 days.

Because the survival in the fifth treatment was about 5% below the average of the lowest four and because the highest treatment still had some survivors, this

provided two partial effects for TRAP to fit a curve. However, the legitimacy of this depends on the lower survival in the fifth treatment actually being a significant Se effect, rather than reflecting random variation of background survival. Because there were multiple spawns with 200-500 total larvae tested for each survival value above, this might be expected to be a real effect, but there is insufficient data reported to test this. However, from day 6 through day 30, survival at the fifth treatment was above that in the first and third treatments, indicating this is not an effect level. These later data establish that the highest treatment is best considered an EC_{100} and the fifth treatment an EC_0. So an interpolation was done using 42 mg/kg as an EC_{100}, resulting in a slope of 7.6 and an EC_{10} of 26.3 mg/kg. The interpolation between the EC_0 and EC_{100} resulted in a slightly higher EC_{10} in the previous draft document (24.15 mg/kg) which used a TRAP model to estimate the EC_{10}. A figure is not provided here because this interpolation represents a synthesis of the data not tied to the data for a specific day.

As for the analysis with egg concentrations, the whole-body analysis recognizes the highest treatment as an EC_{100} (16 mg Se/kg dry wt whole body) and the second highest treatment as an EC_0 (7.2 mg Se/kg dry wt whole body). The interpolation method then results in an EC_{10} of 8.6 mg/kg. As for the egg concentration analysis, no plot is given because the EC_0 is not for a specific day or survival value.

Table 2. Survival of Larvae at Day 5 in the 30-day Post-hatch Test

Se in diet, mg/kg dw	Se in water, µg/L	egg, mg/kg dw	adult whole-body (60 d), mg/kg dw	mean survival, %
0.8	0.5	1.8	0.9	92
0.8	7.9	1.8	0.9	93
4.6	10.5	7.3	2.9	90
8.4	10.5	13	4.9	95
16.8	10.1	23	7.2	87
33.3	10.1	42	16	7

Chronic Value:

effect level	egg, mg Se/kg dw	whole body, mg Se/kg dw
EC_{10}	**26.3**	**8.6**

Cleveland, L. et al. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill sunfish (*Lepomis macrochirus*). Aquatic Toxicol. 27:265-280.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*)

Life Stage: juvenile (5 months - waterborne exposure; 3 months - dietary exposure)

Exposure Route: waterborne (60-d) and dietary (90-d) - separate exposures
waterborne - 6:1 selenate:selenite at 0.17, 0.34, 0.68, 1.38, 2.73 mg/L; dietary - seleno-L-methionine in Oregon moist at 1.63, 3.25, 6.5, 13, 26 mg Se/kg dw)

Study Design: Fish were exposed using a flow-through diluter. Each test consisted of an exposure and a depuration phase. Whole body tissue measurements were made at 31 and 60 days of waterborne exposure and at 31, 59 and 90 days of dietary exposure. Mortality and condition factor, K (weight x 10^5/length^3), were reported at selected intervals.

Effects Data: The waterborne exposure (see table below) was determined to have an EC_{20} = 4.07 mg Se/kg dw (1.96-8.44 mg/kg 95% CL). However, because it was a water-only exposure, it was not considered in the derivation of the FCV. These data nevertheless provide evidence that exposure route influences the tissue concentration toxicity threshold, although the mechanistic explanation for this phenomenon is lacking.

A mortality effect level for the dietary exposure could not be calculated because the highest selenium whole body concentration (13.4 mg Se/kg dw) only had 17.5% mortality. The middle selenium concentration did have 22.5% mortality. Cleveland et al. reported a significant decrease in K between 4.7 and 7.7 mg/kg dw (see table below).

Waterborne Exposure Study

Measured selenium in water (:g/L)	**60-d measured selenium in whole body (mg/kg dw)**	**60-d mortality (%)**	**Condition factor (K)**
20 (control)	1.1	10	1.5
160	2.8	12.5	1.5
330	4	22.5	1.6
640	5.3	52.5	1.5
1120	9.8	70	1.6
2800	14.7*	97.5	NA

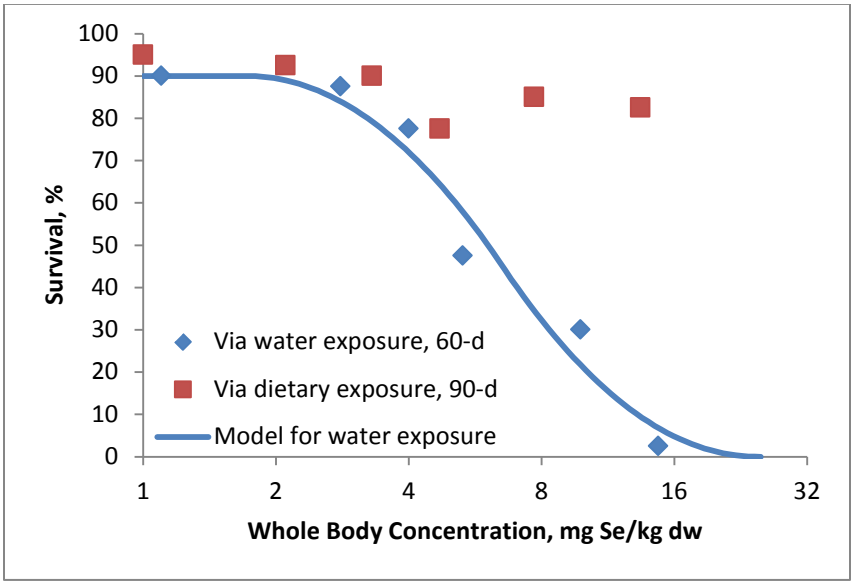
*[a] 30-d measurement because all fish were dead at 60 days in this concentration.

Dietary Exposure Study

Measured selenium in food (mg/kg ww)	90-d measured selenium in whole body (mg/kg dw)	90-d mortality (%)	Condition factor (K)
0.68 (control)	1	5	1.3
2.3	2.1	7.5	1.3
3.5	3.3	10	1.3
6.6	4.7	22.5	1.3
12.7	7.7	15	1.2
25	13.4	17.5	1.2

Discussion

The study demonstrates the influence of exposure route on the potency of a given tissue concentration, as shown in the figure. The TRAP threshold sigmoid concentration-response curve for the water-only exposure yields an EC50 of 6.5 mg Se/kg dw WB. In contrast, higher whole-body concentrations acquired via diet did not yield significant effects and cannot support a TRAP-fitted concentration-response curve or EC estimate. Examination of the graph indicates that the water-only concentration-response curve would need to be shifted to the right a minimum of 4-fold (or possibly more) to be able to fit the (lack of) effects observed in the dietary study. This supports the decision to derive the criteria only from studies relying on the environmentally relevant exposure route, diet.



Survival at 60-days (for water exposure) or 90-days (for dietary exposure) versus whole-body concentration.

Chronic Value: Given (a) the very slight reduction in K (1.3 to 1.2 between 4.7 and 7.7 mg Se/kg dw WB, with no further reduction at 13.4 mg Se/kg dw WB) and uncertain relevance of growth data, and (b) no apparent concentration-related effect on mortality between 4.7 and 13.4 mg Se/kg dw WB, the NOAEC is interpreted to be 13.4 mg Se/kg dw for this study; and the chronic value is >13.4 mg Se/kg dw whole body.

Lemly, A.D. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicol. 27:133-158.

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile 50-70 mm)

Exposure Route: Waterborne and dietary
Water
1:1 selenite:selenate in stock at pH 2; metered in to reach 5 :g/L
Diet
seleno-L-methionine in TetraMin (5 mg/kg dw)

Test Duration: 180 days

Study Design: Fish were exposed (treatment and control) under intermittent flow-through conditions for 180 days. Tests were run at 4° and 20°C with biological (histological, hematological, metabolic and survival) and selenium measurements made at 0, 60, 120 and 180 days. Fish were fed at a rate of 3% body weight per day. All treatments were initiated at 20°C and then decreased in the cold treatment at a rate of 2°C per week for 8 weeks to reach 4°C and then maintained at that temperature for the remainder of the 180 days.

Effects Data : In the 20°C test, fish accumulated 6 mg/kg dw selenium (whole-body) with no significant effect on survival (4.3% and 7.4% mortality in control and treatment, respectively). In the 4°C test, fish exposed to selenium accumulated 7.9 mg/kg dw (whole-body) selenium and had significant mortality after 120 (33.6%) and 180 days (40.4%) relative to control (3.9%). Several hematological measurements were significantly different in both the warm and cold selenium exposures relative to controls. Both warm and cold selenium treatments also had greater O_2 consumption than controls. Fish lipid content in the cold Se treatment decreased more than the cold control; lipid content did not decrease in either the warm control or the warm Se treatment (see summary tables below). The results suggest significant mortality occurs in juvenile bluegill during winter months when tissue concentrations reach 7.91 mg/kg dw and lipid levels decrease to 6 percent.

Chronic Value: 20°C, >6 mg Se/kg whole-body; 4°C, <7.91 mg Se/kg dw whole body

Comments: See “Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies” in this appendix after presentation of the McIntyre et al. (2008) study.

Mean Concentration of Selenium in Tissues, Cumulative Survival*, Percent Lipid Content and Oxygen Consumption in Juvenile Bluegill

day	cold - Se control				cold + Se				warm - Se control				warm + Se			
	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]
0	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98
60	1	97.1	12.5	58	5.8	92.9	10	63	1.2	95.7	13.3	98	5.8	100	13.3	103
120	1.1	97.1	11.5	57	7.9	66.4	6	81	1.1	95.7	13.4	100	6	96.7	13.4	120
180	1.4	97.1	10.5	57	7.9	59.6	6	78	1.2	95.7	13.6	100	6	92.6	13.5	120

[a] whole body Se tissue concentration, mg/kg dw
[b] oxygen consumption, mg/kg/hr

* Cumulative Survival: In this experiment, 240 juvenile bluegill were placed in three 400-L fiberglass tanks, 80 in each, and exposed to each control and treatment for a period of 180 days. Ten fish were removed at random from each treatment replicate on days 0, 60, 120, and 180 for selenium, histological, hematological, and metabolic measurements.

Replicate and Average Whole-body concentrations (mg/kg dry weight) of selenium in juvenile bluegill*

	day 0				day 60				day 120				day 180			
replicate	1	2	3	mean	1	2	3	mean	1	2	3	mean	1	2	3	mean
c+Se	0.87	1.21	0.95	1.01	6.30	5.49	5.76	5.85	8.36	7.31	7.85	7.84	7.53	8.01	8.19	7.91
w+Se	1.17	0.96	0.90	1.01	5.61	6.19	5.43	5.74	6.37	5.92	5.50	5.93	5.48	5.72	6.02	5.74
c-Se	0.89			0.89	0.97			0.97	1.01			1.01	1.10			1.10
w-Se	0.99			0.99	1.12			1.12	0.99			0.99	0.96			0.96

* Each value is for a composite sample made from 5 fish.

The Kaplan-Meier estimator was used to calculate survival at time *t*

$$\hat{S}(t) = \frac{\prod r(t_i) - d_i}{r(t_i)}$$

where $r(t_i)$ is the number of fish alive just before time t_i, i.e. the number at risk, and d_i is the number of deaths in the interval $I_i = [t_i, t_{i+1}]$. The 95% confidence interval for such estimate (Venables and Ripley 2002) was computed as

$$\exp\left\{-\hat{H}(t)\exp\left[\pm k_\alpha \frac{\text{s.e.}\left(\hat{H}(t)\right)}{\hat{H}(t)}\right]\right\}$$

where

$$\hat{H}(t) = \sum \frac{d_j}{r(t_j)} \qquad \text{and } j \# i$$

The following table lists the estimates of survival in the cold + Se treatment at 60, 120 and 180 days. The term n.event is the number of deaths at a given interval; n.risk is the number of organisms alive at the beginning of the interval; survival is computed by the Kaplan-Meier estimator.

Time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
60	210	15	0.929	0.0178	0.884	0.956
120	165	47	0.664	0.0350	0.590	0.728
180	88	9	0.596	0.0381	0.517	0.666

Hematological Measurements in Juvenile Bluegill Sunfish (*indicates significantly different from control)

Warm Exposure	day 0		day 60		day 120		day 180	
blood parameter	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se
total erythrocyte, 10^6/ml	2.95	2.92	2.96	2.93	2.99	2.95	2.96	2.89
% mature	85	86	86	93*	86	94*	85	94*
nuclear shadows, 10^4/ml	0.95	0.86	0.97	2.05*	0.83	2.38*	0.91	2.30*
total leucocytes, 10^4/ml	17.22	17.41	16.90	17.55	16.73	17.62	17.05	17.36
% lymphocytes	23	25	20	23	19	26	21	22
% neutrophils	15	13	14	15	17	19	17	16
hematocrit, %	37	36	37	29*	36	29*	38	28*
MCHC (mean corpuscular hemoglobin conc.)	23	25	25	19*	25	18*	25	17*
Cold Exposure	day 0		day 60		day 120		day 180	
blood parameter	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se
total erythrocyte, 10^6/ml	2.91	2.93	2.97	2.90	3.01	2.95	3.00	2.99

% mature	84	82	87	95*	85	96*	85	97*
nuclear shadows, 10^4/ml	0.86	0.84	0.83	2.30*	0.89	2.49*	0.90	2.36
total leucocytes, 10^4/ml	16.48	16.88	16.79	16.91	16.80	16.74	16.96	16.63
% lymphocytes	17	16	16	17	19	15	19	18
% neutrophils	13	12	15	11	15	12	12	14
hematocrit, %	39	37	40	30*	41	28*	39	27*
MCHC (mean corpuscular hemoglobin conc.)	26	25	25	18*	22	17*	23	17*
MCV (mean corpuscular volume)	182	171	188	146*	180	135*	185	130*

McIntyre et al. 2008. Effect of Selenium on Juvenile Bluegill Sunfish at Reduced Temperatures. US EPA, Health and Ecological Criteria Division. EPA-822-R-08-020

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); juvenile; average length 47 mm, average weight 1 g

Exposure Route: Waterborne and dietary

Water

1:1 selenite:selenate; For exposure systems (ES) 1 and 3, fish were exposed to a control and a series of 6 nominal concentrations, 1.25, 2.5, 5, 10, 20 and 40 µg Se/L. For ES2, fish were exposed to a control and one nominal concentration, 5 µg Se/L.

Diet

For ES1 and ES3, fish were fed a series of six concentrations of selenium and a background control in *Lumbriculus variegatus*. The measured selenium concentrations in the *L. variegatus* treatments in ES1 were: 2.3 (control), 4.5, 5.3, 7.5, 14.2, 25.7 and 34.9 mg Se/kg dw; in ES3: 2.2 (control), 4.2, 5.0, 7.2, 15.2, 25.4 and 46.7 mg Se/kg dw. Fish were fed worms at a rate of 4% of the current biomass in each fish tank. Selenium was accumulated in *L. variegatus* by feeding the worms in separate tanks a series of six concentrations of selenized-yeast diluted with nutritional yeast: 1.7, 3.3, 6.7, 13.3, 26.7 and 53.5 mg Se/kg dw. Control worms were fed nutritional yeast only. Each tank was additionally exposed to the associated aqueous concentration selenium, e.g., the worms fed the 1.7 mg Se/kg dw selenized yeast were exposed to 1.25 :g Se/L, the worms fed the 3.3 mg Se/kg dw selenized yeast were exposed to 2.5 :g Se/L, and so on. For ES2, fish were fed TetraMin spiked with seleno-L-methionine at a nominal concentration of 5 mg/kg dw and at a rate of 3% of the current biomass in each tank.

Test Duration: 182 days

Study Design: Juvenile bluegill were exposed concurrently to selenium using three separate exposure systems, ES1, ES2 and ES3. In ES1 and ES3, 100 fish were exposed to each of 6 selenium treatments (low through high treatments are referred to as Treatments 1 through 6) and two controls in 200 L carboys under flow-through conditions. Each treatment consisted of an aqueous selenium concentration and an associated dietary selenium concentration, e.g., the fish in the lowest ES1 treatment were exposed to 1.25 :g Se/L and fed worms containing 4.5 mg Se/kg dw (see Exposure Route for other treatment concentrations). Temperature was controlled in each system through the immersion of the carboys in a temperature-controlled water bath and by controlling the temperature of the dilution water being added to the carboys. The temperature in ES1 was maintained at 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The only difference between ES1 and ES3 was temperature was decreased 2°C/week until it reached 9°C (test day 65) at which point temperature was maintained until test termination (test day 182).

The exposure of ES2 was similar to ES1 and ES3 in that 100 juvenile bluegill were exposed to treatment in 200 L carboys under flow-through conditions. The

ES2 selenium treatment consisted of two replicates of 5 µg Se/L waterborne and 5 mg Se/kg dw diet (Tetramin). Two controls were maintained with ES2. The temperature regime for ES2 was identical to ES1.

Observations on fish behavior and mortality were checked daily. Total selenium was measured in each fish tank weekly and selenium speciation was measured monthly in each fish tank. Whole body total selenium was measured in the worms from each tank (2 replicate 5 g samples) on test days 0, 30, 60, 112 and 182 and in the bluegill from each tank (3 replicates of 3-fish composites - total 9 fish) on test days 0, 7, 30, 60, 112 and 182. The standard length and weight of each fish was measured on each sample day. Lipid content was measured in fish at day 0 and from each treatment at test termination.

Effects Data:

Selenium increased in bluegill as the exposure concentrations increased (see following table). No meaningful mortality was observed in ES2. The number of fish that died in ES2 during the 182 day test was two fish in one treatment replicate and none in the other treatment replicate; no deaths were reported in ES2 controls. Significant mortality of juvenile bluegill was observed in ES1 and ES3. After 182 days, a total of 24 and 68 fish died in Treatments 5 and 6, respectively in ES1; and a total of 38 and 61 fish died in Treatments 5 and 6, respectively in ES3. See table below for mortalities in all treatments. Estimates of bluegill survival were adjusted for the removal of individuals from the test population. Individuals were removed from the experiments before test completion, for sampling tissue concentrations or because they suffered accidental deaths unrelated to selenium toxicity. For such data, it was necessary to account for the reduction in number of individuals at risk of death due to selenium over time. If $r(t_i)$ is the number of individuals at risk just before time t_i and d_i is the number of deaths in the interval, $I_i = [t_i, t_{i+1})$, then survival (S) at time t can be estimated as

$$\hat{S}(t) = \prod \frac{r(t_i) - d_i}{r(t_i)}$$

The product (P) was calculated for each period in which one or more deaths occur. The equation is the Kaplan-Meier estimator (Venables and Ripley 2002). This correction was applied to calculate the proportion of survival in treatments with ten or more deaths (10% mortality). The table below provides the adjusted proportion and surviving bluegill in each treatment along with the concentration of selenium in bluegill at test termination. The values in this table were used to calculate the EC_{20} and EC_{10} values using the TEAM software. Growth and lipid content of the bluegill was not negatively affected by the selenium exposures.

Measured total selenium concentrations in bluegill sunfish for all treatments and controls in Exposure System 1, 2 and 3.

Total Selenium in Whole Body Bluegill Tissue, mg/kg dw

ES1	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.43 (0.31)	2.48 (0.11)	2.43 (0.18)	2.64 (0.06)	2.72 (0.07)	3.27 (0.27)	4.27 (0.44)
30	2.10 (0.21)	2.85 (0.10)	3.10 (0.04)	2.94 (0.13)	4.24 (0.22)	6.62 (0.23)	10.21 (0.36)
60	2.11 (0.02)	2.70 (0.20)	3.07 (0.05)	3.69 (0.25)	5.21 (0.30)	8.62 (0.45)	12.66 (0.45)
112	1.98 (0.04)	3.16 (0.11)	3.41 (0.08)	3.99 (0.26)	6.42 (0.05)	11.60 (0.43)	
182	2.08 (0.10)	2.56 (0.21)	3.15 (0.25)	4.02 (0.21)	6.72 (0.09)	10.71 (0.55)	

ES3	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.50 (0.10)	2.60 (0.29)	2.38 (0.10)	2.82 (0.20)	3.19 (0.33)	4.29 (0.20)	6.13 (0.62)
30	2.24 (0.41)	2.44 (0.26)	2.70 (0.16)	3.13 (0.10)	3.95 (0.16)	6.06 (0.36)	11.07 (0.92)
60	2.70 (0.22)	2.88 (0.08)	3.04 (0.39)	3.79 (0.24)	5.54 (0.21)	9.50 (0.91)	15.14 (0.96)
112	2.16 (0.14)	2.49 (0.10)	3.10 (0.12)	3.64 (0.16)	6.54 (0.21)	11.50 (0.25)	17.24 (0.30)
182	1.67 (0.21)	3.20 (0.27)	3.83 (0.47)	5.48 (0.24)	9.38 (0.63)	16.01 (0.30)	

ES2	Control	5A	5B
Test Day	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.19 (0.19)	3.55 (0.25)	3.08 (0.50)
30	2.49 (0.15)	7.05 (0.76)	7.51 (1.18)
60	1.53 (0.03)	8.23 (1.55)	8.09 (0.67)
112	1.57 (0.01)	8.97 (1.28)	9.45 (1.73)
182	1.38 (0.06)	9.41 (1.63)	10.61 (0.38)

Total number of deaths in ES1 and ES3 Treatments throughout the experiment's duration (182 days). Both ES1 and ES3 had two control tanks.

Treatment	ES1	ES3
Control (#1, #2)	0, 7	1, 1
1	5	0
2	1	1
3	0	0
4	3	3
5	24	38
6	68	61

The concentration of selenium in bluegill and the adjusted proportion of surviving fish at the end of the 182 day exposure.

	ES1		ES3	
Treatment	$[Se]_{tissue}$, mg/kg dw	surv	$[Se]_{tissue}$, mg/kg dw	surv
control	2.08	0.962	1.67	0.988
1	2.56	0.988	3.20	1.000
2	3.15	0.984	3.83	0.988
3	4.02	1.000	5.48	1.000
4	6.72	0.962	9.38	0.960
5	10.71	0.497	16.01	0.435
6	12.66	0.075	17.24	0.168

Chronic Value: The NOAEC for bluegill in ES2 was calculated as the geometric mean of the concentration of bluegill in the two replicates at the end of the exposure period, 9.992 mg Se/kg dw whole body. The chronic value for ES2 is therefore >9.992 mg Se/kg dw whole body. The EC_{20} and EC_{10} values for ES1 and ES3 are given in the following table.

	ES1 (4°C)	ES3 (9°C)
	Whole body	Whole body
EC_{20} mg Se/kg dw	9.78	14.64
EC_{10} mg Se/kg dw	9.27	14.00

Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies of Lemly (1993a) and McIntyre et al. (2008)

The Lemly (1993a) and McIntyre et al. (2008) cold-temperature juvenile bluegill studies are summarized on the previous pages. This discussion compares and contrasts these studies.

Both studies indicated that juvenile bluegill are more sensitive to selenium at lower temperature than at higher temperature. For a 4°C temperature regime, the EC_{10} of 9.27 mg Se/kg dw WB obtained with McIntyre's selenized yeast-worm-fish dietary bioaccumulation system is somewhat similar to the threshold of 5.85 mg Se/kg dw WB estimated from the time course of bioaccumulation and mortality in Lemly's single treatment with seleno-L-methionine in TetraMin. These chronic values differ by a factor of 1.58.

The difference in diet does not appear to explain the modest difference in results; however, since McIntyre's other 4°C experiment (Exposure System ES2), which used Lemly's seleno-L-methionine in TetraMin diet, experienced no significant toxicity, whereas Lemly's similarly exposed fish experienced 40 percent mortality by the end of the test. In addition to the difference in observed mortalities, Lemly's bluegill in the 4°C selenium exposure decreased in both lipid content and body condition over the 180 days whereas no decreases in these measurements were observed in the McIntyre et al. study, although the fish used in both studies were of comparable size and body condition at test initiation: 47 mm average standard length (range 44 to 54 mm) and a body condition index (100 x fish weight/standard length) of 3.2 in ES2 compared to 50 to 70 mm total length and a body condition factor of 3.9 in Lemly.

There are several possible reasons why such results could differ between studies. (1) ES2 maintained exposure at 20°C for the first 30 days of exposure before decreasing the temperature compared to 7 days in the Lemly study. (2) Lemly measured O_2 consumption by removing and reintroducing test fish to the test tanks, which was not done by McIntyre et al. (3) The two studies differed in photoperiod – Lemly "began with a 16:10 h light/dark photoperiod which was gradually reversed to 10:16" (sic) whereas McIntyre et al. used a fixed photoperiod of 16:8. (4) Some genetic differences between the tested batches of organisms may be expected, reflecting different origins, despite the similarities in their starting size and condition.

The modification to maintain 20°C for 30 days was to allow a longer period of time for the fish to accumulate selenium during a warmer condition prior to decreasing the temperature. This did result in shortening the exposure in ES2 at 4°C by 19 days (103 days at 4°C) compared to 122 days at 4°C in Lemly's study. However, as the majority of deaths in Lemly's study occurred between in the middle 60 days of the 180-day test, the slightly shorter cold period in the McIntyre study would not explain the differences in mortalities.

As stated above, Lemly removed fish (N = 15) from each treatment for oxygen consumption measurement and then returned these fish to the exposure tanks. There is the possibility that the fish removed from the cold plus selenium treatment were sufficiently stressed by the exposure conditions that the additional handling stress contributed to the mortality observed in this treatment. Between test days 60 and 180, 56 fish died Lemly's cold plus selenium treatment. Even if stress due to handling affected all the fish used in the oxygen consumption measurements (up to 30 fish), it does not explain all the mortality that was observed and therefore does not explain the difference between the two studies.

Both Lemly (1993) and McIntyre et al. (2008) showed reduced survival of juvenile bluegill exposed to elevated selenium under lab-simulated winter conditions, albeit at somewhat different concentrations. But only Lemly, not McIntyre et al., found the decreased survival to be accompanied by loss of lipid and body

condition. It was hypothesized that the decrease in EC_{10} observed by Lemly (1993) in the cold water treatment between 60-180 days was attributed to "winter stress syndrome" (WSS). WSS is hypothesized to occur in warmwater fish species because the presence of a stressor places additional metabolic costs on exposed organisms. These stresses can be better tolerated during periods of warm weather and active feeding. However, during the winter months, feeding and activity levels decrease but the metabolic costs of the stressor remain. As a result, fishes deplete their lipid stores, resulting in lower condition factors and increased susceptibility to mortality (Lemly 1996). Lemly noted three conditions that must be met simultaneously in order for WSS to occur: 1) a significant metabolic stressor must be present, 2) cold water temperatures must be present, and 3) fish must respond by reducing activity and feeding (Lemly 1996).

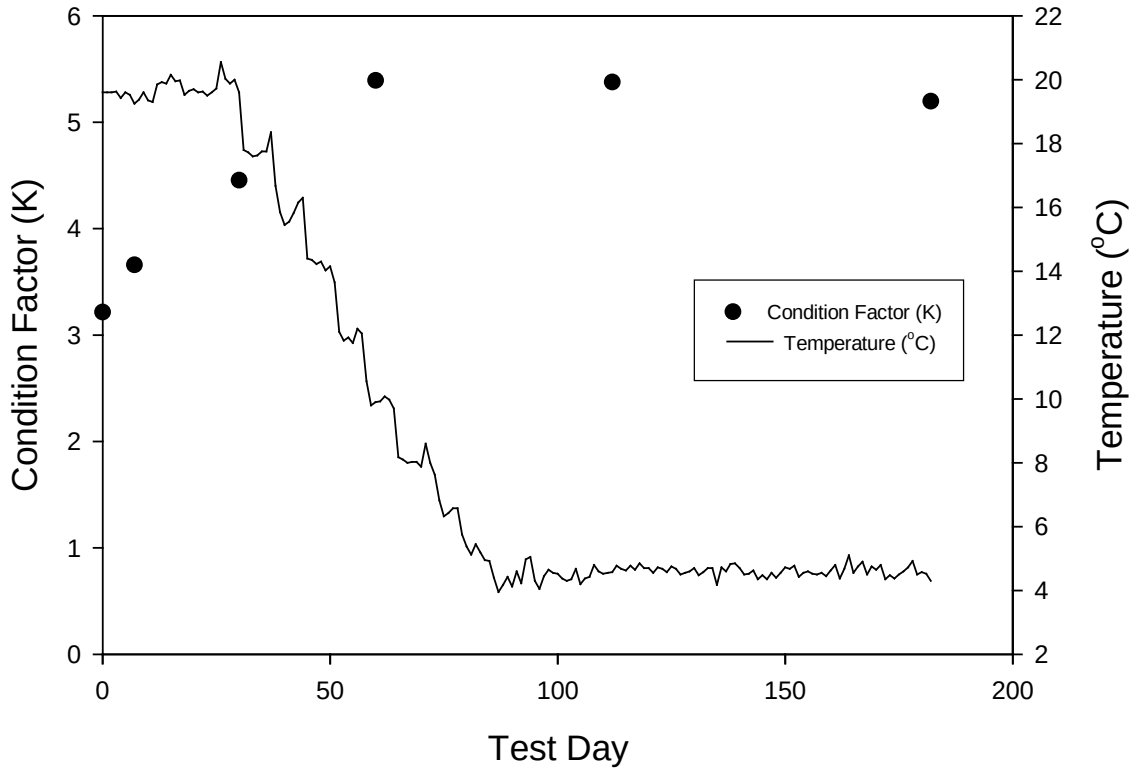
Several other studies have reported decreased feeding and activity levels for several fish species. McCollum et al. (2003) observed decreased overwinter feeding, and subsequent weight loss, of white crappie. Parrish et al. (2004) observed overwinter weight loss among mature, but not juvenile, salmon in a laboratory study in experimental raceways. Current speed, and by extension prey delivery rate, was the most important factor regulating overwinter feeding and growth. Eckmann (2004) observed overwinter reductions in feeding, weight, and lipid levels in yellow perch, but not in ruffe. Sogard and Olla (2000) observed walleye pollock could mitigate the effects of overwinter lipid depletion by moving to colder waters, where reduced metabolism allowed them to conserve energy. In all of these studies, fish continued to feed during the winter, but feeding rates decreased. The increase in weight among ruffe was attributed to its ability to feed on benthos in the dark during the winter months, suggesting that feeding reduction during winter may be more pronounced for species dependent on vision to feed. This was supported by Bennett and Janz (2007a), who observed that burbot, which rely primarily on smell while feeding on benthic invertebrates, experienced significant overwinter increases in weight and lipids in all sites, while northern pike, which rely primarily on vision while feeding on zooplankton, experienced slight but non-significant increases in weight and length.

WSS has not been definitively confirmed or refuted, although it has been investigated in the field. Bennett and Janz (2007a) observed no evidence of WSS for juvenile northern pike or burbot. Lengths, weights, and lipids increased for both species, particularly the olfactory feeding burbots, in the spring compared to the previous fall. Overall weights and lipids were higher in the low and high exposure lakes than the reference lake, possibly because of nitrogen limitation in the reference lake coupled with relatively low stressor concentrations in the exposed lakes. In a separate study, overwinter weights and lipids remained similar or increased in northern pike and burbot at both reference and exposure sites, while overwinter weights and lipids decreased at the exposure site for slimy sculpin (Bennett and Janz 2007b). However, this study neither supports nor refutes the WSS hypothesis, because stressor concentrations at the exposure site were not significantly different than at the reference sites, and the weight decrease in sculpin was attributed to higher turbidity at the exposure site, which inhibited food acquisition. In a final field test of WSS fathead minnow, creek chub, and white suckers were collected from reference and exposure sites (Driedger et al. 2009). Stressor levels at exposure sites were high, as whole body Se concentrations in fathead minnow ranged from 11-42 mg/kg dw. All three species either gained or maintained weight overwinter at all sites, indicating that active feeding occurred overwinter. Overall weights at exposure sites were higher, likely because of nutrient limitation at the reference sites, which confounded the ability to fully test the WSS hypothesis.

These results suggest that fish species responses to cold temperatures vary by species and environment. Many species lose weight, but this can be partially explained by the impact of low light levels on consumption levels, especially in northern latitudes where overwinter light limitation is pronounced. Field tests found no evidence of WSS, but were confounded by low stressor levels, nutrient limitation at reference sites, or both.

It may then be questioned whether the fixed photoperiod alone could account for the differences in the results of the two studies. More explicitly, did the longer light period in McIntyre et al. photoperiod allow the fish to feed more than the fish exposed to the shorter light period in the Lemly study, such that lipid and body condition in the McIntyre et al. fish were maintained and therefore not susceptible to "winter stress syndrome." The effects of photoperiod on fish and other ectotherms are well-documented. Temperature-independent seasonal changes in fish have been reported for growth and food conversion efficiency (Biswas and Takeuchi 2003; Jonassen et al. 2000; Simensen et al. 2000), feeding behavior (Volkoff and Peter 2006), metabolic rate (Evans 1984), and reproduction (Koger et al. 1999; Scott 1979). Some of these studies have found conflicting results on the effect of photoperiod on growth (Fuchs 1978; Jonassen et al. 2000; Simensen et al. 2000). Coupled with temperature being a dominant factor in controlling physiological functions in temperate-zone fish as indicated by a 3 to 4-fold fluctuation in metabolic activities over 10°C (Brett 1970; Fry 1971), it is difficult to use literature findings to explain the difference in the two bluegill studies. In field studies of fish at northern latitudes (Eckmann 2004), reduced light resulted in weight loss not though a bioenergetics interaction with cold temperatures, but by inhibiting feeding ability of visual, but not non-visual predators. If this mechanism applies to bluegill, then photoperiod is less likely to play a major role in the difference in results, as the overwinter light:dark cycle (8:16) should have been sufficiently long for the bluegill in Lemly (1993) to feed.

Observational recordings of the feeding behavior in McIntyre et al. noted that in both control replicates and in both treatment replicates the feeding of the juvenile bluegill went from active to not active on test day 78 when temperatures were decreased from 6.6 to 5.8°C. The feeding observations are reflected in a gradual slight decrease in the body condition factor (K) after test day 60 in the figure below. Although food intake was not quantified during the study, the lack of growth indicated in K suggests feeding markedly decreased as the temperature declined, as shown in the figure. Body condition decreased much more in the Lemly's cold plus selenium exposed fish after test day 60 (approximately 50%) but K in his cold-without-selenium exposure decreased only slightly, similar to McIntyre et al. Therefore it is not possible to determine if the greater decrease in K and in lipid content in Lemly's cold plus selenium treatment was due to decreased feeding because of a shorter photoperiod or because the bluegill fish population used in his study were more sensitive to selenium in cold conditions. McIntyre et al. obtained bluegill from Osage Catfisheries in Missouri whereas Lemly collected fish from ponds (assumed to be near Blacksburg, Virginia, not stated in paper). The fish obtained from Missouri, a location with colder winters than Virginia, may have been better adapted for withstanding colder winter temperatures than Lemly's fish and therefore were less sensitive to "winter stress syndrome" as induced by selenium exposure. Similarly, different populations of a species can have varying sensitivities to stressors. Furthermore, the relative difference in the Lemly and McIntyre et al. results is slightly **less** than Delos (2001) found to be typical when equivalent toxicity tests of the same species are compared. There should thus be no expectation that the two study results should agree more closely than they do.



Relationship between body condition factor (K) and temperature in juvenile bluegill fed a diet of Se-enriched TetraMin in the McIntyre et al. (2008) study.

Carolina Power & Light. 1997. Largemouth Bass Selenium Bioassay- Report. Carolina Power & Light Company, Environmental Services Section, 3932 New Hill, North Carolina. December 1997

Test Organism: Largemouth bass (*Micropterus salmoides*)

Exposure Route: Laboratory; dietary exposure only; DL-selenomethionine added to an artificial diet. Adult largemouth bass obtained from a commercial supplier were fed several months prior to spawning a series of selenium concentrations in the artificial diet.

Test duration: Embryo-larval monitoring through swim-up stage.

Study Design: Dietary exposure studies were conducted in 1995 and in 1996. In 1995, the measured dietary Se concentrations were 0.9 (control), 2.9, 7.5 and 11.2 mg Se/kg dw: in 1996, they were 26.7, 53.1 and 78.4 mg Se/kg dw. Parent fish were fed to satiation twice per day. Approximately 100 eggs from each spawn were transferred to each of 2 to 4 incubation cups. Eggs and larvae were monitored for mortality and deformities up to the larval swim-up stage. Selenium was measured in the liver, muscle and gonad tissues of the parent fish. All live deformed larvae at swim-up stage were considered as mortalities in the analyses.

Effects Data: Over the two year period, 56 successful spawns were obtained across all dietary treatments. Live larval fish with deformities (kyphosis, scoliosis, jaw gap, and lordosis) and edema at swim-up stage were considered mortalities for data analysis. The average concentration of selenium in ovaries ranged from 3.1 mg/kg dw in the control to 77.6 mg/kg dw in the high dietary treatment (Table 1). Larval survival generally decreased as the selenium concentration in the ovary increased (Table 1; Figure 1). A plot of the percent survival of larval largemouth bass as a function of the selenium concentration in the parental female ovary shows two groups of data; one at background survival with considerable variability (mean 90.3%, standard deviation 10.9%) and one with <10% survival, with most of the data being at 0% survival. Due to inadequate partial effects, a TRAP interpolation was used to estimate an EC_{10} value. Based on a risk management decision that the LOEC cannot be any higher than the lowest concentration with 0% survival (32.9 mg/kg) and that any ECx should be below this, this establishes the higher concentration point for the interpolation (an EC_{100} of 32.9 mg/kg) and requires that the highest 4 NOECs not be considered in setting the EC_0. The lower concentration point for the interpolation is therefore set here to 24.6, the next highest NOEC with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg (and a steep slope of 16).

An EC_{10} for the muscle tissue in Table 1 was not determined due to uncertainty in the values. The authors of this report also measured selenium in the ovaries and muscle tissues of largemouth bass collected from Mayo Reservoir (Table 2). There was a considerable difference in the proportion of selenium in the ovaries to the muscle tissues between the largemouth bass collected from the bioassay study and the field collected largemouth bass. The ratio of Se in ovaries to muscle in the laboratory fish was approximately 3.3 whereas it was 1.1 in the field collected fish. With the exception of mountain whitefish, the ovary to muscle ratio observed in the laboratory fish is also considerably higher than other

species (see Appendix B Table B-3). Based on this uncertainty in the muscle concentrations in the laboratory fish, an EC_{10} for this tissue was not calculated. The effect concentration based on the ovary selenium concentrations are not considered uncertain because these concentrations represent the direct exposure of selenium to the larvae from which the effect was observed.

Effect Concentration: **26.3** mg/kg dw in ovaries

Table 1. Selenium concentrations in the diet, ovary and muscle tissues and the percent mortality and deformities.

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
0.9 ± 0.1 (0.7 – 1.3)	6	1.62	5.38	3.1	75.5	95.3
	12	1.77	7.34		99.7	
	13	2.01	3.51		96.2	
	26	2.27	5.74		88.9	
	34	1.18	1.58		99.5	
	35	1.28	1.36		96.8	
	3	1.534	2.09		98.8	
	4	1.583	1.85		100	
	10 (2F)	1.15	2.11		97	
	13	1.181	1.86		97.1	
	14	1.341	1.40		98.4	
2.9 ± 0.5 (2.1 – 3.8)	9	2.075	9.59	8.8	84.9	94.8
	12	1.853	8.03		100	
	15	2.026	9.73		98.5	
	18	3.134	7.66		95.9	
7.5 ± 0.6 (6.3 – 8.4)	1	2.741	8.43	10.8	75	85.8
	2	3.737	25.15		63.9	
	5	5.709	15.31		90.6	
	7	3.468	1.20		79.1	
	8	2.545	6.78		95	
	16	7.302	8.25		96.8	
	19	4.776	10.20		100	
11.2 ± 1.4 (9.3 – 14.1)	6	4.521	35.44	25.0	91.5	88.7
	11	6.044	15.08		77.9	
	17	4.882	24.59		96.7	
26.7 ± 1.7 (23.6 – 29.5)	2	7.52	37.14	40.0	91.2	18.3
	5	12.42	44.67		0	
	11	9.73	34.26		75.9	
	16	10.1	35.58		0	
	17	5.74	33.48		9.9	
	19	11.74	48.24		0	
	36	10.21	35.81		6.3	
	37	14.12	37.88		0	
	51	11.68	32.95		0	

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
	52	11.16	59.89		0	
53.1 ± 4.8 (45.5 – 61.9)	22	18.15	46.22	61.0	0	0
	25	21.07	70.45		0	
	30	25.02	81.62		0	
	31	16.63	54.99		0	
	32	14.3	53.96		0	
	41	17.73	51.48		0	
	48 (2F)	26.25	84.31		0	
	50 (2F)	11.66	32.87		0	
	55	18.36	73.33		0	
78.4 ± 4.3 (73.2 – 87.0)	4 (2F)	12.6	66.81	77.6	66	5.5
	7	17.24	56.98		0	
	8	20.36	86.49		0	
	10	19.59	65.99		0	
	18	22.52	72.35		0	
	21	18.58	71.89		0	
	24	22.08	62.44		0	
	28	29.15	99.02		0	
	38	58.2	52.37		0	
	44	17.7	102.82		0	
	47	24.14	88.15		0	
	49	18.94	105.29		0	

[a] ± standard error; range of concentrations in parentheses.

Table 2. Se concentrations in muscle and ovary of field-collected (Mayo Reservoir) female largemouth bass.

Date	Se Muscle (mg/kg dw)	Se Ovary (mg/kg dw)	Ovary to Muscle Ratio
05/10/95	8.48	14.79	1.74
05/10/95	8.48	14.79	1.74
05/09/95	7.29	8.35	1.15
04/21/94	15	19	1.27
04/20/94	15	15	1.00
04/22/94	12	14	1.17
04/22/94	10	18	1.80
04/25/94	18	15	0.83
04/25/94	18	15	0.83
04/27/94	11	12	1.09
04/27/94	11	9.4	0.85
04/27/94	13	10	0.77
05/04/94	11	11	1.00
			Median Ratio 1.09

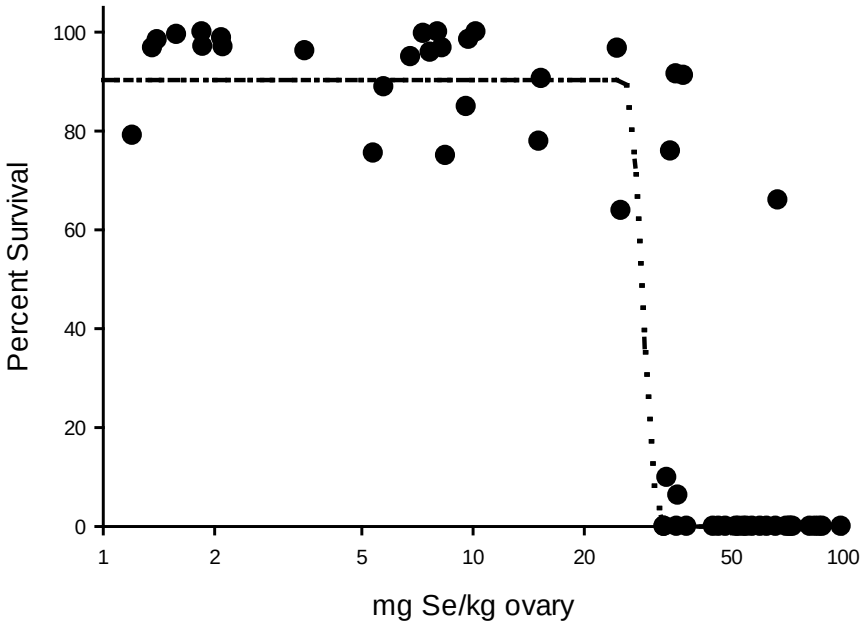


Figure 1. Largemouth bass larval survival relative to Se in ovary. TRAP interpolation was used to estimate the EC_{10} value. The higher concentration point for the interpolation was set at 32.9 mg/kg (EC_{100}) and the lower concentration point for the interpolation was set at 24.6 (NOEC) with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg and a steep slope of 16.

APPENDIX D: SUMMARY STUDIES OF NON-REPRODUCTIVE EFFECTS

1.0 STUDIES OF NON-REPRODUCTIVE EFFECTS

1.1 Acipenseridae

1.1.1 Acipenser transmontanus (white sturgeon)

Juvenile white sturgeon were exposed for 8 weeks to a series of 5 concentrations of seleno-L-methionine added to an artificial diet (Tashjian et al. 2006). Survival was not affected by selenium treatment with a mean survival rate of 99% across all groups. Fish fed the highest three dietary treatments of selenium, 41.7, 89.8 and 191.1 mg Se/kg dw, exhibited significant declines in growth assessed by body weight measurements. The EC_{10} for reduction in body weight is 15.08 mg Se/kg dw in whole body or 27.76 mg Se/kg dw muscle; the EC_{20} is 17.82 mg Se/kg dw in whole body or 32.53 mg Se/kg dw muscle tissue. The criterion values derived in this document that are based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study.

1.2 Cyprinidae

1.2.1 Pogonichthys macrolepidotus (Sacramento splittail)

Teh et al. (2004) exposed juvenile Sacramento splittail (7 months-old) to 8 levels of dietary selenium, 0.4 (no added selenium), 0.7, 1.4, 2.7, 6.6, 12.6, 26.0, and 57.6 mg/kg. Selenium was added to the diet via selenized yeast which was diluted with Torula yeast (inactive) to attain the target levels. Mortality, growth, histopathology, deformities and selenium content in muscle and liver were observed or measured after 5 and 9 months of exposure. The appearance of deformities was the most sensitive endpoint. The authors determined the occurrence of deformities was higher in fish fed 6.6 and 12.6 mg Se/kg in their diet; however, such pathology was examined for only 15 of the 120 individuals per treatment, and a consistent concentration-response relationship did not occur (i.e., no deformities in the high concentration). The lack of a concentration-response relationship for the incidence of deformities has also been observed in another study. Crane et al. (1992) exposed a European species of perch, *Perca fluviatilis* to three aqueous and dietary selenium treatments in experimental ponds for 288 days up through spawning. Crane et al. (1992) found an increased occurrence of deformities in embryos and larvae in the lowest selenium treatment relative to the control, but a decrease in the middle treatment. No hatching occurred in the high treatment. Teh et al. (2004) proposed several physiological mechanisms to explain the lack of a dose-response relationship, but it appears that the underlying mechanism is not understood at this time. Toxicity tests with unusual dose-response relationships are typically not considered for criteria derivation, but since another assay (Crane et al. 1992) observed a similar relationship, the Teh et al. (2004) study with *P. macrolepidotus* is included. Using prevalence of deformities as the endpoint, the NOEC, LOEC and MATC (chronic value) in *muscle tissue* are 10.1, 15.1 and 12.34 mg Se/kg dw, respectively. The critieron value in muscle tissue, based on the reproductive EC_{10}, is 11.8 mg Se/kg dw. Appendix C provides further details on the study results and an approximate estimate of their relationship to egg-ovary and whole-body concentrations. Teh et al. (2004) is the only study in which deformities developed in fish that were not exposed to selenium from their mothers' ovaries. The selenium criterion values derived based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study, considering the non-reproductive muscle MATC of 12.3 mg Se/kg dw is greater than the reproductive muscle criterion of 11.8 mg Se/kg dw.

1.2.2 Pimephales promelas (fathead minnows)

Non-reproductive chronic values for fathead minnows were derived from two laboratory-based studies. These studies (Bennett et al. 1986 and Dobbs et al. 1996) involved exposing algae to selenium (either as

sodium selenite or sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fathead minnows. In the Bennett et al. (1986) study, larval fathead minnows were fed control rotifers (cultured in chambers without selenium containing algae) or selenium-contaminated rotifers (cultured in chambers with selenium containing algae previously exposed to sodium selenite in the water) in three separate experiments lasting 9 to 30 days. The different experiments were distinguished by 1) the day selenium-laden rotifers were first fed; 2) the day selenium-laden rotifers were last fed; and 3) the age of larvae at experiment termination. The results from the three experiments reported by Bennett et al. (1986) were conflicting. Larval growth was significantly reduced at larval whole-body selenium concentrations of 43.0 mg Se/kg dw in the first experiment and 51.7 mg Se/kg dw in the second experiment, but was slightly but not significantly reduced at 61.1 mg Se/kg dw in the third experiment (see Appendix C). Following the approach of Section 7.1.1, the geometric mean of these three values, 51.40 mg Se/kg dw, is the chronic value for this study.

Dobbs et al. (1996) used a test system similar to that of Bennett et al (1986) (described above). Larval fathead minnows were exposed to the same concentrations of sodium selenate in the water as their prey (rotifers), but also received additional selenium from the consumption of the selenium-contaminated rotifers. In this study, the fathead minnows did not grow well at concentrations exceeding 108.1 µg Se/L in water, and they survived only to 11 days at selenium concentrations equal to or greater than 393.0 µg/L in the water (75 mg Se/kg dw in the diet, i.e., rotifers). The LOEC for retarded growth (larval fish dry weight) in this study was <73 mg Se/kg dw tissue.

A third laboratory study, by Ogle and Knight (1989), examined the chronic effects of elevated foodborne selenium on growth and reproduction of fathead minnows. Juvenile fathead minnows were fed a purified diet mix spiked with inorganic and organic selenium in the following percentages: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine. The pre-spawning exposure lasted 105 days using progeny of adult fathead minnows originally obtained from the Columbia National Fishery Research Laboratory, as well as those obtained from a commercial fish supplier. After the 105 day exposure period, a single male and female pair from each of the respective treatment replicates were isolated and inspected for spawning activity for 30 days following the first spawning event of that pair. There was no effect from selenium on any of the reproductive parameters measured, including larval survival, at the dietary concentrations tested (5.2 to 29.5 mg Se/kg dw food). Sub-samples of larvae from each brood were maintained for 14 days post-hatch and exhibited >87.4 percent survival. The pre-spawning adult fish fed a mean dietary level of 20.3 mg Se/kg dw exhibited a significant reduction in growth compared to controls (16 percent reduction), whereas a nonsignificant reduction in growth (7 percent) occurred in the fish fed 15.2 mg Se/kg dw. The chronic value, as determined by the geometric mean of the NOEC and the LOEC measured at 98 days post-test initiation, was 17.57 mg Se/kg expressed as the above dietary concentrations, and 5.961 mg Se/kg dw as fathead minnow whole-body tissue. The concentration-response relationship, as indicated by the study data presented in Appendix E, was uniformly shallow; not resembling the sharp sigmoidal function characteristic of most selenium response curves.

Since Ogle and Knight reported that food in the higher selenium concentrations remained uneaten and fish were observed to reject the food containing the higher selenium concentrations, the authors suggested that the decreased growth was caused by a reduced palatability of the seleniferous food items, which contained unnatural percentages of inorganic selenium (Fan et al. 2002). This is a common observation also noted by Hilton and Hodson (1983) and Hilton et al. (1980) and apparent in Coughlan and Velte (1989). It is here interpreted to be an artifact of unrealistic spiking of the diet with inorganic selenium in this early experimental protocol. That is, in the real world it is not expected that avoidance of food items that were unpalatable because of excessive selenium would be either a mechanism by which selenium causes effects or a mechanism by which organisms can avoid exposure. (See Janz et al. (2010) for a more complete discussion of selenium's mechanism of toxicity.) Given the no observed effect on larval

survival and the apparent non-toxicological effect on growth in the Ogle and Knight study, a chronic value for this study is not included.

1.3 Catostomidae

1.3.1 Xyrauchen texanus (razorback sucker)

Two non-reproductive endpoint studies have been done with the endangered razorback sucker. In the first study, Beyers and Sodergren (2001a) exposed larval razorback suckers for 28 days to a range of aqueous selenate concentrations (6.12, 25.4, 50.6, 98.9, and 190.6 μg/L) and respectively fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw). Reflecting the lack of effects on survival and growth in any exposure, the chronic value for this study, based on selenium measured in the larvae at the end of the test, is >12.9 mg Se/kg dw.

In a second study, Beyers and Sodergren (2001b) exposed larval razorback suckers to a control water and three different site waters containing varying concentrations of selenium for 28 days. Two treatments were tested within each water type: fish fed rotifers cultured in the same water type (site diet) and fish fed rotifers cultured in control water. There were no reductions in survival or growth in fish exposed to both the site water and site diet compared to fish exposed to control water and control diet. There were, however, reductions in growth of fish exposed to site water/site food compared to the same site water and control food. The authors did not attribute the effect on larval growth by the diet to selenium and cited several lines of evidence, including: (1) there was not a dose-response relationship in the concentration of selenium in the food (rotifers) and growth, nor in the concentration of selenium in the fish larvae and growth across the three water types; and (2) water from the De Beque site promoted a significant reduction in the growth of fish exposed to site water/site food relative to site water/control food, but contained low levels of selenium in the water (<1 μg/L) and in food (2.10 mg/kg dw) typically lower than those that have been found to elicit effects. The chronic value for this study is >42 mg Se/kg dw based on the whole body concentration of selenium in the larval razorback suckers exposed to North Pond site water.

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the razorback sucker (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results, of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). Summaries of each of these two studies as well as a third study with razorback suckers (Hamilton et al. 2005d) are presented in Appendix E.

Due to the confounding results, lack of dose-response within and among related studies, and the uncertainty of the effect of other inorganic contaminants on larval response to the various dietary and waterborne treatments, the data from these three studies for razorback sucker (Hamilton et al. 2001a,b; Hamilton et al. 2005d) have not been included. A more detailed explanation of why these studies were not included is given in Appendix E. Because of the vastly different results between the Beyers and Sodergren studies and Hamilton et al. studies and the inability to resolve the differences, SMCV and GMCV were not calculated for the razorback sucker.

1.3.2 Catostomus latipinnis (flannelmouth sucker)

Beyers and Sodergren (2001a) exposed flannelmouth sucker larvae to a range of aqueous selenate concentrations (<1, 25.4, 50.6, 98.9, and 190.6 µg/L) and fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively). There were no survival or growth effects observed after the 28 day exposure. The chronic value based on the concentration of selenium measured in the larvae exposed to the highest test concentration was >10.2 mg Se/kg dw.

1.4 Salmonidae

1.4.1 Oncorhynchus tshawytscha (Chinook salmon)

Hamilton et al. (1990) conducted a 90-day growth and survival study with swim-up larvae fed one of two different diets. The first diet consisted of Oregon moist™ pellets where over half of the salmon meal was replaced with meal from selenium-laden mosquitofish (*Gambusia affinis*) collected from the San Luis Drain, CA (SLD diet). The second diet was prepared by replacing half the salmon meal in the Oregon moist™ pellets with meal from low-selenium mosquitofish (i.e., the same relatively uncontaminated mosquitofish that were used in the control diet) and spiked with seleno-DL-methionine (SeMe diet). Analysis of the trace element composition in the two different diets indicated that while selenium was the most toxic element in the SLD diet, concentrations of boron, chromium, iron and strontium in the high-selenium mosquitofish replacement diet (SLD diet type) were slightly elevated compared to the replacement diet. These trace elements were, however, only 1.2 (e.g., iron) to 2.0 times (e.g., chromium) higher in the SLD diet than the SeMe diet, which contained the following measured concentrations (dry weight basis) in the food: 10 mg boron/kg, 2.8 mg chromium/kg, 776 mg iron/kg, and 48.9 mg strontium/kg.

During the test, survival of control Chinook salmon larvae (consuming food at approximately 3 mg Se/kg dw) was 99 percent up to 60 days post-test initiation. Between 60 and 90 days of exposure, however, the control survival declined to 66.7% in the SLD test and to 72.5% in the test using the SeMe diet, indicating compromised health. Therefore, only data collected up to 60 days post-test initiation were considered for analysis. Nevertheless, there remains the possibility that even at 60 days, the control organisms were not healthy, although overt signs of stress did not appear until later.

For the SeMe diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 7.355 mg Se/kg dw and an EC_{20} of 10.47 mg Se/kg dw. For the SLD diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 11.14 mg Se/kg dw and an EC_{20} of 15.73 mg Se/kg dw. Note: The San Luis Drain mosquitofish (comprising the Chinook salmon's SLD diet) were not tested for contaminants other than certain key elements. Because the San Luis Drain receives irrigation drainage from the greater San Joaquin Valley, there is a possibility that the SLD diet might have contained elevated levels of pesticides, possibly a confounding factor, although the SLD diet was less toxic than the SeMe diet.

1.4.2 Oncorhynchus mykiss (rainbow trout)

Hilton and Hodson (1983) reared juvenile rainbow trout on either a high (25 percent) or low (11 percent) available carbohydrate diet supplemented with sodium selenite for 16 weeks. Body weights, feed: gain ratios, and total mortalities were followed throughout the exposure every 28 days. Tissues (livers and kidneys) were extracted for selenium analysis after 16 weeks. By the end of the exposure, fish fed diets (low carbohydrate and high carbohydrate) with the highest selenium concentrations (11.4 and 11.8 mg Se/kg dw food, respectively) exhibited a 45 to 48 percent reduction in body weight (expressed as kg per 100 fish) compared to control fish. The authors attributed such results to food avoidance. With only two dietary exposure concentrations and a control, these data were not amenable to regression analysis. The MATC for growth of juvenile rainbow trout relative to the concentrations of selenium in liver tissue of trout reared on the high carbohydrate seleniferous dietary type is the geometric mean (GM) of 21.00 mg Se/kg dw liver (NOEC) and 71.7 mg Se/kg dw liver (LOEC), or 38.80 mg Se/kg dw liver. The calculated MATC for the same group of experimental fish exposed to selenium in the low carbohydrate diet is 43.5 mg Se/kg dw liver tissue, which is the same MATC for trout exposed for an additional 4 weeks based on the occurrence of nephrocalcinosis in kidneys (see Hicks et al. 1984; Appendix C).

Hilton et al. (1980) employed a similar test design to that of Hilton and Hodson (1983) to examine the narrow window at which selenium changes from an essential nutrient to a toxicant affecting juvenile rainbow trout. The food consisted of a casein-Torula yeast diet supplemented with selenium as sodium selenite. As discussed previously for the Ogle and Knight (1989) study with fathead minnow, this represents an unrealistic fraction of inorganic selenium in the diet. The experiment lasted for 20 weeks. During this time, the trout were fed to satiation 3 to 4 times per day, 6 days per week, with one feeding on the seventh day. Organs (liver and kidney) and carcasses were analyzed for selenium from fish sacrificed at 4 and 16 weeks. No gross histopathological or physiological effects were detected in the fish, although trout raised on the highest dietary level of selenium (13.06 mg Se/kg dw food) had a significantly lower body weight (wet basis), a higher feed:gain ratio, and higher number of mortalities (10.7; expressed as number per 10,000 fish days). The MATC for growth and survival of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue is the geometric mean of the NOEC (40 mg Se/kg dw liver) and the LOEC (100 mg Se/kg dw liver), or 63.25 mg Se/kg dw, both of which hinge on accepting dietary spiking entirely with inorganic selenium as an acceptable experimental protocol.

The non-reproductive GMCV for *Oncorhynchus* (both rainbow trout and Chinook salmon) is 9.052 mg Se/kg dw whole body based on the EC_{10} value derived from the Hamilton et al. (1990) study with Chinook salmon. The NOEC values for the rainbow trout studies conducted by Hilton and Hodson (1983), Hilton et al. (1980), and Hicks et al. (1984) were not used in the GMCV calculation because of the large difference between the NOEC and the LOEC values. If adult fish contained whole-body selenium concentrations equal to 9.052 mg Se/kg dw, their egg-ovary concentrations would be estimated to be 21.5 mg Se/kg dw when translated using the factor 2.37. The criterion values derived based on reproductive endpoints are protective of the endpoint measured.

1.5 Moronidae

1.5.1 Morone saxitilis (striped bass)

A non-reproductive chronic value for selenium was determined from a laboratory dietary exposure conducted using yearling striped bass (Coughlan and Velte 1989). During the experiment, the bass were fed contaminated red shiners (38.6 mg Se/kg dw whole body) from Belews Lake, NC (treated fish) or golden shiners with low levels of selenium (1.3 mg/kg dw whole body) purchased from a commercial supplier (control fish). The test was conducted in soft well water and lasted up to 80 days. During the experiment, all fish were fed to satiation 3 times per day. Control fish grew well and behaved normally.

Treated fish behaved lethargically, grew poorly due to a significant reduction in appetite, and showed histological damage, all eventually leading to the death of animals. The final selenium concentration in muscle of treated striped bass averaged from 16.2 to 18.5 mg/kg dw tissue (assuming 78.4 percent moisture content), which was 3.4 to 3.6 times higher than the final selenium concentrations in control striped bass, which averaged 5.10 mg/kg dw tissue. The chronic value for this species was determined to be <16.2 mg Se/kg dw in muscle tissue.

1.6 Centrarchidae

1.6.1 Lepomis macrochirus (bluegill)

Bryson et al. (1985b) conducted juvenile survival toxicity tests using hatchery bluegill and various forms of selenium spiked to an artificial diet as well as a diet consisting of zooplankton collected from Hyco Reservoir. There was no effect on length or weight of the juvenile bluegill after 60 days of exposure. The highest concentration of selenium measured in whole body of the juveniles in these tests was in the seleno-DL-cysteine-2X treatment (3.74 mg Se/kg dw).

Cleveland et al. (1993) performed a 90-day diet-only laboratory exposure in which juvenile bluegill were fed a range of selenomethionine concentrations added to Oregon moist™ pellets. The authors observed no significant effects on survival, but did report a very small but apparently statistically significant decrease in the condition factor, K, from 1.3 at four concentrations between 1.0 and 4.7 mg Se/kg dw whole body, to 1.2 at the two concentrations 7.7 and 13.4 mg Se/kg dw whole body. The condition factor (weight x 10^5/length^3) is intended to reflect a fish's reserves. In contrast to the studies of Ogle and Knight (1989), Hilton and Hodson (1983), and Hilton et al. (1989), which appear to have involved an inorganic selenium food palatability problem, this study did not use inorganic selenium in the diet. Nevertheless, given that the reduction in K (1.3 to 1.2) is slight and shows no increasing effect between 7.7 and 13.4 mg Se/kg dw, thus not yielding a sigmoidal concentration-response curve to support an EC_{10} calculation, the chronic value for this study was estimated at >13.4 mg Se/kg dw in whole body tissue.

Data from Lemly (1993a) indicate that over-wintering fish may be more susceptible to the effects of waterborne and dietary selenium due to increased sensitivity at low temperature. The author exposed juvenile bluegill in the laboratory to a single elevated exposure level, waterborne (1:1 selenite:selenate; nominal 5 µg Se/L) and foodborne (seleno-L-methionine in TetraMin; nominal 5 mg Se/kg dw food) selenium for 180 days. Tests with a control and the treated fish were run at 4°C and 20°C with biological and selenium measurements made every 60 days. Survival and whole-body lipid content were unaffected at 20°C (whole-body selenium concentrations equal to 6 mg/kg dw, the sole treatment exposure) when compared to control fish. Thus, at 20°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium based on survival was >6 mg/kg dw in whole-body tissue. Fish exposed to the combination low-level waterborne and dietary selenium at 4°C exhibited significantly elevated mortality (40.4 percent) relative to controls (2.9 percent), and exhibited significantly greater oxygen consumption and reduced lipid content, which are indicative of stress. At 4°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium was <7.91 mg Se/kg dw in whole body based on mortality and tissue measurements at the end of the test (180 days), and 5.85 mg Se/kg dw in whole body based on mortality at 180 days and tissue measurements at 60 days. The increase in the concentration of whole-body selenium between Day 60 and 180 at 4°C was apparently due to reductions in body weight caused by loss of lipid (comparatively low in selenium) while body burden in other tissues remained relatively constant. If this concentration of selenium in tissues occurs in sensitive overwintering fish in nature, a concentration of 5.85 mg/kg dw (the selenium tissue concentration in the 4ºC exposure after 60 days) in fish collected during the summer or fall months could be considered a threshold concentration for the selenium-sensitive fish during the winter months. Therefore, this study's chronic value for the threshold concentration prior to winter stress is 5.85 mg Se/kg dw in whole body tissue.

McIntyre et al. (2008) also investigated the toxicity of selenium to juvenile bluegill under cold temperature conditions in the laboratory. Whereas relative to the control, Lemly (1993a) tested only one exposure level, 5 mg Se/kg in the diet and 5 µg Se/L and one low temperature regime, 4°C, McIntyre et al. (2008) evaluated a range of diet and water concentrations, two types of diet, and two low-temperature regimes. The goal of the study was to determine EC_{10} and EC_{20} values for selenium exposure to juvenile bluegill in 4°C and 9°C low-temperature regimes. Three separate exposure systems were run concurrently for 182 days. Two systems exposed juvenile bluegill to a series of six aqueous and dietary selenium treatments and a control; one exposure system (ES1) with a cold temperature regime (4°C), and one (ES3) with a cool temperature regime (9°C), both using a yeast-worm-fish food chain bioaccumulation system. That is, graded levels of selenized-yeast in ES1 and ES3 were fed to the oligochaete, *Lumbriculus variegatus*, which in turn was fed to bluegill. The third exposure system (ES2) used diet and exposure conditions similar to Lemly's 4°C treatment, i.e., nominal 5 µg Se/L in the water and nominal 5 mg Se/kg dw food (seleno-L-methionine in TetraMin). The cold temperature regime for ES1 and ES2 was 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The cool temperature regime (ES3) was similar except when the temperature reached 9°C (test day 65), it was maintained until test termination (test day 182).

At the end of the 182 day exposure in the ES2 (with Lemly's diet and temperature), the bluegill accumulated an average (geometric mean) whole body concentration of 9.99 mg/kg dw with no meaningful mortality in the treatment or control. Significant mortality of juvenile bluegill was observed in the two highest treatments in the cold (ES1) and cool (ES3) *Lumbriculus*-fed tests. No effects on body weight or condition factor were observed. The EC_{10} and EC_{20} values for the cold treatment (ES1) are 9.27 and 9.78 mg Se/kg dw in whole body, respectively. The EC_{10} and EC_{20} values for the cool treatment (ES3) are slightly higher at 14.00 and 14.64 mg Se/kg dw in whole body, respectively.

The design and the results of the McIntyre et al. (2008) study have similarities and differences with Lemly (1993a), as presented in detail with comparisons and contrasts in Appendix C. Both studies found juvenile bluegill were more sensitive in a cold-temperature regime than in a cool (McIntyre et al.) or a warm regime (Lemly). The effect levels determined for the cold temperature regime differed by a factor of 1.58 (ES1 of McIntyre et al., 9.27 mg Se/kg; Lemly, 5.85 mg Se/kg), a difference rather typical of chronic studies conducted in different laboratories using different fish populations (Delos 2001) and similar to the 1.51 factor difference between two EC_{10}s of Hamilton et al. (1990) for chinook salmon.

The difference in the effect levels of the McIntyre ES2 exposure (>9.99 mg/kg) and the Lemly study (5.85 mg/kg) could have been due to the fitness of the fish entering the cold regime. The condition factor, K, in the ES2 selenium-exposed bluegill increased from 3.2 at the start of the exposure to 5.2 at day 60 (approximately 10°C at day 60) and decreased only slightly through over 100 days of 4°C exposure (see figure in bluegill summary in Appendix C). In contrast, K in the Lemly selenium-exposed fish decreased approximately 50% after 120 days of exposure. Shoup and Wahl (2011) conducted an overwinter exposure study with bluegill in which they fed and starved young of year bluegill (the larger size similar to the McIntyre and Lemly fish) under two temperature regimes, 4°C (harsh winter) and 9°C (mild winter) for 140 days and a 10 h light:14 h dark photoperiod. The juvenile bluegill in the Shoup and Wahl study ate in both temperature regimes. The 4°C exposed fish consumed 0.4-0.8% of their body weight/day and their K was not significantly different at the end of the test compared to the start. The Shoup and Wahl results only provide an indication that cold-exposed fish under a winter photoperiod feed and can maintain K.

The mortality observed in the Lemly laboratory study does not appear to be consistent with field observations. The occurrence of mortality in the field at the concentrations Lemly (1993a) reported to cause mortality in his lab was not observed in the Lemly (1993b) field study of centrarchid deformities in Belews Lake. In that field study, Lemly (1993b) found larval centrarchid deformities at concentrations ranging from 12-80 mg Se/kg dw WB. If juvenile mortality occurred at concentrations lower than those found to induce larval deformities and at concentrations as low as Lemly (1993a) reported in the lab (EC_{40} = 7.91 mg Se/kg WB), then centrarchids would likely not have been present in Belews Lake. The observations of Lemly (1993b) are evidence that larval deformity, not juvenile mortality, is the more sensitive endpoint.

The Crutchfield and Ferson (2000) predictions and field observations of recovery of bluegill at Hyco Reservoir likewise suggest that significant mortality was unlikely to be occurring at the concentrations Lemly (1993a) reported to cause substantial mortality. During a time period over which Crutchfield (2000) indicated dietary invertebrate concentrations exceeded 20 mg Se/kg dw, Crutchfield and Ferson (2000) indicated that bluegill population growth occurred at rates predicted to be natural for the unimpaired species. In contrast, if the Lemly (1993a) lab EC_{40} of 7.91 mg Se/kg dw whole-body were applicable to this field situation, the mortality associated with the resulting bluegill whole-body concentrations (25 mg Se/kg dw whole-body, assuming a trophic transfer factor of 1.27) would have prevented any recovery.

Selenium-induced cold temperature loss of lipid and body condition, a non-reproductive sublethal effect that Lemly (1993a) observed to accompany juvenile mortality in the laboratory (but which McIntyre et al. (2008) did not observe in a similar study) has also not generally been corroborated by field evidence (Janz 2008). Several studies have measured growth and energy storage indicators in juvenile fish just prior to and just after winter at reference sites and sites with elevated selenium in northern Canada (Bennett and Janz 2007a, b; Kelly and Janz 2008; Driedger et al 2009; Weber et al. 2008). The growth (length, weight, condition factor, muscle RNA:DNA ratio, muscle protein) and energy storage (whole body lipids, whole body triglycerides, liver triglycerides, liver glycogen) indicators for five fish species (northern pike, burbot, fathead minnow, creek chub, white sucker) measured just after winter were similar or greater than those measured just before winter at the selenium exposed sites. The slimy sculpin did show a decrease in whole body triglycerides, but the reduction was similar at exposed and reference sites.

Given the uncertainty in the occurrence of winter stress, the results of all four cold-temperature (4°C and 9°C) juvenile-survival lab studies were combined per the standard procedure described in the U.S.EPA Ambient Water Quality Criteria Guidelines, to determine the non-reproductive SMCV for bluegill. The SMCV for the combined 4°C and 9°C tests is 9.33 mg Se/kg dw whole body, based on the four chronic values: (a) the Lemly (1993a) concentration prior to winter stress (5.85 mg Se/kg dw whole body), (b) the McIntyre et al. (2008) ES1 EC_{10} (9.27 mg Se/kg dw whole body), (c) the McIntyre et al. (2008) ES2 NOEC (>9.992 mg Se/kg dw whole body), and the McIntyre et al. (2008) ES3 EC_{10} of 14.00 mg Se/kg dw whole body. This value is not less than the reproductive endpoint-based whole-body criterion concentration of 8.5 mg Se/kg dw. The studies of Bryson et al (1985b) and Cleveland et al. (1993) were not conducted at cold temperatures and were thus not used for these SMCV calculations.

Table D-1. Freshwater Chronic Values from Acceptable Tests - Non-Reproductive Endpoints (Parental Females Not Exposed).
(Same as Table 6.2 in the main document).

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Acipenser transmontanus white sturgeon	Tashjian et al. 2006	dietary (lab) 8 weeks	seleno-L-methionine in artificial diet	EC_{10} juvenile growth	15.08 WB 27.76 M	EC_{10} 15.1 WB 27.8 M	15.1 WB 27.8 M
			seleno-L-methionine in artificial diet	EC_{20} juvenile growth	17.82 WB 32.53 M	EC_{20} 17.8 WB 32.5 M	
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	dietary (lab) 9 months	selenized-yeast	NOEC	10.1 M	10.1 M 15.1 M 12.3 M	10.1 M 15.1 M 12.3 M
				LOEC	15.1 M		
				MATC juvenile deformities (juvenile exposure only)	12.34 M		
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab) 9 to 19 days	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40 WB	51.40 WB 69.83 M	51.40 WB 69.83 M
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab) 8 days	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOEC for larval fish dry weight after 8 d	<73 WBb		
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>12.9 WBb	see text	see text
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001b	dietary and waterborne (lab) 28 days	water: site waters; diet: algae exposed to site water then fed to rotifers which were fed to fish	NOEC for survival and growth	>42 WBb		
Catostomus latipinnis flannelmouth sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab) 28 days	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOEC for survival and growth	>10.2 WB	>10.2 WB	>10.2 WB

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab) 60 days	mosquitofish spiked with seleno-DL-methionine	EC_{10} for juvenile growth	7.355 WB	EC_{10} 9.052 WB EC_{20} 12.83 WB	EC_{10} 9.052 WB
				EC_{20} for juvenile growth	10.47 WB		
			mosquitofish spiked with SLD diet	EC_{10} for juvenile growth	11.14 WB		
				EC_{20} for juvenile growth	15.73 WB		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab) 16 weeks	sodium selenite in food preparation	juvenile growth NOEC	21 Liver	NOAEC 28.98 L LOAEC 84.68 L MATC 49.52 L	
				LOEC	71.7 Liver		
				MATC	38.80 Liver		
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juvenile survival and growth NOEC	40 Liver		
				LOEC	100 Liver		
				MATC	63.25 Liver		
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab) 80 days	Se-laden shiners from Belews Lake, NC	LOEC for survival of yearling bass	<16.2 M[c]	<16.2 M	<16.2 M
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab) 180 days 20 to 4°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOEC for juvenile mortality at 4oC	<7.91 WB	4°C EC_{10}-NOAEC 8.15 WB 4°C EC_{20}-LOAEC 8.80 WB 9°C EC_{10} 14.0 WB 9°C EC_{20} 14.6 WB	4°C & 9°C 9.33 WB
				Threshold prior to "winter stress"	5.85 WB		
		dietary and waterborne (lab) 180 days 20°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC for juvenile mortality at 20oC	>6.0 WB		
Lepomis macrochirus bluegill	McIntyre et al. 2008	dietary and waterborne (lab) 182 days 20 to 4°C (ES1)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES1	9.27 WB		
				EC_{20} juv. survival ES1	9.78 WB		
		dietary and waterborne (lab) 182 days 20 to 9°C (ES3)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES3	14.00 WB		
				EC_{20} juv. survival ES3	14.64 WB		

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
		dietary and waterborne (lab) 182 days 20 to 4°C (ES2)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC juv. surv. ES2	>9.992 WB		
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary (lab) 60 days	seleno-DL-cysteine	NOEC for juvenile growth	>3.74 WBb		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary (lab) 90 days	seleno-L-methionine	NOEC for juvenile survival	>13.4 WBb		

[a] All chronic values reported in this table are based on the measured concentration of selenium in whole body (WB), muscle (M) or liver (L) tissues.

[b] Chronic value not used in SMCV calculation (see text).

[c] Tissue value converted from ww to dw. See Appendix C for conversion.

APPENDIX E: OTHER DATA

1.0 SELENITE

Additional data on the lethal and sublethal effects of selenium on aquatic species are presented in Table E-1. Bringmann and Kuhn (1959a,b, 1976, 1977a, 1979, 1980b, 1981), Jakubczak et al. (1981), and Patrick et al. (1975) reported the concentrations of selenite that caused incipient inhibition (defined variously, such as the concentration resulting in a 3% reduction in growth) for algae, bacteria, and protozoans (Table E-1). Although incipient inhibition might be statistically significant, its ecological importance is unknown. Albertano and Pinto (1986) found the growth of three red algal species was inhibited at selenite concentrations that ranged from 790 to 3,958 μg/L.

2.0 SELENATE

Dunbar et al. (1983) exposed fed *D. magna* to selenate for seven days and obtained an LC_{50} of 1,870 μg/L. This value is in the range of the 48-hr EC_{50}s in Table E-1.

Watenpaugh and Beitinger (1985a) found that fathead minnows did not avoid 11,200 μg/L selenate during 30-minute exposures (Table E-1). These authors also reported (1985b) a 24-hr LC_{50} of 82,000 μg/L for the same species and they found (1985c) that the thermal tolerance of the species was reduced by 22,200 μg/L. Westerman and Birge (1978) exposed channel catfish embryos and newly hatched fry for 8.5 to 9 days to an unspecified concentration of selenate. Albinism was observed in 12.1 to 36.9% of the fry during the five years of such exposures. Pyron and Beitinger (1989) also investigated fathead minnows, and after a 24-hr exposure, no effect on reproductive behavior was found at 36,000 μg/L, but when adults were exposed to 20,000 μg/L selenate for 24-hr, edema was observed for their larvae.

The respiratory rate of the eastern oyster, *Crassostrea virginica*, was unaffected by exposure to selenate at 400 μg/L for 14 days (Fowler et al. 1981). Embryos of the striped bass were quite tolerant to selenate in dilute salt water (Klauda 1985a, b). There was a 93% successful hatch of embryos at 200,000 μg/L, but 50% of 72-day-old juveniles died after four days at 87,000 μg/L. Exposure of juvenile fish for up to 65 days to concentrations of selenate between 39 and 1,360 μg/L caused developmental anomalies and pathological lesions.

Table E-1. Other Data on Effects of Selenium on Aquatic Organisms

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	96 hr	Incipient inhibition (river water)	2,500	Bringmann and Kuhn 1959a,b
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	Decreased dry weight and chlorophyll a	75	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 12-21[b]	10-100	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 11,164[c]	150	Foe and Knight, Manuscript
Alga, *Chrysochromulina breviturrita*	Selenious acid	-	30 days	Increased growth	320	Wehr and Brown 1985
Red alga, *Cyanidium caldarium*	Selenious acid	-	20 days	Inhibited growth	3,958	Albertano and Pinto 1986
Red alga, *Cyanidioschyzon merolae*	Seleniousacid	-	20 days	Inhibited growth	3,140	Albertano and Pinto 1986
Red alga, *Galdieria sulphuraria*	Seleniousacid	-	20 days	Inhibited growth	790	Albertano and Pinto 1986
Algae (diatoms), Mixed population	Sodium selenite	-	18 days	Inhibited growth	11,000	Patrick et al. 1975
Bacterium, *Escherichia coli*	Sodium selenite	-	-	Incipient inhibition	90,000	Bringmann and Kuhn 1959a
Bacterium, *Pseudomonus putida*	Sodium selenite	-	16 hr	Incipient inhibition	11,400 (11,200)	Bringmann and Kuhn 1976; 1977a; 1979; 1980b
Protozoan, *Entosiphon sulcatum*	Sodium selenite	-	72 hr	Incipient inhibition	1.8 (1.9)	Bringmann 1978; Bringmann and Kuhn 1979; 1980b; 1981
Protozoan, *Microreqma heterostoma*	Sodium selenite	-	28 hr	Incipient inhibition	183,000	Bringmann and Kuhn 1959b
Protozoan, *Chilomonas paramecium*	Sodium selenite	-	48 hr	Incipient inhibition	62	Bringmann and Kuhn 1981; Bringmann et al. 1980

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Protozoan, *Uronema parduezi*	Sodium selenite	-	20 hr	Incipient inhibition	118	Bringmann and Kuhn 1980a; 1981
Snail, *Lymnaea stagnalis*	Sodium selenite	-	7.5 days	LT50	3,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenite	-	48 hr	EC50 (river water)	2,500	Bringmann and Kuhn 1959a,b
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	LC50	16,000	Bringmann and Kuhn 1977a
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	EC50 (swimming)	9.9	Bringmann and Kuhn 1977b
Cladoceran, *Daphnia magna*	Sodium selenite	329	48 hr 96 hr 14 days	EC50 (fed)	710 430 430	Halter et al. 1980
Cladoceran (<24 hr), *Daphnia magna*	Sodium selenite	-	48 hr 21 days	EC50 (fed)	685 160	Adams and Heidolph 1985
Cladoceran (5th instar), *Daphnia magna*	Sodium selenite	-	48 hr	LC50 (fed)	680	Johnston 1987
Cladoceran, *Daphnia magna*	Selenious acid	220[d]	48 hr	LC50 (fed)	1,200	Kimball, Manuscript
Cladoceran (preadult), *Daphnia pulex*	Sodium selenite	42	24 hr	Did not reduce oxygen consumption or filtering rate	>498	Reading and Buikema 1980
Ostracod, *Cyclocypris* sp.	Sodium selenite	100.8	48 hr	LC50	130,000	Owsley 1984
Amphipod, *Hyalella azteca*	Sodium selenite	329	14 days	LC50 (fed)	70	Halter et al. 1980
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	48 hr	LC50	623	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodiumselenite	133	10 days	LC50 (fed)	312	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	24 days	LOEC reproduction (static-renewal)	200	Brasher and Ogle 1993
Midge (first instar), *Chironomus riparius*	Sodium selenite	134	48 h	LC50	7,950	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenite	40-48	48 h	LC50	14,600	Ingersoll et al. 1990
Coho salmon (fry), *Oncorhynchus kisutch*	Sodium selenite	325	43 days	LC50	160	Adams 1976
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	LC50	460	Adams 1976

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	Reduced growth	250	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	330	5 days	LC50	2,700 2,750	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	48 days	LC50	500	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	96 days	LC50	280	Adams 1976
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	200	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	4.7 µg/g dw (whole-body)	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	BCF = 23	100	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC growth (dietary only exposure)	>9.96 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC survival (dietary only exposure)	5.34 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	9 days	LC50	7,020	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	7,200 5,410	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	8,200 6,920	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	41 days	LOAEC (Reduced hatch of eyed embryos)	26	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	50 wk	Decreased iron in blood and red cell volume	53	Hodson et al. 1980
Rainbow trout (fertilized egg), *Oncorhynchus mykiss*	Sodium selenite	135	44 wk	BCF = 33.2 BCF = 21.1	53	Hodson et al. 1980
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	120 hr	Did not reduce survival or time to hatch	10,000	Klaverkamp et al. 1983b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	-	90 days	Chronic value for survival	14	Mayer et al. 1986
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	LC50	55.2[e]	Hunn et al. 1987
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	MATC survival	31.48	Hunn et al. 1987
Rainbow trout (egg), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 17.5 BCF = 3.5	0.4 45.6	Hodson et al. 1986
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 3.1 BCF = 3.0	0.4 45.6	Hodson et al. 1986
Rainbow trout (sac-fry), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 13.1 BCF = 1.6	0.4 45.6	Hodson et al. 1986
Rainbow trout (swim-up fry) *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 80.3 BCF = 20.2	0.4 45.6	Hodson et al. 1986
Northern pike, *Esox lucius*	Sodium selenite	10.2	76 hr	LC50	11,100	Klaverkamp et al. 1983a
Goldfish, *Carassius auratus*	Selenium dioxide	157	14 days	LC50	6,300	Cardwell et al. 1976a,b
Goldfish, *Carassius auratus*	Sodium selenite	-	10 days	Mortality	5,000	Ellis 1937; Ellis et al. 1937
Goldfish, *Carassius auratus*	Sodium selenite	-	46 days	Gradual anorexia and mortality	2,000	Ellis et al. 1937
Goldfish, *Carassius auratus*	Selenium dioxide	-	7 days	LC50	12,000	Weir and Hine 1970
Goldfish, *Carassius auratus*	Selenium dioxide	-	48 hr	Conditional avoidance	250	Weir and Hine 1970
Fathead minnow, *Pimephales promelas*	Selenium dioxide	157	9 days	LC50	2,100	Cardwell et al. 1976a,b
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	96 hr	LC50 (fed)	1,000	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	14 days	LC50 (fed)	600	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Selenious acid	220[d]	8 days	LC50 (fed)	420	Kimball, Manuscript
Creek chub, *Semotilus atromaculatus*	Selenium dioxide	-	48 hr	Mortality	∃12,000	Kim et al. 1977
Bluegill, *Lepomis macrochirus*	Sodium selenite	318	48 days	LC50	400	Adams 1976
Bluegill, *Lepomis macrochirus*	Selenium dioxide	157	14 days	LC50	12,500	Cardwell et al. 1976a,b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	16	323 days	MATC larval survival (dietary only exposure)	19.75 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	25 and 200	120 days	No mortality	>10	Lemly 1982
Largemouth bass (juvenile), *Micropterus salmoides*	Sodium selenite	25 and 200	120 days	No mortality	10	Lemly 1982
Yellow perch, *Perca flavescens*	Sodium selenite	10.2	10 days	LC50	4,800	Klaverkamp et al. 1983a,b
African clawed frog, *Xenopus laevis*	Sodium selenite	-	7 days	LC50	1,520	Browne and Dumont 1980
African clawed frog, *Xenopus laevis*	Sodium selenite	-	1-7 days	Cellular damage	2,000	Browne and Dumont 1980
Selenium (VI)						
Alga, *Chrysochromulina breviturrita*	-	-	30 days	Increased growth	50	Wehr and Brown 1985
Rotifer, *Brachionus calyciflorus*	Sodium selenate	120	96 hr	EC20 Growth (dry weight)	42.36 (µg/g dw)	Dobbs et al. 1996
Snail, *Lymnaea stagnalis*	Sodium selenate	-	6 days	LT50	15,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenate	129.5	7 days	LC50 (fed)	1,870	Dunbar et al. 1983
Cladoceran (juvenile), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	550	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	750	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	90 hr	42% of organisms had visible changes in gut morphology	250	Johnston 1989
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	48 hr	LC50	2378	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	10 days	LC50 (fed)	627	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	24 days	LOEC reproduction (static renewal)	>700	Brasher and Ogle 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	18 (SO_4=3.4)	10 days	LC50 (fed)	43	Borgmann et al. 2005
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	124 (SO_4=32)	10 days	LC50 (fed)	371	Borgmann et al. 2005
Midge (first instar), *Chironomus riparius*	Sodium selenate	134	48 h	LC50	16,200	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenate	40-48	48 h	LC50	10,500	Ingersoll et al. 1990
Rainbow trout (embryo, larva), *Oncorhynchus mykiss*	Sodium selenate	104 (92-110)	28 days	EC50 (death and deformity)	5,000 (4,180) (5,170)	Birge 1978; Birge and Black 1977; Birge et al. 1980
Goldfish (embryo, larva), *Carrassius auratus*	Sodium selenate	195	7 days	EC50 (death and deformity)	8,780	Birge 1978
Goldfish, *Carassius auratus*	Sodium selenate	-	24 hr	BCF = 1.42 BCF = 1.15 BCF = 1.47 BCF = 0.88 BCF = 1.54	0.45 0.9 1.35 2.25 4.5	Sharma and Davis 1980
Fathead minnow, *Pimephales promelas*	Sodium selenate	337.9	48 days	LC50	2,000	Adams 1976
Fathead minnow, *Pimephales promelas*	Sodium selenate	338	48 days	LC50	1,100	Adams 1976
Fathead minnow, *Pimephales promelas*	-	51	30 min	No avoidance	11,200	Watenpaugh and Beitinger 1985a
Fathead minnow, *Pimephales promelas*	-	-	24 hr	LC50	82,000	Watenpaugh and Beitinger 1985b
Fathead minnow, *Pimephales promelas*	-	-	24 hr	Reduced thermal tolerance	22,200	Watenpaugh and Beitinger 1985c
Fathead minnow, *Pimephales promelas*	Sodium selenate	44-49	7 days	Chronic value - growth Chronic value-growth Chronic value-survival	1,739 561 2,000	Norberg-King 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	No effect on reproductive behavior	36,000	Pyron and Beitinger 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	Edema in larvae produced from adults exposed to Selenium VI	20,000	Pyron and Beitinger 1989

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Channel catfish (embryo, fry), *Ictalurus punctatus*	Sodium selenate	90	8.5-9 days	Induced albinism	-	Westerman and Birge 1978
Narrow-mouthed toad (embryo, larva), *Gastrophryne carolinensis*	Sodium selenate	195	7 days	EC50 (death and deformity)	90	Birge 1978; Birge and Black 1977; Birge et al. 1979a
Organo-selenium						
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	16	323 days	MATC larval survival (dietary only exposure)	20.83 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	283	90 days	EC20 survival (dietary only exposure)	>13.4 µg/g dw (food)	Cleveland et al. 1993
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	53.83 µg Se/g dw (liver)	Reash et al. 1999
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	23.38 µg Se/g dw (ovaries)	Reash et al. 1999
Redear sunfish (adult), *Lepomis microlophus*	Selenium	-	field	LOEC Adverse histopathological alterations	<38.15 µg Se/g dw	Sorensen 1988
Selenium Mixtures						
Phytoplankton, Mixed population	Selenium	-	field	Reduced growth rates	18	Riedel et al. 1991
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC growth	115.2 µg Se/L	Ingersoll et al. 1990
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC productivity	21.59 µg/g dw (whole-body)	Ingersoll et al. 1990
Midge (<24-hr), *Chironomus riparius*	Selenite-Selenate mixture	138	30 days	MATC emergence	503.6	Ingersoll et al. 1990
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	NOEC survival	340	Cleveland et al. 1993
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	EC20 survival	4.07 µg/g dw (whole body)	Cleveland et al. 1993

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Anaerobic bacterium, *Methanococcus vannielli*	Sodium selenite	-	110 hr	Stimulated growth	79.01	Jones and Stadtman 1977
Bacterium, *Vibrio fisheri*	Sodium selenite	-	5 min	50% decrease in light output (Microtox7)	68,420	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenite	32	14 days	5-12% increase in growth	10-10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenite	32	14 days	23% increase in growth	100-10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenite	32	20 days	Increased growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b
Diatom, *Skeletonema costatum*	Selenium dioxide	-	5 days	BCF = 18,000 BCF = 16,000 BCF = 10,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Chaetoceros muelleri*	Selenium dioxide	-	6 days	BCF = 337,000 BCF = 65,000 BCF = 5,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Phaeodactylum tricornutum*	Selenium dioxide	-	8 days	BCF = 109,000 BCF = 27,000 BCF = 7,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Thallassiosira aestivalis*	Selenium oxide	29-30	72 hr	No effect on cell morphology	78.96	Thomas et al. 1980a
Brown alga, *Fucus spiralis*	Sodium selenite	-	60 days	1355% increase in growth of thalli	2.605	Fries 1982
Red alga, *Porphyridium cruentum*	Sodium selenite	32	27 days	Increase growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Selenium (VI)						
Bacterium, *Vibrio fisheri*	Sodium selenate	-	15 min	50% decrease in light output (Microtox7)	3,129,288	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenate	32	14 days	No effect on rate of cell	10-1,000	Wheeler et al. 1982
Green alga, *Chlorella* sp.	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10-100	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	71% reduction in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	16% decrease in rate of cell population growth	100	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	50% decrease in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Brown alga, *Fucus spiralis*	Sodium selenate	-	60 days	160% increase in growth rate of thalli	2.605	Fries 1982
Red alga, *Porphridium cruentum*	Sodium selenate	32	14 days	23-35% reduction in rate of cell population growth	10-1,000	Wheeler et al. 1982
Red alga, *Porphyridium cruentum*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Eastern oyster (adult), *Crassostrea virginica*	Sodium selenate	34	14 days	No significant effect on respiration rate of gill tissue	400	Fowler et al. 1981
Striped bass (embryo), *Morone saxatilis*	Sodium selenate	7.2-7.5	4 days	93% successful hatch and survive	200,000	Klauda 1985a,b
Striped bass (larva), *Morone saxatilis*	Sodium selenate	4.0-5.0	4 days	LC50 (control survival= 77%)	13,020	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	9-65 days	Significant incidence of development anomalies of lower jaw	39-1,360	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	45 days	Significant incidence of severe blood cytopathology	1,290	Klauda 1985a,b

[a] Concentration of selenium, not the chemical. Units are µg selenium/L of water unless noted otherwise.
[b] Converted from dry weight to wet weight basis (see Guidelines).
[c] Growth of algae was inhibited.
[d] From Smith et al. (1976).
[e] Calculated from the published data using probit analysis and allowing for 8.9% spontaneous mortality.

3.0 OTHER DATA - ENDANGERED SPECIES

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the endangered species, razorback sucker, *Xyrauchen texanus* (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results in the context of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). A summary of each of these two studies is presented below.

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1996 (Hamilton et al. 2001a; also Hamilton et al. 2005 a,b,c)

This study was initiated with 5-day old razorback sucker larvae spawned from adults (first time spawners) which were previously held (9 months) in three different locations along the Colorado River that contained varying levels of selenium: Horsethief (the designated reference site which receives water pumped directly from the Colorado River near Fruita, CO, and where dissolved selenium concentrations in water ranged from <1.6 to 3.9 µg/L during the period of exposure), Adobe Creek (low level selenium contamination - dissolved selenium concentrations in water ranged from 1.5 to 11.6 µg/L; avg. = 3.8 µg/L), and North Pond (high level selenium contamination - dissolved selenium concentrations in water ranged from 3.8 to 19.6 µg/L; avg. = 9.5 µg/L). The selenium content in eggs from three Horsethief females ranged from 5.8 to 6.6 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 3.4 to 5.0 mg Se/kg dw. The selenium content in the eggs from three Adobe Creek females ranged from 38.0 to 54.5 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 11.5 to 12.9 mg Se/kg dw. The selenium content in the eggs from three North Pond females ranged from 34.3 to 37.2 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 14.1 to 17.3 mg Se/kg dw. The selenium content in eggs from one of three hatchery brood stock females was 7.1 mg Se/kg dw, and the selenium content in muscle plugs of two of three hatchery brood stock females at spawning ranged from 2.6 to 13.8 mg Se/kg dw. The razorback sucker larvae spawned from fish hatchery brood stock (older, previously spawned females) and held in Colorado River (Horsethief) water were used as an additional reference group of test fish.

The experimental groups were subdivided into those receiving reference water (hatchery water; 24-Road Fish Hatchery) or site water (Table E-2). They were further subdivided into those receiving a daily ration of reference food (brine shrimp) or zooplankton (predominantly cladocerans and copepods) collected from each site where their parents were exposed for the previous 9 months. A total of 60 larvae from each of the four adult sources (Horsethief, Adobe Creek, North Pond, Brood Stock held in different ponds at Horsethief) were exposed to each treatment (2 replicates x 3 spawns x 10 fish/beaker). The larvae were held in beakers containing 800 ml of test water. Fifty percent of the test water was renewed daily.

Table E-2. Treatment conditions during the 30-day larval study.

Source of Larvae	Treatments	Se in food (mg/kg dw)	Dissolved Se in water (µg/L)
Horsethief Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9
Adobe Creek Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	5.5
	Site food: Reference water	20	< 1.6
	Site food: Site water	20	5.5
North Pond Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	10.7
	Site food: Reference water	39	<1.6
	Site food: Site water	39	10.7
Hatchery raised Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9

Growth, survival and development were evaluated amongst treatment groups for up to 30 days in the treatment conditions. Each treatment group was fed once daily after renewal. Test waters were collected every day from each site as grab samples for the renewal. A small portion of this water was retained at 3- and 7-day intervals for an analysis of total and dissolved selenium concentrations. At approximately 2-day intervals, aquatic invertebrates and brine shrimp not used for feeding were sieved from the media for selenium analysis. The number of live fish was recorded daily. After the 30-day exposure period, the surviving fish were sacrificed and measured for total length. At this same time,

approximately four fish from each treatment, when available, were collected as a composite sample and analyzed for total selenium.

After 30 days of exposure in the reference food-reference water treatment, survival of razorback sucker larvae from brood stock and Horsethief adults (89 and 87 percent, respectively) was slightly higher than those from Adobe Creek adults (84 percent) and North Pond adults (75 percent). Corresponding selenium concentrations in larval whole-body tissue were 3.6, 3.3, 7.7 and 9.7 mg Se/kg dw, respectively. Survival was similar or slightly reduced in larvae from all four sources after 30 days of exposure in the reference food-site water treatments; corresponding selenium concentrations in larval whole-body tissue were 5.2, 5.1, 12.7 and 15.2 mg Se/kg dw, respectively. In contrast, none of the larvae spawned from parents from Horsethief, Adobe Creek, or North Pond survived to 30 days when fed zooplankton collected from the three sites, irrespective of the water type they were exposed to (i.e., reference or site). Only the larvae from brood stock adults, which were fed zooplankton from the Horsethief site for this treatment, survived, and even these larvae suffered substantial mortality (40 and 60 percent respectively). The mean selenium concentrations in whole-body tissue of larvae from brood stock adults after the 30-day exposures were 5.4 mg Se/kg dw (site food-reference water treatment) and 6.9 mg Se/kg dw (site food-site water treatment).

Several inconsistencies were observed that indicate selenium may not be solely responsible for the effect on larval survival. Larval survival in the Adobe Creek treatment group exposed to reference water (<1.6 µg/L) and reference food (2.7 mg Se/kg dw) was 84 percent, similar to survival of larvae from brood stock (89 percent). The selenium concentration in the larvae from this Adobe Creek treatment group after 30 days was higher (7.7 mg/kg dw) than that of the brood stock fish (5.4 mg Se/kg dw) in the reference water (<1.6 µg/L) and site food (5.6 mg Se/kg dw) treatment, which had a 30-day survival of 62 percent. Also, the time to 50 percent mortality between the site food treatments, where most mortality occurred, was not related to selenium concentration in the diet or in the larvae.

Although the larvae from brood stock held at Horsethief and the larvae from the first-time spawning adults held at Horsethief that were used for the 9 month exposure received the same site food, no larvae from the latter group survived the 30 day exposure. Concentrations of selenium in the larvae of these two treatment groups were essentially the same between days 6 and 12 of the exposure (8.1 to 8.9 mg Se/kg dw). During this same general time frame (6 to 7 days of exposure), larvae from Adobe Creek and North Pond adults apparently tolerated up to 32 and 39 mg Se/kg dw in tissue, respectively, without any increase in mortality when exposed to reference food and reference water. Larvae grown out under hatchery conditions from adults in the Horsethief and Adobe Creek treatments also did not differ in total deformities compared to larvae from brood stock. There was also no difference between treatments (brood stock, Horsethief, Adobe Creek, and North pond) in percent egg viability, percent hatchability,

percent embryos with deformities, and percent mortality of deformed embryos and larvae from a separate test initiated with eggs in the same study (Hamilton et al. 2005b).

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1997 (Hamilton et al. 2001b)

In a similar 30-day larval study conducted by the authors in the following year (1997), razorback sucker larvae from a single hatchery brood stock female (11 mg Se/kg dw muscle) were subjected to the sixteen different combined water and dietary exposure conditions described in the earlier (1996) study. The female parent was held at Horsethief Canyon State Wildlife Area before spawning. The larvae were held in beakers containing 800 ml of test water as before; fifty percent of the test water was renewed daily. Specific treatment conditions for the 1997 30-day larval study are listed in Table E-3.

Table E-3. Treatment conditions during the 30-day larval study.

Water Treatments	Se in food (mg/kg dw)	Se in water (µg/L)
Reference food (brine shrimp): Reference water (24-Road Hatchery)	3.2	< 1
Reference food: Site water (Horsethief)	6.0	1.6
Reference food: Site water (Adobe Creek)	32.4	3.4
Reference food: Site water (North Pond)	52.5	13.3
Horsethief food: Reference water	3.2	< 1
Horsethief food: Site water (Horsethief)	6.0	1.6
Horsethief food: Site water (Adobe Creek)	32.4	3.4
Horsethief food: Site water (North Pond)	52.5	13.3
Adobe Creek food: Reference water	3.2	< 1
Adobe Creek food: Site water (Horsethief)	6.0	1.6
Adobe Creek food: Site water (Adobe Creek)	32.4	3.4
Adobe Creek food: Site water (North Pond)	52.5	13.3
North Pond food: Reference water	3.2	< 1
North Pond food: Site water (Horsethief)	6.0	1.6
North Pond food: Site water (Adobe Creek)	32.4	3.4
North Pond food: Site water (North Pond)	52.5	13.3

After 30 days of exposure in this study, there was also good survival of razorback sucker larvae fed reference food (brine shrimp) and held in reference water or water from Horsethief (83 and 81 percent, respectively). The survival of these larvae was significantly greater than survival of larvae fed brine shrimp and held in water from North Pond (52 percent). Corresponding selenium concentrations in larval whole-body tissue after 10 days were 6.3, 6.7, and 11 mg Se/kg dw, respectively. The average concentrations of selenium in the water for the three treatments were <1, 1.6, and 13.3 µg Se/L. After 30

days the mean selenium concentrations in these larvae were 5.2, 5.2, and 16 mg Se/kg dw, respectively. Survival was markedly reduced (0 to 30 percent survival) in the remaining treatments where larvae were fed zooplankton from the various sites. Complete mortality was experienced by larvae exposed to Horsethief food and reference water treatment after 30 days.

Similar to the previous study, several inconsistencies in results suggested that selenium may not have been solely responsible for the effect on larval survival. The most notable inconsistency was that the greatest effect on larval survival (percent survival or time to 50 percent mortality) was from exposure to Horsethief food, the food with the lowest selenium contamination.

The authors of the above two studies (Hamilton et al. 2001a,b) make a strong argument that some of the inconsistency in response observed in their studies between larvae fed reference and site diets may be related to the difference in arsenic concentration between the two diets. The arsenic concentration measured in the brine shrimp used in the reference diet was 24 mg total As/kg dw (measured in the second larval study) versus between 6 and 7.5 mg total As/kg dw measured in the zooplankton from the various sites. In their publication (Hamilton et al. 2005c), the authors cite several studies reporting an ameliorating effect of arsenic against the toxicity of a variety of forms of selenium in various animals (Dubois et al. 1940, Hoffman et al. 1992, Klug et al. 1949, Levander 1977, Moxon 1938, Thapar et al. 1969). In terms of the survival of larvae from Horsethief, Adobe Creek and North Pond adults when fed the reference diet, the authors propose that the arsenic concentrations in the brine shrimp diet may have resulted in an antagonistic interaction with selenium and reduced adverse effects in larvae. Such hypothesis is questionable, because their studies included diets spiked with inorganic arsenic salts, whereas the arsenic in brine shrimp (and other natural diets), is most likely predominantly organic arsenic (US EPA 2003). Additionally, in a separate but related study by the same authors (Hamilton et al. 2005d), larval razorback sucker spawned from one female at the Ouray Native Fish Facility were fed zooplankton from six sites (S1, S3, S4, S5, SR, and NR) adjacent to the Green River, Utah at four different initial ages (5, 10, 24, and 28 day old larvae) for 20 to 25 days. The selenium concentrations in zooplankton from the S1 reference site ranged from 2.3 to 3.5 mg Se/kg dw (dissolved Se in water <0.6 to <1.1 µg/L). The concentrations in zooplankton from sites S3 and S4 were slightly higher (range 2.4 to 6.7 mg Se/kg dw; water, 0.3-0.8 µg/L), substantially elevated at S5 (12- 26 mg Se/kg dw; water, 0.6-3.1 µg/L), and highest at SR and NR (44-94 mg Se/kg dw; water, 14-107 µg/L). All larvae in the test initiated when they were 5 days old (study 1) died after 25 days of exposure. Median time to death was shortest in fish fed zooplankton from the reference site (S1) and longest for SR and NR. Interestingly, the concentration of arsenic measured in zooplankton collected from S1 was 12 mg As/kg dw, half that of the brine shrimp used in the above study (Hamilton et al. 2001b), which did not appear to antagonize the toxicity of the

selenium in the diet in this test. In this and the previous two studies, additional inorganic contaminants such as vanadium and strontium were elevated in the zooplankton fed to the larval razorback sucker.

De Riu, D., L. Jang-Won, Huang, S., Monielloa, G., and Hung, S. 2014. Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon. Aquat. Toxicol. 148:65-73.

Test Organisms: Green sturgeon (*Acipenser medirostris*)
White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Three different concentrations of L-selenomethionine were added to an artificial diet mixture: nominal concentrations of 0 (control), 50, 100, and 200 mg SeMet/kg (measured: 2.2 mg/kg Se in control diet (no added Se) and 19.7, 40.1 and 77.7 mg/kg Se in the three treatment diets).

Test Duration: 8 weeks

Study Design: Daily rations of the treatment diets (3% BW/d for first 4 weeks and 2% BW/d for second 4 weeks) were fed to the juvenile sturgeon (approximately 30 g). Each of the four dietary treatment consisted of 3 replicate 90 L tanks with 25 juveniles in each tank. Several endpoints were monitored over the 8 week exposure period including survival, percent body weight increase (% BWI), and hepatosomatic index (HSI).

Effects Data: White sturgeon had no mortalities through the highest dietary treatment. Green sturgeon juveniles had 0%, 7.7% and 23.1% mortality with the three dietary treatments (see table below). %BWI had a greater response to selenium concentration in juvenile tissues than HSI (see table below). Of note is the relatively high concentration of Se in the whole body and muscle tissues of the juvenile sturgeon in the control treatment (both species). The reason for the relatively high Se control concentrations was not due to accumulation of Se from the artificial diet because the concentration of Se remained relatively constant over the 8 week exposure.

Chronic Value: TRAP analysis (threshold sigmoid nonlinear regression) of the green sturgeon survival data resulted in a whole body EC_{10} value of 28.93 mg/kg dw. EC_{10} values were lower for % BWI and HSI using TRAP. For % BWI, the whole body EC_{10} value for green sturgeon was 16.36 mg/kg dw, and for white sturgeon, 23.94 mg/kg dw. For HSI, the whole body EC_{10} value for green sturgeon was 10.86 mg/kg dw (with a very wide 95% confidence interval, 1.842-64.08 mg/kg dw), and for white sturgeon there were no discernible effects.

Selenium in Juvenile Sturgeon Tissues and Endpoints Measured at end of Eight Week Exposure

Green Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	7.1	8.4	100	6.6	2
19.7	22.8	31.1	100	2.6	1.3
40.1	27.8	37	92.3	0.8	0.8
77.7	34.3	36.8	76.9	-1	0.9

White Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	5.6	9.2	100	4.2	2.6
19.7	20.1	27	100	4.2	3.6
40.1	31.8	41.3	100	2.8	3
77.7	47.1	57.9	100	1	2.2

4.0 OTHER DATA – CHRONIC STUDIES WITH FISH SPECIES

Some chronic studies met the requirements of an acceptable chronic test but were excluded from being included in the data set used for criterion derivation for a variety of reasons. Summaries of these studies are provided below.

Vidal, D., S.M. Bay and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol.49:71-75.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*)

Exposure Route: Dietary only
Selenomethionine was added to dry fish food; the measured dietary concentrations were 4.6, 12 and 18 µg Se/g dw. The measured selenium in the control diet was 0.23 µg Se/g dw.

Test Duration: 90 days

Study Design: Each of the three dietary treatments and control had 5 replicates, each replicate contained 12 to 16 larval rainbow trout that were 27 days old at initiation. Each fish was fed an average of 10 mg/d for 30 days; 25 mg/d on days 30-60; and 40 mg/d thereafter. Fish were sampled on days 30, 60 and 90 for length, weight, selenium, hepatic GSH and thiobarbituric acid-reactive substances (TBARS) measurements.

Effects Data: The authors reported significant decreases in weight and length after the 90-day exposure (Table E-4). There were no significant differences in the hepatic lipid peroxidation and hepatic GSH to GSSH ratios among the treatments. The authors found significant differences in weight and length in the 4.6 and 12 µg Se/g dw dietary treatments, but not the 18 µg Se/g dw treatment. Based on larval trout body burden, the authors reported an LOEC of 1.20 µg/g ww, the concentration of Se in fish fed the 12 µg Se/g dw dietary treatment. The Se concentration in larval rainbow trout associated with the lowest dietary treatment that showed significant decreases in larval weight and length was 0.58 µg Se/g ww or 2.06 µg Se/g dw based on 71.8% moisture in whole body rainbow trout (NCBP).

Chronic Value: The data from this study was not used to calculate a chronic value for selenium due to several inconsistencies. The significant decreases in length and weight observed in the two lowest concentrations were not observed in the highest dietary treatment. The Se concentrations in the larval rainbow trout were irregular with the 60-day concentrations being considerably higher than the 90-day concentrations. The authors explain this observation to rapid growth in the fish causing dilution of the Se body burden. However, the increase in fish weight from 30 to 60 days was similar to the 60 to 90 day increase and the 60 day Se concentrations increased from day 30. Also, the Se concentration in the control fish went from below detection on day 0 to 0.46 µg/g ww on day 30; to 1.24 µg/g ww on day 60; and to 0.31 µg/g ww on day 90. The 60-day measured Se in the control fish (1.24 µg/g ww) was more than twice the concentration of Se in the fish with lowest concentration showing effects (0.58 µg/g ww).

Table E-4. Mean (SD) rainbow trout growth after four SeMet dietary treatments.

test day	Treatment, µg/g dw	weight, g	fork length, cm	[Se] whole body, µg/g ww	[Se] whole body, µg/g dw**
0	control	0.37 (0.30)	3.14 (0.41)	ND	ND
30	control	1.33 (0.92)	4.66 (0.41)	0.46 (0.20)	1.63
	4.6	1.25 (0.21)	4.84 (0.29)	1.05 (0.77)	3.72
	12	1.33 (0.30)	5.09 (0.46)	1.81 (1.04)	6.42
	18	1.31 (0.37)	4.97 (0.50)	1.60 (0.93)	5.67
60	control	2.96 (0.92)	6.91 (0.56)	1.24 (0.54)	4.40
	4.6	2.33 (0.63)	6.69 (0.67)	1.70 (0.72)	6.03
	12	2.52 (0.38)	6.88 (0.35)	1.83 (0.94)	6.49
	18	2.59 (0.24)	6.92 (0.24)	2.62 (1.22)	9.29
90	control	5.17 (1.09)	7.70 (0.33)	0.31 (0.20)	1.09
	4.6	3.45 (0.35)*	6.93 (0.19)*	0.58 (0.21)	2.06
	12	3.45 (0.35)*	6.84 (0.68)*	1.20 (0.21)*	4.25
	18	3.82 (0.62)	7.37 (0.62)	1.41 (0.27)*	5.00

* Significantly different than the control.

** ww converted to dw using 71.8% moisture for whole body rainbow trout (NCBP).

Pilgrim, N. 2009. Multigenerational Effects of Selenium in Rainbow Trout, Brook Trout, and Cutthroat Trout. Master's Thesis. University of Lethbridge.

Test Organisms: Rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*) and brook trout (*Salvelinus fontinalis*)

Exposure Route: Dietary only
Selenomethionine added to trout chow and gelatin. Two dietary treatment levels, nominal Se concentrations, 15 (low) and 40 (high) mg/kg.

Test Duration: Rainbow trout were fed the experimental diets from August - December 2009, brook trout July - November 2010, and cutthroat trout December 2010 - April 2011.

Study Design: Fish were obtained from a fish hatchery brood stock. Mature females and were fed the experimental diets in 710 L tanks. Spawning was stimulated by injecting Ovaprim® into the females. Eggs were fertilized and incubated at the fish hatchery until the eye spots were visible. A portion of the eyed stage larvae from each treatment was shipped to the University of Lethbridge Aquatic Research Facility for the swim-up stage of the experiment conducted in gravel bed flumes. Endpoints measured included percent survival in the first (spawned eggs to eyed eggs) and second (eyed eggs to yolk-absorbed fry) stages of development, swim-up success, and malformations (spinal, craniofacial and finfold deformities and edema).

Effects Data: Selenium affected larval survival, swim-up success and the percent of malformations in larvae in one or more of the three species tested (see table below). Visual inspection of plots of the replicate data in Pilgrim (2009) showed considerable variation between the endpoints and selenium in eggs. The distribution of selenium among the tissues was markedly inconsistent with other studies that have used these species. For example, the amount of selenium in the eggs was 8 and 18 times greater than the concentration in the respective muscle tissues in cutthroat and rainbow trout. Median ratios (egg Se:muscle Se) calculated for rainbow trout (Casey and Siwik 2000; Holm et al. 2005) and cutthroat trout (Golder 2005; Kennedy et al. 2000; Rudolph et al. 2007) were 1.9 and 1.8, respectively. Due to the considerable variation in the concentration response of the replicate data and anomalous selenium distribution, these data were not included in the data set to derive the criterion.

Table E-5. Mean selenium concentrations in the diet and selected tissues and selected endpoints measured in rainbow trout (RN), brook trout (BK) and cutthroat trout (CT).
Adapted from Table 3.1 in Pilgrim (2009).

		Tissue, mg/kg ww			Survival, %			
Species	**Diet ww**	**Muscle**	**Liver**	**Egg**	**Stage 1**	**Stage 2**	**Swim-up success**	**Total malformations, %**
	1.47	0.21	3.77	1.17	82.36	61.56	57.18	10
	12.7	0.51	6.53	4.30	77.86	48.64	73.83	9.86
RBT	35.2	0.74	17.21	13.0	54.72	30.33	27.45	29.63
	1.47	0.23	0.72	0.81	86.3	82.68	84	21.3
	12.7	1.14	7.23	5.01	71.37	88.72	83.42	23.93
BK	35.2	3.41	20.4	8.15	71.37	44.63	50.11	24.23
	1.47	0.31	1.00	2.02	61.41	61.87	55.3	6.13
	12.7	0.93	6.00	9.80	30.65	14.75	21.71	48.06
CT	35.2	2.05	14.4	18.0	21.99	0	0.08	NA

Formation Environmental. 2012. Appendix E – Yellowstone Cutthroat Trout Adult Laboratory Reproduction Studies. Technical Support Document: Proposed Site-Specific Selenium Criterion, Sage and Crow Creeks, Idaho. Prepared for J.R. Simplot Company. January 2012.

Test Organism: Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*)

Exposure Route: Field collected. Adult female and male Yellowstone cutthroat trout were collected at five field sites from four streams near the Smokey Canyon mine. In addition Yellowstone cutthroat trout eggs were obtained from a hatchery as method controls.

Test Duration: Test duration was from hatch through 15 days post swim up, and averaged 55-56 days for larvae hatched from field collected fish and 64 days for larvae hatched from laboratory collected fish.

Study Design: Eggs were collected from 15 ripe females at five sites from four streams upstream and downstream of the Smokey Canyon mine. This included one selenium impacted stream downstream of the mine, Sage Creek (LSV), one site along Crow Creek upstream of Sage Creek (CC-150) and one site along Crow Creek downstream of Sage Creek (CC-350), and in sites within the reference streams Deer Creek (DC), and South Fork Tincup Creek (SFTC). Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. In addition, eggs were collected from 16 ripe females obtained from Henry's Lake hatchery (HL) to serve as method controls. Hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. For field and hatchery fish, Se was measured in adult fish (whole body) and in eggs of field collected females.

A target of approximately 600 fertilized eggs from each female (were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15 day post swim up feeding trial.

Endpoints measured in the laboratory were hatch, survival (hatch to swim up, and hatch through 15 days post swim up), and deformities. Deformities were combined as assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Eggs failed to hatch for one of the field treatments (SFTC-1), and six of the hatchery treatments, resulting in a final dataset of eggs fertilized from 14 field collected fish and 10 hatchery fish. Se concentrations in eggs obtained from field collected females ranged from 11.4 mg/kg in Deer Creek through 47.6 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in eggs obtained from Henry's Lake hatchery fish ranged from 0.83 mg/kg – 3.23 mg/kg (Table E-6). Se concentrations in whole body tissue samples obtained from field collected females ranged from 8.17 mg/kg in Deer Creek through 25.7 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in whole body tissue samples obtained from Henry's Lake hatchery fish ranged from 0.23-0.91 mg/kg (Table E-6).

Table E-6. Yellowstone cutthroat trout selenium concentrations, survival, and deformity data from hatch to test end.

Sample ID[a]	Egg Se mg/kg	WB[b] Se mg/kg	# Free From Deformities	# Assessed For Deformities	# Died	# Survived	# Assessed + # Died
CC-150/001	17.6	16.3	22	182	33	182	215
CC-350/001	27.9	20.7	14	138	120	138	258
CC-350/002	29.7	19.4	143	602	83	602	685
CC-350/003	22.3	17.0	73	330	36	330	366
CC-350/004	14.6	16.7	149	480	19	480	499
CC-350/005	47.6	25.7	91	392	71	392	463
DC/001	22	8.17	95	275	30	275	305
DC/002	15.4	9.07	133	465	26	465	491
DC/003	11.4	8.63	59	380	39	380	419
DC/004	12.7	16.6	7	38	23	38	61
HL/002	2.03	0.45	5	39	10	39	49
HL/003	2.48	0.44	121	302	19	302	321
HL/004	1.36	0.36	154	416	20	416	436
HL/006	0.83	0.36	21	244	103	244	347
HL/007	2.26	0.44	120	404	18	404	422
HL/008	1.87	0.28	147	412	37	412	449
HL/011	3.23	0.31	69	296	22	296	318
HL/012	1.58	0.23	112	454	27	454	481
HL/013	1.93	0.72	148	483	24	483	507
HL/015	2.06	0.91	0	36	6	36	42
LSV2C/001	40.1	19.4	2	200	536	0	536[c]
LSV2C/002	30.0	21.0	40	319	105	319	424
LSV2C/003	35.6	18.6	92	487	138	487	625
LSV2C/004	30.5	22.5	107	476	75	476	551

a – CC – Crow Creek; DC – Deer Creek; LSV2C – Sage Creek; HL – Henry's Lake (Hatchery)
b – whole body
c – does not include the 200 fish assessed that were dead prior to assessment, as all fish for that treatment died during the swim up stage in this sample.

Figure E-1 is a plot of % free from deformities versus egg concentration. The previous draft used TRAP to estimate an effect level for these data but after further review it was concluded these data just do not demonstrate any clear effect of Se and therefore inappropriate for analysis by TRAP. There is no obvious trend, especially one that is substantial relative to the data variability. The correlation coefficient for these data is not significant and a t-test of the two data clusters is likewise not significant. The survival data also do not show a useful trend, especially one suitable for EC10 estimation. Although no effect concentration was determined for this test, the data do not contradict the other cutthroat trout datasets in that there are no effects up to 30 mg/kg and of the three points in excess of 30 mg/kg, one did show 100% mortality. The data are consistent with *Oncorhynchus* not being one of the four most sensitive genera.

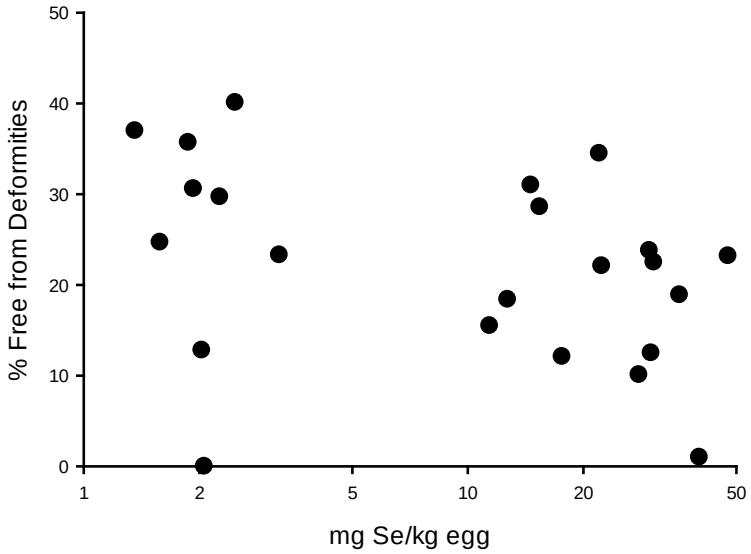


Figure E-1. Plot of percent free from deformities relative to the concentration of selenium in cutthroat trout eggs.

Effect Concentration: NA

Deng, X. 2005. Early life stages of Sacramento splittail (*Pogonichthys macrolepidotus*) and selenium toxicity to splittail embryos, juveniles and adults. Doctoral dissertation, University of California, Davis.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*)

Exposure Route: Dietary only
Four concentrations of selenium in the fish diet (0.6, 17.3, 33.0, and 70.1 mg/g) were created by mixing different proportions of selenized and Torula yeast. A different batch of selenized yeast was used in the adult exposure.

Test duration: 24 weeks

Study Design: Fourteen adult fishes were placed in each circular tank (92 cm diameter, 33 cm height) and fed one of the four diets. Each diet was provided to fishes in three tanks. The twelve tanks were arranged in three rows. Each row had all four treatment concentrations with randomly assigned positions. Thus, the experiment had a randomized block design. Adult splittail fishes were obtained from the Tracy Pump Station (U.S. Bureau of Reclamation, Tracy, CA). After 12 and 24 weeks of exposure, blood samples were collected, the liver, gonad, kidney and white muscle were dissected, and liver and gonad were weighed to calculate hepatosomatic and gonadosomatic indices. Stages of ovarian and testicular development were determined from histological studies.

Effects Data: No mortality occurred throughout the experiment. Fish in control, 17.3, and 33.0 mg/g treatments exhibited normal behavior. Fish exposed to 70.1 mg/g in did not consume as much food as fishes exposed to lower selenium concentrations, and displayed abnormal behaviors. Splittail adults were less sensitive to dietary selenium than juveniles. Relative to control, no changes in body weight, total length, GSI, and condition factor were observed in fishes exposed to selenium concentrations in food up to 33 mg/g. In general, tissue concentrations in fishes exposed to selenium were higher than in the control, but differences in selenium concentrations among them were often small and not significant (Table E-7). Percentages of ovaries with atretic follicles increased with higher concentrations of selenium in their diet: 30% in control, 45.5% in the 17.3 mg Se/g, and 100% in the 33.0, and 70.1 mg/g treatments. The average concentration of selenium in ovaries of fish exposed to 17.3 mg/g in their diet was 6.5 mg/g. This low effect level, though, is disputable because of the very low number of ovaries analyzed, the occurrence of atresia in 30% of ovaries in control, and the lack of significant differences in concentrations of selenium in ovaries among treatments exposed to elevated levels of this element.

Table E-7. Mean concentration of selenium in ovaries (SE).[‡]

	Diet Concentration (mg Se/g)			
	0.6	17.3	33.0	70.1
[Se] in ovary (mg/g dw)	4.4 (0.57)	6.5 (1.0)	8.3 (0.14)	8.9 (0.46)

[‡] Values estimated from Figure 4 in Deng (2005) (pg. 111)

de Rosemond, K. Liber and A. Rosaasen. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker. Bull. Environ. Contam. Toxicol. 74: 1134-1142.

Test Organism: White Sucker (*Catostomus commersoni*)

Exposure Route: Field collected.
In June, 2002, eggs were collected from 4 females from Island Lake (exposed site); milt was obtained from 2 males. Island Lake is downstream from Cluff Lake uranium mine located in northern Saskatchewan. Selenium concentrations in Island lake range from 1 to 11 µg/L and in recent years have been typically 4-5 µg/L. No fish/eggs were collected from a reference site.

Test duration: Through the end of yolk absorption by the larvae; 33 days post-fertilization.

Study Design: Individual batches of eggs were fertilized in the field with milt and water-hardened. Eggs were air transported to the laboratory in Saskatoon for testing. 200 eggs were randomly selected from each clutch and then separated into groups of 100 which were placed into individual test chambers (n = 8).

On test day 30 (3 days prior to test termination), all fish larvae that exhibited macroscopic deformities (e.g., kyphosis, lordosis, scoliosis and edema) were removed, photographed and preserved. At test termination, (day 33), 40 larvae from each female whites sucker were evaluated for deformities using a microscope.

Effects Data: Although all four females were collected from the exposed site, selenium concentrations in eggs were grouped into two low (Fish 2 and 3 in Table E-8) and two high (Fish 1 and 4 in Table E-8). Larval mortality and developmental deformities were not related to selenium concentrations in eggs (Table E-8). The data suggest that embryo/larval effects are not observed at concentrations in eggs reaching 40.3 mg/kg dw (geometric mean of the two high selenium concentrations in eggs). However, because a reference condition with low selenium exposure was not established, it is not appropriate to estimate an effect concentration for this study. Note: the average percent moisture for the four clutches of eggs was 92.6%.

Effect Concentration: NA

Table E-8. Embryo/larval endpoints for eggs from four female white sucker collected from Island Lake in June 2002.

Measurement	Fish 1	Fish 2	Fish 3	Fish 4
Successfully hatched larvae[a]	161	140	176	141
Deformed larvae[b]	21	25	16	13
Dead larvae[c]	6	14	6	4
Macroscopic deformities , %				
Embryological[d]	6.8	6.4	5.7	1.4
Developmental[e]	6.2	11.4	3.4	7.8
Microscopic deformities, %				
Developmental[f]	7.5	5	2.5	7.5
Total developmental deformities, %[g]	13.7	16.4	5.9	15.3
[Se] eggs mg/kg ww[h]	2.7	0.7	0.6	3.2
[Se] eggs mg/kg dw[h]	33.6	9.4	8.4	48.3

[a] Initial number was 200 per fish

[b] Total number of deformed larvae throughout study; includes embryological and macroscopic deformities

[c] Total number of larvae that died throughout study.

[d] Percent of curled deformities that appeared in embryonic fish; deformities were evident immediately after embryos hatched.

[e] Percent of deformities that were designated developmental; deformities became evident as larvae grew and absorbed yolk sac (after experimental day 15).

[f] Percent of microscopic developmental deformities that were evident in the 40 fish examined per female white sucker.

[g] The estimated percentage of offspring that had microscopic and macroscopic developmental deformities combined.

[h] Selenium concentration measured in a subsample of embryos collected on test day 0.

Ogle, R.S. and A.W. Knight. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). Arch. Environ. Contam. Toxicol. 18:795-803.

Test Organism: Fathead minnows (*Pimephales promelas*; juvenile, 59 to 61 d old)

Exposure Route: Dietary only
Purified diet mix spiked with inorganic and organic selenium: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine, homogenized in dextrin.

Test Treatments: Completely randomized block design (2 blocks); 4 replicates per block (n = 8 replicates total per treatment). Actual mean total selenium levels in each exposure treatment were: 0.4 (control), 5.2, 10.2, 15.2, 20.3, and 29.5 mg/kg dw. Fish used in the first randomized block (F_2 generation fish) were progeny from F_1 generation originally obtained from the Columbia National Fishery Research Laboratory, some of which were used in an initial range-finding experiment. Fish obtained from a commercial supplier were used in the second randomized block. The prepared diet was extruded into 1.5 mm pellets which were air-blown dried to 5 percent moisture content and crushed and sieved so that only particles retained by an 11.8 mesh/cm sieve were used in the study. The amount of selenium in water that leached from the food during the experiment averaged only 0.8 μg/L.

Test Duration: 105 days, F_2 generation (block one) and commercial fish (block two);
14 days F_3 generation

Study Design: Ten fish were randomly placed in each cell per block (n = 8x10, or 80 fish total per treatment). Fish were fed twice daily at 6 percent body weight per day, with wastes and uneaten food removed 30 min. after each feeding. Test tanks were flushed with two tank volumes of fresh test water after each feeding (solution renewal). Growth (as wet weight) was determined every two weeks by bulk weighing, and one fish from two of the cells per treatment in a given block (n = 4 total per treatment) was removed for selenium (whole-body) analysis. After 105 days of exposure, a single male and female fish from each treatment replicate (n = 4 breeding pairs per treatment in a given block, or 8 breeding pairs per treatment total) were placed in 250 ml beakers and inspected for spawning activity for 30 days following the first spawning event for that pair (each pair being one replicate). Gonads and muscle tissue were dissected for selenium analysis from these fish at the end of the 30 days spawning period. The spawning substrates were inspected daily for eggs to determine fertility and viability. Samples of not more than 50 eggs from each spawn were incubated in flowing, aerated water and inspected for percent hatch determination. Ten larvae from each incubated brood were transferred to separate glass test chambers and maintained (48 h renewal; fed brine shrimp twice daily) for 14 days to determine percent larval survival.

Effects Data: There was no effect of selenium on any of the reproductive parameters measured at the dietary concentrations tested. Percent hatch and percent larval survival were very high (>87.4 percent) and essentially equal for all of the treatments. Growth of pre-spawning adults was affected by the selenium exposure (Table E-9).

Table E-9. Effects on fathead minnow growth after 98 days of exposure to dietary selenium.

Measured mean selenium in diet, mg/kg dw	Whole-body selenium, mg/kg dw	Mean fish weight, g ww
0.4	1.76	1.30
5.2	2.78	1.24
10.2	3.42	1.20
15.2	5.40	1.21
20.3	6.58	1.09
29.5	7.46	0.94

Chronic Value: An EC value could not be calculated for these data because the data did not meet the minimum requirements for analysis.

GEI Consultants. 2008. Maternal Transfer of Selenium in Fathead Minnows, with Modeling of Ovary Tissue to Whole Body Concentrations.

Test Organism: Fathead Minnow (*Pimephales promelas*)

Exposure Route: Field collected.
Gravid adult fathead minnows were collected from creeks with a wide range of surface water selenium concentrations near the city of Denver, CO during the 2006 summer breeding season.

Sites
Low selenium exposure:

- Sand Creek at Colfax. In 2002, aqueous selenium averaged 0.9 µg/L.

Moderate to high selenium exposure:

- Sand Creek downstream of refinery
- East Tollgate Creek
- Mainstem Tollgate Creek

Control fish – no field exposure

- Laboratory-reared fish from Aquatic BioSystems

Test duration: Embryo-larval test was 48 hours post hatch.

Study Design: Field collected adult fish were either field dissected for selenium measurement in paired tissues or transported live back to the laboratory in coolers with site water. Fish were transported to the laboratory where mating pairs were bred in individual chambers containing spawning substrates. Eggs were removed from the spawning substrate and reared in a standard Falcon dish with lab water. Eggs were screened under a dissecting microscope for viability. Dead eggs were removed and numbers recorded on a datasheet. Three separate breeding experiments were conducted.

Upon hatching, larvae were moved to standard bioassay cups containing lab water and maintained in the laboratory incubator at 25ºC. Larvae were maintained via static conditions in exposure cups for 48 hours post-hatch without food to ensure full absorption of the yolk sac before they were fixed in formalin. Deformity assessment was performed on fixed embryos using a dissection microscope. Test endpoints consisted of egg production, fertilization success, mortality, and deformities (includes edema and skeletal, craniofacial and finfold malformations). The authors used a graduated severity index (GSI) for deformities in which larvae were scored 0 (normal), 1 (slight), 2 (moderate), and 3 (severe) based on the level of defect.

Effects Data: All fish successfully spawned except those collected from Sand Creek downstream from the refinery. These fish had visible parasites and were only used in the ovary-to-whole body selenium analysis. A suite of metal and metalloids were measured in fish samples from each location. Fish collected from East Tollgate Creek had higher concentrations of 9 of the 15 metals that were

measured in fish from at least one site. Aluminum and iron showed the highest difference with an approximate 10-fold increase in the East Tollgate Creek fish.

Only the first brood of each mating pair was used for the analysis because effects appeared to be muted in subsequent broods. The lower response in the second brood was thought to be due to clearing of selenium in the oocytes. There was poor correlation between egg fertilization ($R^2 = 0.13$) and embryo mortality ($R^2 = 0.18$) data with whole body selenium concentrations in the adult fish (see Table E-10 for summary data; see Table E-11 for individual brood data). Neither the fraction of embryos surviving nor fertilization rate as a function of the concentration of selenium in maternal fathead minnows was suitable for estimating EC values. Although there were low survival and fertilization rates at some higher selenium concentrations, these responses were quite varied and did not follow a defined concentration-response relationship (Figure E-2).

Of the 9 broods from fish collected at the three exposed sites only one brood (from East Tollgate Creek) had deformities greater than 10%. The fathead minnow females that produced the brood with the greatest number of deformities and highest GSI also had the second highest concentration of whole body selenium, 46.4 mg/kg dw (Table E-12; Figures E-3 and E-4). Approximately half of the larvae from this brood exhibited some sort of malformation. Similar to the embryo parameters, EC values were not able to be estimated for any of the 4 malformation parameters.

The authors used probit analysis and TRAP to determine effect levels for each of the embryonic and larval endpoints (Table E-13). Although there is an indication of effect due to selenium exposure in both the embryonic and larval endpoints, there is too much variation in the responses observed with the embryos and insufficient response observed with the larvae to derive a reasonable estimate of effect levels. Therefore, no effect level was determined for this study.

Effect Concentration: Unable to determine due to high variability or insufficient response.

Table E-10. Mean fathead minnow first brood embryo and larval parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

Parameter	Site			
	Con	SCC	TGC	ETC
n (number of breeding pairs)	10	3	3	4
WB Se concentration (mg/kg dw)	2.86 ± 0.18	9.17 ± 0.46	35.87 ± 3.73	44.53 ± 2.41
Egg fertilization (%)	84.75 ± 3.32	23.99 ± 22.45	63.42 ± 31.82	59.6 ± 22.26
Embryo mortality (%)	22.03 ± 3.34	89.04 ± 9.70	46.40 ± 26.86	50.76 ± 23.63
Mean spawn size (# of eggs per spawn)	129 ± 23	318 ± 63	162 ± 61	317 ± 158
Total larva evaluated (total # of broods)	957	89	281	254
Mean brood GSI score	4.85 ± 1.22	8.88 ± 8.88	14.88 ± 4.63	21.75 ± 9.53
Larval craniofacial defects (%)	2.64 ± 0.90	4.65 ± 4.65	6.26 ± 3.63	18.48 ± 13.84
Larval skeletal defects (%)	4.74 ± 0.89	9.30 ± 9.30	6.21 ± 1.48	19.62 ± 12.11
Larval finfold defects (%)	2.19 ± 0.78	4.07 ± 4.07	5.71 ± 3.08	17.23 ± 14.48
Larval edema (%)	3.89 ± 1.01	5.23 ± 5.23	6.26 ± 3.63	20.32 ± 12.93
Larval length (mm)	4.90 ± 0.05	4.97 ± 0.12	4.83 ± 0.14	4.90 ± 0.07

Table E-11. Fathead minnow first brood embryo parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); for site acronyms see Table E-9.

Brood	Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-1a-1	CON		2.90	19	0.79	0.96
T-1f-1	CON		3.24	238	0.77	0.88
T-1f-1	CON		1.94	19	0.63	0.73
T-2a-1	CON		2.25	135	0.98	0.98
T-3a-1	CON		2.71	154	0.68	0.72
T-3b-1	CON		2.64	90	0.90	0.95
T-3d-1	CON		3.67	76	0.70	0.71
T-4d-1	CON		3.43	199	0.85	0.91
T-5d-1	CON		3.33	149	0.73	0.87
T-6d-1	CON		2.52	183	0.76	0.78
T-2b-1	SCC		9.92	395	0.00	0.00
T-4a-1	SCC		8.35	193	0.03	0.03
T-6a-1	SCC		9.25	340	0.30	0.69
T-2a-1	TGC		32.29	132	0.83	0.91
T-3a-1	TGC		43.33	79	0.00	0.00
T-4a-1	TGC		31.99	262	0.77	1.00
T-1f-1	ETC		39.76	141	0.52	0.70

Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-3b-1	ETC	47.47	208	0.88	0.92
T-5a-1	ETC	46.37	634	0.07	0.17

Table E-12. Fathead minnow first brood larval malformations and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total Larvae	Spinal Incidence	%larvae w/o spinal deformity	%larvae w/o craniofacial deformity	%larvae w/o finfold deformity	%larvae w/o edema	Total GSI Score
T-1f-1	CON	1.94	11	9	91	100	100	100	1
T-2a-1	CON	2.25	141	3	97	99	98	96	24
T-6d-1	CON	2.52	117	2	98	99	99	97	16
T-3b-1	CON	2.64	81	4	96	98	99	98	12
T-3a-1	CON	2.71	96	1	99	100	100	100	1
T-1a-1	CON	2.90	14	7	93	93	93	93	10
T-1f-1	CON	3.24	189	8	92	98	98	94	53
T-5d-1	CON	3.33	95	4	96	97	99	98	20
T-4d-1	CON	3.43	164	3	97	98	99	96	28
T-3d-1	CON	3.67	49	6	94	92	94	90	29
T-4a-1	SCC	8.35	3	0	100	100	100	100	0
T-6a-1	SCC	9.25	86	19	81	91	92	90	71
T-4a-1	TGC	31.99	190	5	95	97	97	97	41
T-2a-1	TGC	32.29	91	8	92	90	91	90	78
T-1f-1	ETC	39.76	65	5	95	95	98	94	20
T-5a-1	ETC	46.37	39	44	56	54	54	54	152
T-3b-1	ETC	47.47	150	11	89	95	96	91	89

Table E-13. Authors calculation and comparison of fathead minnow larval deformity EC_{10} estimates using probit analysis and TRAP.

Effect	Endpoint	Probit Results WB [Se] mg/kg, dw (±SE)	TRAP Results WB [Se] mg/kg, dw (95% CL)	Probit Results Ovary [Se] mg/kg, dw (±SE)	TRAP Results Ovary [Se] mg/kg, dw (95% CL)
Edema	EC_{10}	39.48 ± 16.21	45.78 (40.95 - 51.20)	52.99 ± 19.99	61.43 (55.04 – 68.55)
Finfold	EC_{10}	68.55 ± 27.26	48.31 (39.41 - 59.21)	87.95 ± 32.16	64.81 (53.01 – 79.24)
Skeletal	EC_{10}	27.80 ± 9.53	46.08 (41.94 - 50.62)	38.67 ± 12.32	61.82 (56.36 – 67.80)
Craniofacial	EC_{10}	53.86 ± 18.77	47.41 (38.92 - 57.76)	70.83 ± 22.84	63.56 (52.37 – 77.16)
All abnormalities	EC_{10}	16.98 ± 5.38	45.50 (41.10 - 50.37)	24.23 ± 7.06	61.06 (55.26 – 67.48)
All abnormalities except edema	EC_{10}	21.35 ± 6.45	45.69 (41.10 - 50.79)	30.32 ± 8.51	61.27 (55.23 – 67.97)

Figure E-2. The fraction total survival of embryos (top left), fraction of embryos successfully fertilized (right), survival adjusted for fertilization (bottom) versus maternal whole body selenium concentration. Bottom figure EC10=35.2 mg/kg Se dw WB.

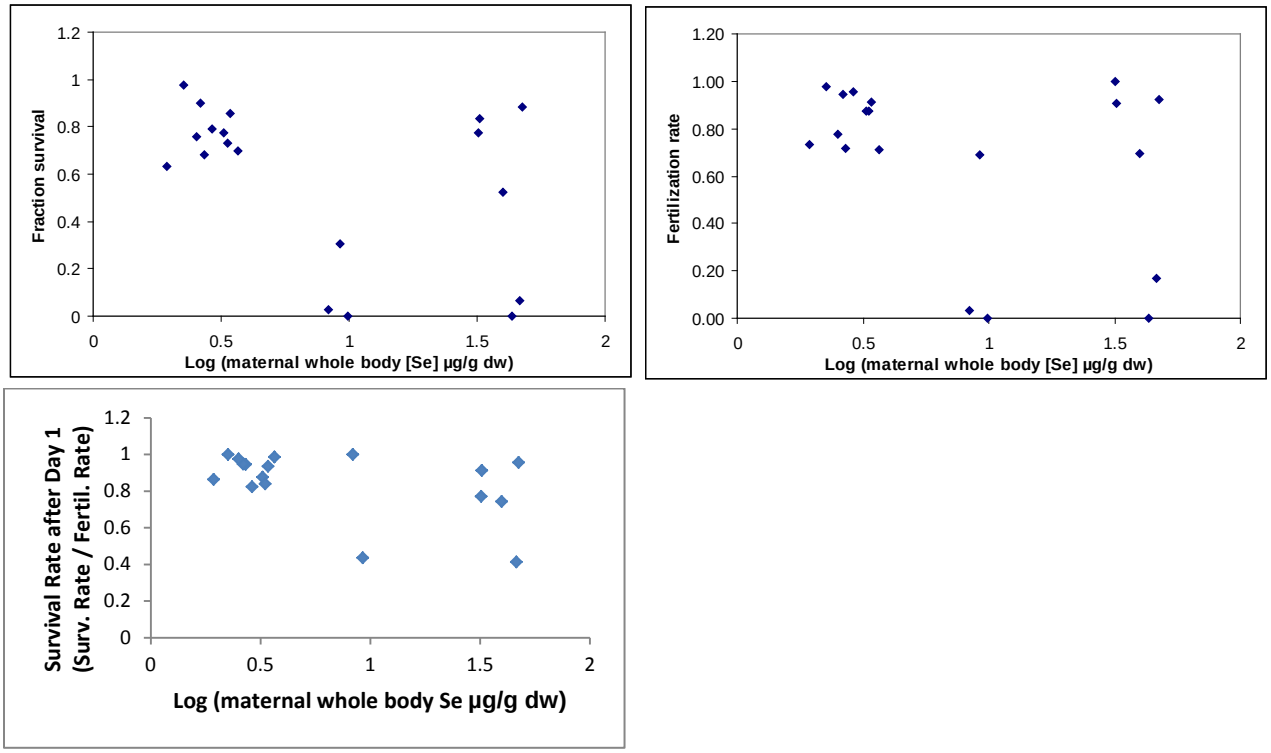


Figure E-3. Percent 2-day post-hatch larvae without edema (A), finfold deformity (B), craniofacial deformity (C), and spinal deformity (D) relative to maternal whole body selenium concentration. EC10s: 61.4 – 64.8 mg/kg dw WB.

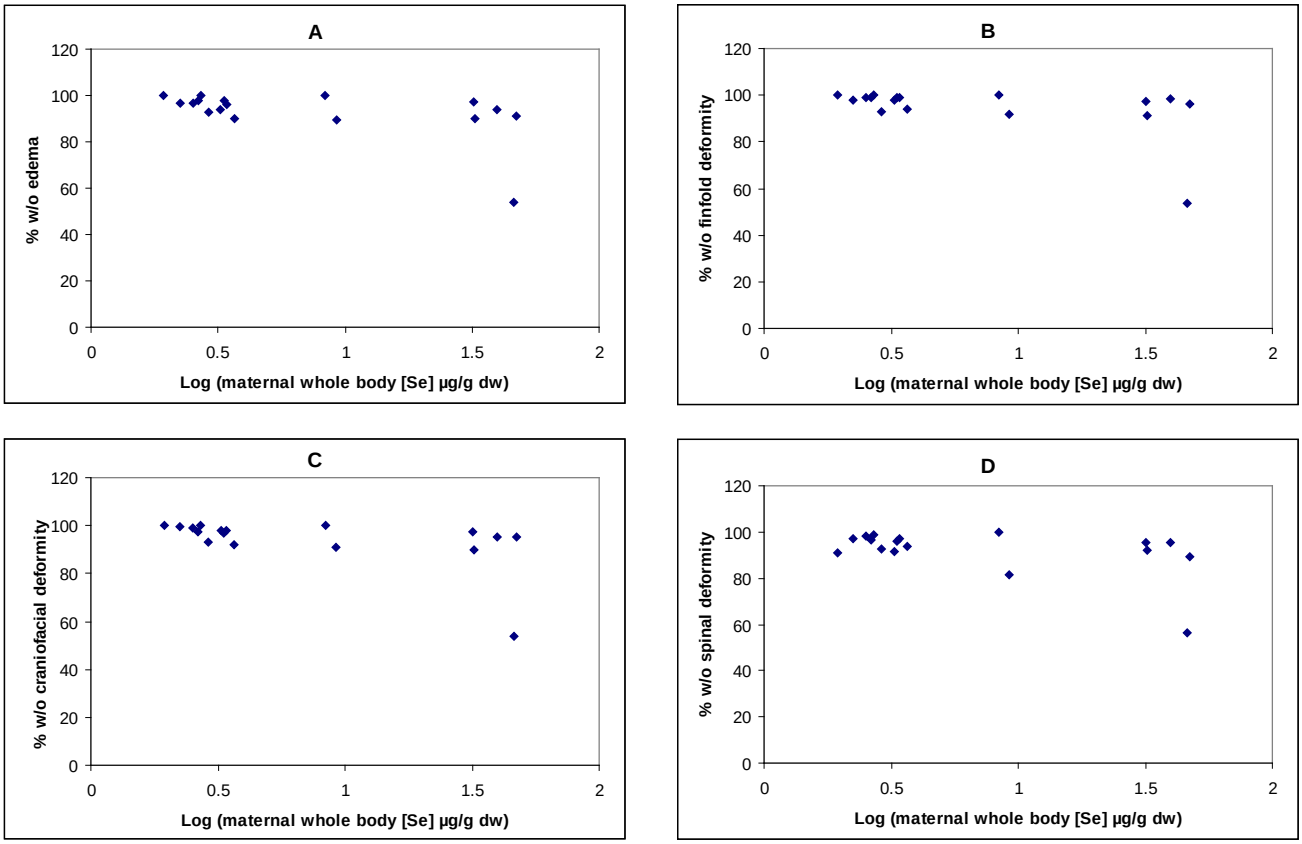
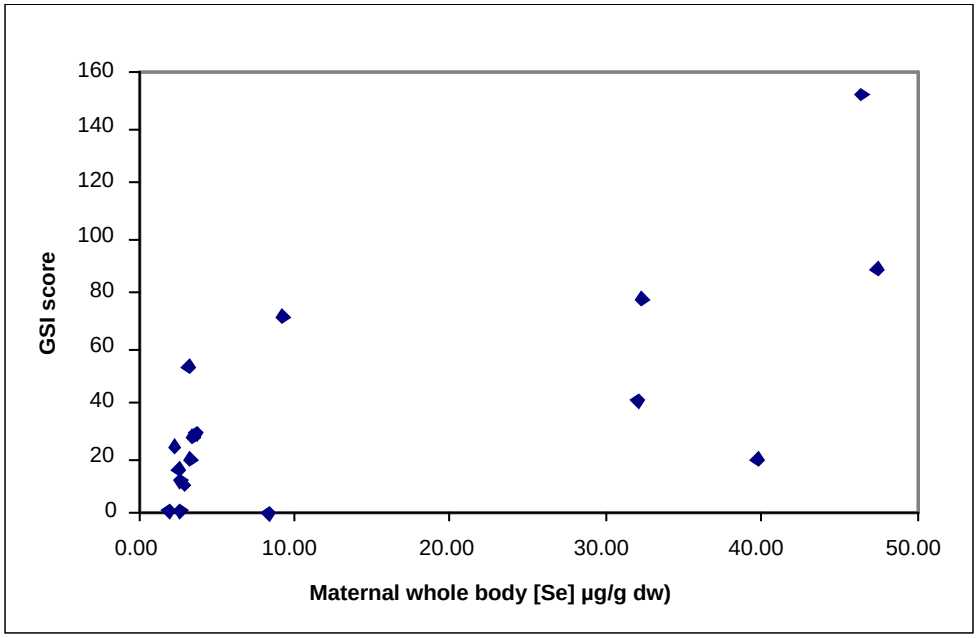


Figure E-4. Percent 2-day post-hatch larvae Graduated Severity Index (GSI) relative to maternal whole body selenium concentration



4.1 Evaluation of zebrafish (Danio rerio) and native cyprinid sensitivity to selenium

Overview:

Two new studies on zebrafish (*Danio rerio*), Thomas and Janz (2014), Thomas (2014), and Penglase et al. (2014), were made available to EPA by David Janz, one of the external peer reviewers. Thomas (2014) and Thomas and Janz (2014) were the original dissertation and peer reviewed paper, respectively, of the same body of work. The apparent sensitivity of the zebrafish to selenium relative to other species in the EPA selenium criteria document was the subject of several public commenters, as well as Dr. Janz in the comments received by EPA.

EPA calculated an EC10 of 7.004 mg Se/kg egg dw, or approximately 3.5 mg/kg whole body) from the Thomas (2014) and Thomas and Janz (2014) study. EPA was not able to calculate an EC10 from Pengalese et al. (2014). The Thomas (2014) and Thomas and Janz (2014) study is summarized in the following section (Part I). Penglase et al. (2014) is summarized in section 7.1.5 of the main document.

EPA noted that the concentration-response curves for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species. The shallow slope indicates partial effects across the range of test doses, with some individuals being very sensitive, and others being less sensitive than other test species. A typical test signature of the nutritionally essential element selenium is that above a particular concentration there is a precipitous increase in adverse effects, with most test organisms affected within a narrow dose range. Additional issues discovered during the analysis of available information in the literature and supplied by the investigator raised questions of test quality that introduced uncertainty in the results reported. This uncertainty, and the fact that zebrafish may not represent the sensitivity range for cyprinids native to the US (discussed in Part II), led to the decision to include this study qualitatively in the effects characterization.

The paucity and relative insensitivity of the available data for cyprinids (fathead minnow EC10 = < 23.9 mg/kg dw; based on LOEC in ovary) relative to other fish families like centrarchids (sunfish), and salmonids (trout and salmon) caused additional concern. This led EPA to investigate the field significance of the zebrafish EC10 (7.004 mg/kg egg) compared to what we know about cyprinid occurrence in selenium impacted waters. The available studies with native cyprinids indicate that a variety of native cyprinid genera (e.g. chubs, shiners, dace) have stable, diverse populations and are reproducing successfully (based on length frequency data) in selenium impacted waters at whole body concentrations far exceeding our proposed whole body criterion element of 8.0 mg/kg dw. Taken together, the available studies (Hamilton et al. (1998), NAMC (2008), Presser (2013), USGS (2012)), indicate that native cyprinids as a family are not expected to be overly sensitive to selenium when compared with other families of freshwater fish. This is important because zebrafish are non-native, and have only been recently discovered in U.S. waters due to accidental introduction.

EPA believes there is significant uncertainty regarding the actual sensitivity to zebrafish, and therefore proposes inclusion of the zebrafish studies in the effects characterization section, as well as inclusion of a comprehensive analysis of the studies as well as the studies on sensitivity of selenium to native cyprinids (below) in its own technical appendix, and issuing an FRN soliciting additional studies or information on zebrafish, as well as native cyprinids.

4.1.1 Part I. Chronic summary of Thomas (2014) and Thomas and Janz (2014)

Thomas, J.K. 2014. Effects of Dietary and in ovo Selenomethionine Exposure in Zebrafish (*Danio rerio*). Dissertation. University of Saskatchewan, Saskatoon, Canada.

Thomas and Janz, D.M. 2014. *In ovo* exposure to selenomethionine via maternal transfer increases developmental toxicities and impairs swim performance in F1 generation zebrafish (*Danio rerio*). Aquatic Toxicol. 152:20-29.

Test Organism: Zebrafish (*Danio rerio*)

Exposure Route: Dietary only
Selenomethionine spiked into Nutrafin® basic flake food

Test Treatments: Control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw).

Test Duration: 90 days

Study Design: Adult zebrafish were fed a control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw) for 60 days, followed by an additional 30-40 days with equal rations (2.5%) of control or SeMet-spiked diets and clean chironomids. After 90 days of feeding exposure, adult fish from each exposure group were bred 3-4 times and embryos were collected and used to assess a number of different effects including larval survival and deformities. Eggs from each treatment were pooled from which replicate samples were collected for selenium measurement, larval survival and deformity assessment

Effects Data: The authors presented mortality and deformities in the F1 generation graphically for days up to 6 days post fertilization (dpf). The bar graphics were initially converted to numeric values using a length measuring tool in GIMP (GNU Image Manipulation Program). EC10 values for both mortality and deformities were very low with deformities being slightly lower. Upon request, the authors provided a table of the number of deformities in observed in 2-6 days post fertilization (dpf) fish larvae for each replicate pool of eggs (Table E-14) (David Janz, pers. comm.). TRAP analysis of these data produced a very low EC10 of 7.0 mg/kg egg Se dw. The concentration-response curve in Figure E-5 is extremely shallow compared to similar tests on other species, such that the apparent sensitivity of zebrafish relative to other species depends on what level of effect is considered. A comparison of egg-ovary zebrafish concentration-response curves for survival and deformities with well-founded concentration-response curves for other species is presented in Figure E-6. The shallow survival and deformity slopes for the zebrafish stand out as atypical for a selenium response. Note the EC50 values for the zebrafish are very similar to the EC50 values for the majority of other fish species and the zebrafish EC90 is similar to the EC90 of the least sensitive fish, Dolly Varden.

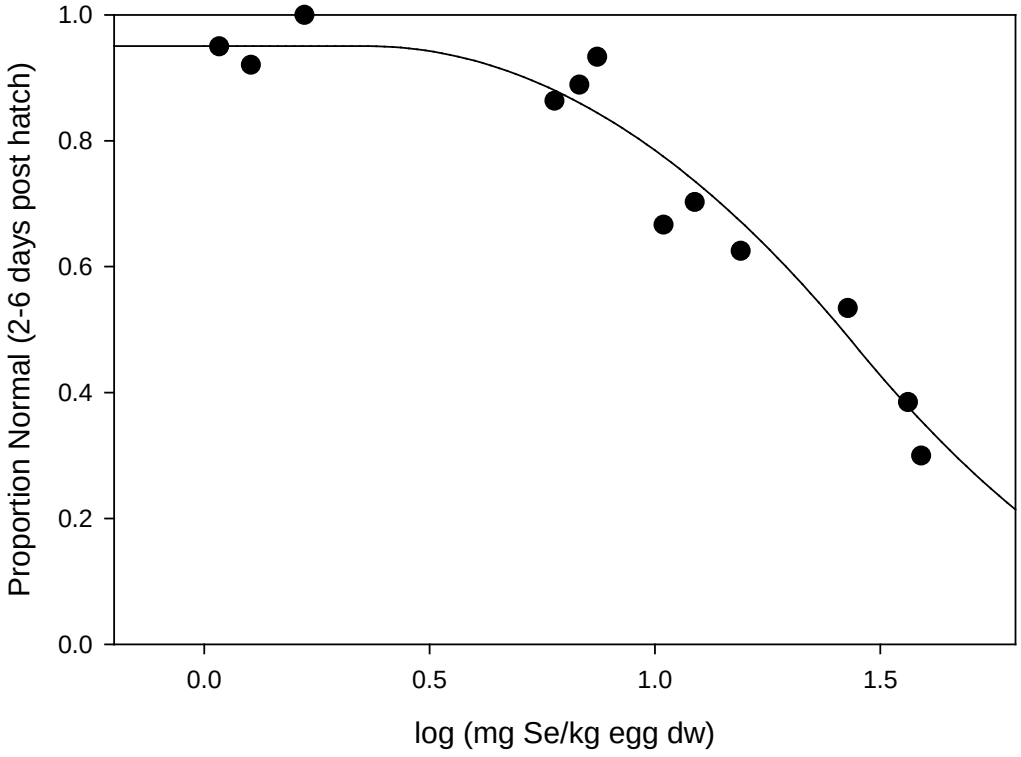
A GMCV based on this test has not been included in the Sensitivity Distribution for several reasons. Although the deformity and survival EC50s are within the range observed for a number of other species, the concentration-response curves

for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species (Figure E-6). Furthermore, if the concentration-response curves are log-symmetrical, as generally has been assumed in estimating EC10s, the projected EC90s for zebrafish would place it among the least sensitive known species**,** indicating greater variability among individuals within this one species than among individuals across the entire class of other fishes represented in the figure. The implication of such a shallow concentration-response curve is that this species has exceptional genetic diversity with respect to selenium tolerance, such that populations could adapt to very high or very low selenium concentrations. The field significance of its exceptionally low EC10 is thus uncertain. The low EC10 might or might not have some relationship to the selenium deficiency reported by Hook (2008) in substantial portions of its home range in the Ganges and Brahmaputra basins in India and Bangladesh.

An assessment of the relative sensitivity of cyprinids using both field and laboratory data is provided in the following section (Part II).

Table E-14. Selenium concentrations in zebrafish eggs and deformities in 2-6 dpf larvae.

Se in eggs, mg.kg dw	Total	Deformed	% Deformity
1.67	35	0	0.00
1.27	63	5	7.94
1.08	40	2	5.00
5.99	44	6	13.64
7.45	45	3	6.67
6.80	36	4	11.11
12.26	37	11	29.73
10.46	39	13	33.33
15.51	48	18	37.50
38.98	30	21	70.00
36.44	65	40	61.54
26.81	88	41	46.59



Parameter Summary:					
Parameter	**Initial**	**Final**	**Std. Error**	**95%LCL**	**95%UCL**
LogX50	1.45	1.4421	0.0408	1.3632	1.5247
Standard Deviation	0.44	0.4421	0.0586	0.3514	0.5964
Y0	0.95	0.9503	0.0184	0.9	0.9799

Effect Concentration Summary:			
%Effect	**ECx**	**95%LCL**	**95%UCL**
90	65.15	45.28	93.73
50	27.79	23.08	33.47
20	11.12	8.647	14.29
10	7.004	4.884	10.04
5	5.053	3.208	7.958

Figure E-5. Tolerance distribution model (triangular distribution model shape) of the proportion of normal zebrafish larvae (1-fraction with deformities) vs. the logarithm of concentration of selenium in zebrafish eggs.

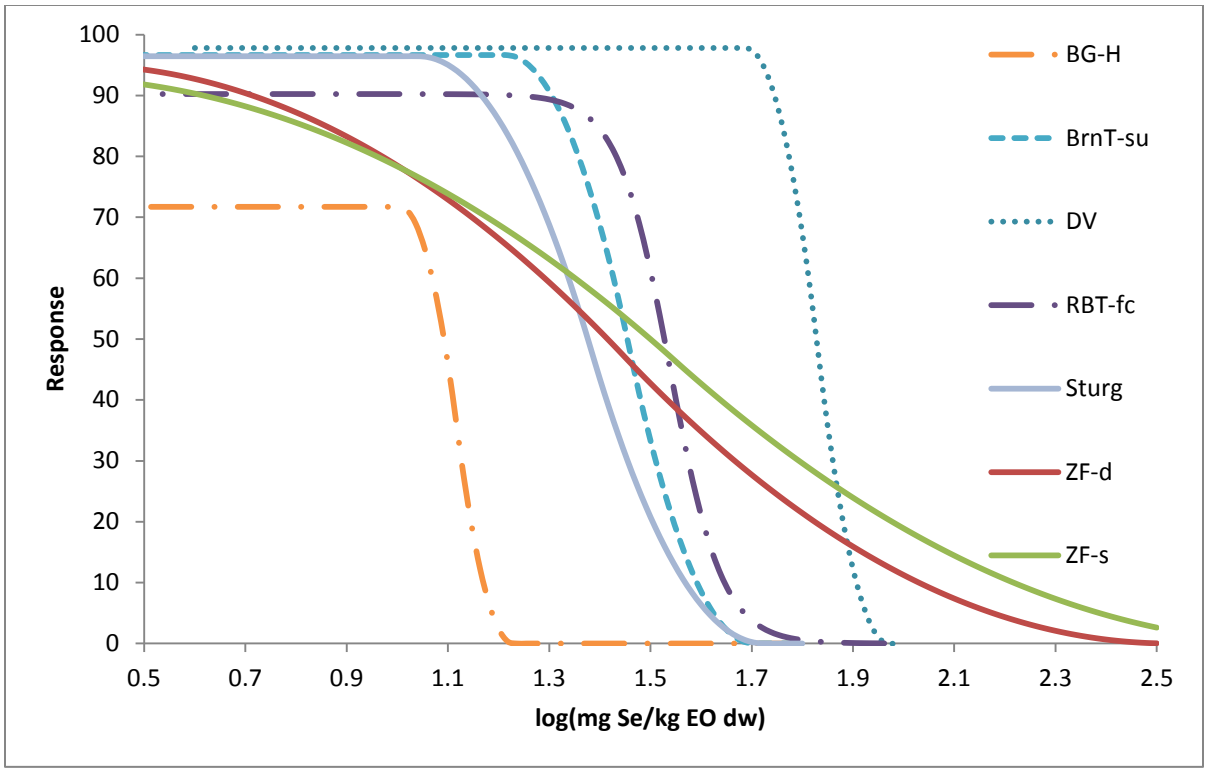


Figure E-6. Thomas and Janz (2014) zebrafish concentration-response curves for deformities and survival, ZF-d and ZF-s, compared with representative concentration-response curves for other species spanning the full range of EC10s.
BG-H: bluegill, Hermanutz et al. (1992, 1996); BrnT-su: brown trout survival to swim-up (Formation 2011); DV: Dolly Varden, Golder (2009; RBT-fc: rainbow trout facial-cranial deformities, Holm (2002) and Holm et al. (2003, 2005); Sturg: sturgeon deformities, Linville (2006).

4.1.2 Part II - Evaluating Sensitivity of Cyprinids (Cyprinidae) to Selenium from Field and Laboratory Data

Background:

The draft selenium criteria document is based on reproductive effects (mortality deformities) to larval fish following maternal exposure. These chronic tests are based primarily on species from the families salmonidae and centrarchidae. There is a paucity of data for a number of fish families used for development of selenium criteria. This limitation in data is particularly notable for the family cyprinidae ("minnows"), because it is comprised of approximately 180 general and is one of the most diverse families in North America. A recent toxicity test with zebrafish (*Danio rerio*), discussed above in Part 1, indicated that some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. This study was very different than all previous studies

examining larval effects in that the slope was very shallow, whereas the slopes for all other species were steep (see Figure E-6).

This analysis considers the results of the zebrafish laboratory survival study and several field collection studies, which evaluated cyprinid abundance and diversity in watersheds impacted by selenium, to compare the sensitivity of the zebrafish evaluated by Thomas (2014) and Thomas and Janz (2014) to native cyprinid populations. Available water and whole body tissue selenium concentrations (> 8.0 mg/kg dw), were compared to the translated egg-ovary to whole body zebrafish EC10 values (~ 3.5 mg/kg dw) to evaluate the relative sensitivity of native cyprinids to the non-native zebrafish test outcome.

Executive Summary:

The occurrence and effect of selenium on native cyprinids were evaluated based on the results of field studies conducted in four aquatic systems (CO, NC, UT, and WV) having elevated selenium concentrations. The objective of this evaluation was to compare the sensitivity of native cyprinid populations with the results of a recent toxicity test with zebrafish (*Danio rerio*) (Thomas (2014), Thomas and Janz (2014)) that suggests some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. The following set of analyses evaluated studies of widely-distributed native cyprinid species occurring in waters impacted by selenium from various sources and the relationships between whole body tissue levels, (and water concentrations where available) and impacts from selenium via toxicity or population metrics.

Cyprinid genera representing many species native to the US were found to be present in waters with selenium concentrations exceeding the current national criteria value (5µg/L). Cyprinid species present in the four studies examined represent 169 of the approximately 180 species present (at the genus level) in the United States. Abundance and diversity at sites impacted by selenium (water concentrations > 5.0 µg/L) were found to be no different than at sites in the Arkansas River, Colorado with low selenium concentrations (3.0-3.5 µg/L) watershed, with the exception of one location where extremely high selenium concentrations (Wildhorse Creek, CO; approximately 413 µg Se/L) were detected.
Whole body tissue concentrations within several widely distributed cyprinid genera exceeded the proposed whole body tissue element of 8.0 mg/kg dw and had sustainable reproducing populations, as indicated by length frequency analysis and occurrence data for the four studies. When evaluated by itself, the influence of selenium whole-body concentration in reducing family Cyprinidae densities was not statistically significant ($R^2 = 0.02$; $p = 0.51$). Rather, substrate characteristics of the waterbodies sampled had the strongest influence. In contrast, when evaluated by itself, the influence of selenium whole-body concentration in reducing family Centrarchidae densities was significant ($R^2 = 0.53$; $p = 0.02$).

In spite of the potential for confounding factors, GEI (2008) obtained parallel results at a different location, Dixon Creek and Canadian River in Texas, affected by refiner effluent selenium. Again, selenium whole-body selenium had no relationship to cyprinid density ($R^2 = 0.00$) but was a significant negative factor for centrarchid density ($R^2 = 0.41$, $p = 0.003$). And in the Sand Creek Drainage, CO, GEI found no negative association between fathead minnow densities and selenium concentrations of 3-26 mg Se/kg whole-body dw and 8-45 mg Se/kg ovary dw.

These findings suggest that native cyprinids are less sensitive than centrarchids, and are thus likely to be protected by a national criterion based heavily on centrarchid and salmonid sensitivity. Based on these available data, native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species, and much greater than indicated by the non-native zebrafish test outcome. It is therefore expected that the proposed selenium criterion will be protective of native cyprinids occurring throughout the United States.

Laboratory Exposures:

1. Chronic Toxicity and Hazard Assessment of an Inorganic Mixture Simulating Irrigation Drainwater to Razorback Sucker and Bonytail. Hamilton et al. (2000). USGS CERC Laboratory

Toxic effects from inorganics associated with irrigation activities, and possibly contributing to the decline of endangered fish in the middle Green River, Utah were investigated. Two 90-day chronic toxicity studies were conducted with two endangered fish, razorback sucker (*Xyrauchen texanus*) and bonytail chub (*Gila elegans*). Swim-up larvae were exposed in a reconstituted water simulating the middle Green River. The inorganic mixtures were tested at 1X, 2X, 4X, 8X, and 16X the measured environmental concentrations of the evaluated inorganic constituents (2 ug/L arsenic, 630 ug/L boron, 10 ug/L copper, 5 ug/L molybdenum, 51 ug/L selenate, 8 ug/L selenite, 33 mg/L uranium, 2 ug/L vanadium, and 20 ug/L zinc).

Bonytail chub survival was 95% or greater at 30, 60, and 90 days except for the 16X treatment (1232 ug/L Se), whereas growth was reduced after 30, 60, and 90 days at the 8X treatment (532 ug/L Se). Swimming performance of bonytail chub was reduced after 90 days of exposure at the 8X treatment. Whole-body residues of copper, selenium, and zinc increased in a concentration-response manner, but did not increase at 90 days of exposure at the 8X treatment for most species tested, and at lower treatment concentrations for the bonytail chub. Mean whole body selenium residues at the 8X treatment were 23.3, 16.7, and 9.4 mg/kg Se dw at 30, 60 and 90 days respectively. Hamilton et al. (2000) concluded that adverse effects in bonytail chub were associated with whole-body concentrations of 9.4 to 10.8 mg/kg Se dw in this study. One key uncertainty is the effect that the combination of toxic elements, in contrast to selenium alone, had on outcomes measured in this study. However, basing the selenium toxicity

evaluation on exposure to multiple contaminants is expected to provide a more conservative estimate of effect on the bonytail chub (*Gila elegans*) than if selenium is tested alone.

Field Collection Studies

2. Selenium Tissue Thresholds: Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field. Part III: Field Application of Tissue Thresholds: Potential to Predict Population or Community Effects in the Field. NAMC Report (2008).

Field studies were conducted by GEI in the Arkansas River, CO mainstem and selected tributaries between 2005 and 2006 to examine the relationship between selenium concentrations as well as habitat characteristics in surface waters and cyprinid abundance and diversity in the Arkansas River. The data collected for the study included:

1) Seasonal fish and macroinvertebrate (not shown) sampling to determine species composition and the relative abundance of aquatic organisms);
2) Whole-body fish tissue, composite macroinvertebrate tissue (not shown), and water and sediment (not presented) sample collection for the evaluation of Se concentrations in these tissues and the evaluation of bioaccumulation pathways; and
3) Physical habitat measurements (not presented), to determine relationships between the occurrence of biota and their physical environment. Data were collected from fall 2004 to fall 2006 from the Arkansas River, Fountain and Wildhorse Creeks, and the St. Charles River.

Total selenium (dissolved) was measured at 4 sites mainstem and 6 sites on three tributaries of the Arkansas River watershed near Pueblo Colorado (Table E-15). Multiple site visits (6 to 17) to collect water for selenium determination were conducted at the 10 sampling stations between 2005 and 2006.

Table E-15. Selenium water column data: Total Selenium (µg/L, dissolved).

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
AR (Arkansas River)				
AR1 (ARM) Mainstem, in Pueblo below Whitlock WWTP	8 months	15	7.05	3.69
AR2 (ARE) Mainstem below Pueblo WW Reclamation Center and Fountain Creek	12 months	9	10.6	4.06
AR3 (ARB) Mainstem, downstream of Pueblo	10 months	7	8.72	4.0
AR4 (ARN) Mainstem, downstream of St. Charles River	10 months	8	8.81	2.85

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
Arkansas River Tributaries				
WHC (Wildhorse Creek)	6 months	17	418	115
FC (Fountain Creek)				
FCP (Upstream)	12 months	9	3.43 (4.9)*	1.05
FC4 (Downstream)	6 months	12	12.1	4.34
SC (St. Charles River)				
SC1 (Upstream)	6 months	6	3.09 (4.8)*	1.37
SC2 (Mid-Point)	6 months	11	11.7	6.22
SC5 (Downstream)	8 months	13	20.3	13

* Maximum [Se] in FCP and SC1 < 5.0 ug/L, current selenium criterion

Summary of Selenium Concentrations in Water:

1) Total selenium concentrations exceeded the EPA chronic selenium standard of 5 µg/L in surface water samples collected from most locations, with only the upper reaches of the St. Charles River and Fountain Creek having mean selenium concentrations below the EPA chronic selenium standard.
2) Selenium concentrations in water samples from Wildhorse Creek were more than 20X greater than in water samples collected from all other sample locations, with a mean selenium concentration of 418 ± 115 µg/L.
3) The minimum concentration measured in water samples from Wildhorse Creek (315 µg/L) was approximately 7X greater than the maximum selenium concentration measured at other study sites (43.6 µg/L at St. Charles River, SC5).

Selenium in Fish Tissue:

Selenium concentrations in fish tissue (whole body) were measured for three representative cyprinid species (central stoneroller, sand shiner, red shiner), one catostomid (white sucker), and three centrarchids (green sunfish, smallmouth bass, and largemouth bass) (Table E-16).

Table E-16. Mean fish tissue concentrations.
[Average whole body mg/kg dw estimated by eye from graphs in NAMC (2008)].

Sample Site	ARM	ARN	ARE	ARB	WHC	FCP	FC4	SC1	SC2	SC5
Mean water [Se] ug/L	7.0	8.8	10.6	8.7	418	3.43	12.1	3.1	11.7	20.3
Cyprinids										
Sand Shiner	10	10-21	25			10-17	15-21			
Red Shiner		23	42				25			30
Central Stoneroller	8	10-20			18-47	12	14	5	45	33
Centrarchids										
Green Sunfish								12	30	
Largemouth Bass	11-15	14-36	22	26						40
Smallmouth Bass	7		20	20						
Catostomids										
White sucker	8-11	10-24	16-18	14-21	32-33	6-10	24	6-14		47

Summary of selenium in fish tissue:

1) The mean concentrations in all cyprinids across all sites was 21.06 mg/kg dwt; SE = 1.38).
2) For comparison, the mean concentration in all centrarchids across all sites was 19.73 mg/kg dw; SE = 1.32; and the mean concentration in white sucker (catostomids) across all sites was 17.52 mg/kg dw; SE = 1.52.
3) Most mean whole-body Se concentrations were well above the U.S. EPA (2014) proposed chronic tissue criterion element for whole body of 8.13 mg/kg dry weight.

Comparison to national draft fish tissue criteria:

Given that these are waters known to be impacted by selenium there were only a few fish samples (Tables E-17, E-18) that were at or below the proposed whole body criteria element of 8.1:

1) The Arkansas River mainstem (mean water [Se] = 7.05 ug/L), had samples from three species that met the criteria in 2006, central stoneroller, smallmouth bass and white sucker.
2) In the tributaries to the Arkansas River that were sampled, white sucker in both Fountain Creek (mean water [Se] = 3.43 ug/L) and St. Charles River met the whole body criteria in 2004 and 2005, whereas the only cyprinid to meet the proposed whole body criterion was the central stoneroller in 2005.

Cyprinid Abundance and Diversity:

Table E-17. Cyprinid Diversity (native spp. present– excludes carp): NAMC 2008 Study.

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	1/6	3/6
ARB	8.72	6/6	5/6
ARN	8.81	5/6	3/6
ARE	10.6	5/6	4/6
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	5/6	4/6
FC4	12.1	4/6	6/6
Whitehorse Creek (WHC)	413	1/6	1/6
St. Charles River			
SC1	3.09	5/6	5/6
SC2[1]	11.7	4/6	NS
SC5	20.3	6/6	5/6

[1]SC2 only sampled in 2005

Table E-18. Cyprinid Abundance (native spp. present– excludes carp): NAMC 2008 Study

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	8	460
ARE	8.72	643	950
ARB	8.81	697	521
ARN	10.6	446	116
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	746	2352
FC4	12.1	1978	1825
Whitehorse Creek (WHC)[1]	413	926	81
St. Charles River			
SC1	3.09	2920	14583
SC2[2]	11.7	2757	NS
SC5	20.3	3102	2568

[1]Whitehorse Creek comprised 1 species, central stoneroller
[2] SC2 not sampled in 2006

Summary of cyprinid abundance and diversity:

1) Diversity as well as abundance of cyprinids in the tributaries vs the Arkansas River mainstem more likely a function of habitat and/or predator density rather than influence of selenium.
2) Several sites on Wildhorse Creek, Fountain Creek, and the St. Charles River, had substantial changes in the populations of some fish species between sample years 2005 and 2006, with fish that were present in one year in high numbers and with a variety of age classes, either

absent or present in low numbers the other year. These changes are likely to be linked to higher stream flows present in 2006 and significant habitat changes due to beaver activity at some sites. Variable population compositions and numbers of cyprinids are not uncommon in plains streams with highly variable flow regimes and habitat conditions (Schlosser 1987).

3) Based on an evaluation of age class distribution (indicated by length-frequency distribution data), it was concluded that the following sites had viable and reproducing cyprinid populations (NAMC 2008:

> Arkansas River mainstem: The length-frequency data collected for the fish species at these sites indicates multiple age groups present for most of the species at the sites.
> Fountain Creek - Length-frequency analysis of the flathead chubs indicated that the populations are reproducing, with juvenile and older adult fish present in relatively high numbers at both sites and years.
> St. Charles River - Length-frequency analysis of the fish populations indicated that sites had reproducing populations of central stonerollers, fathead minnows, and sand shiners, with juvenile and adult fish collected during both years (GEI 2007a).
> Wildhorse Creek - the age class distribution of central stonerollers was similar between years, indicating a reproducing population that includes both juvenile and adult fish in both years, despite the extremely high [Se] in water.

Relevance/Surrogacy of Arkansas River Cyprinids to all Cyprinid Species in US

Cyprinids captured from the Arkansas River are representative of cyprinid species occurring throughout the US. This conclusion is based on the following lines of evidence:

- Six of the seven cyprinid species (central stoneroller, fathead minnow, flathead chub, longnose dace, red shiner, and sand shiner) captured from the Arkansas River during this investigation are native to the United States;
- Four of the six cyprinid species found in the Arkansas River basin (central stoneroller, fathead minnow, sand shiner and red shiner) are widely distributed throughout the United States (see species specific distribution maps Attachment 1); and,
- Six of the native species present in the Arkansas River Basin are direct surrogates at the genus level for the 142 native cyprinids in North America (Table E-19).

Table E-19. Cyprinid species surrogacy and occurrence in water for native species inhabiting the Arkansas River and select tributaries.

Species	Cyprinid group	# of species represented by genus	[Se] in waterbodies where species occurred	Average tissue concentration or range
Campostoma anomalum Central stoneroller	stonerollers	5 species	3.1-418 ug/L	5-47 mg/kg dw
Pimephales promelas Fathead minnow	Blunthead minnows	4 species	3.1 - 20.3 ug/L	No tissue
Platygabio gracilis Flathead chub	Flathead chub	1 species	3.1 - 20.3 ug/L	No tissue
Rhynichthys cataractae Longnoise dace	dace	9 species	3.1 - 20.3 ug/L	No tissue
Cyprinella lutrensis Red shiner	Satinfin shiners	32 species	3.1 - 20.3 ug/L	23-42 mg/kg dw
Notropis stramineus Sand shiner	Eastern shiners	91 species	3.1 - 20.3 ug/L	10-25 mg/kg dw

Summary cyprinid surrogacy:

Cyprinid species collected from the Arkansas River watershed are representative (at the genus level) of the 142 cyprinid species native to North America. With the exception of one sample location (Whitehorse Creek), the abundance and diversity of cyprinid species present and the occurrence of multiple age classes indicates that cyprinids are successfully surviving and reproducing in the Arkansas River watershed, even with selenium concentrations exceeding 5ug/L in water and 8 mg/kg bw in whole body fish tissue. North American species not represented at the genera level comprise 54 species (mostly chubs – 40 species), many of which are geographically isolated.

3. Observations of cyprinids in NC Reservoirs (Hyco Reservoir and Belews Lake) – (located at end of NAMC 2008 report).

Crutchfield et al. (2000) evaluated long-term water quality data, selenium chemical concentration data collected for sediment, invertebrate and fish tissues, and invertebrate and fish population data collected from the Hyco Reservoir to document the recovery of the aquatic community following the 1990 installation of a dry fly ash pollution abatement system. Since 1973, data have been collected from six locations in the Hyco Reservoir, with varying fly ash exposure. Gamefish including bluegill sunfish and largemouth bass were reproductively extirpated due to high selenium concentrations prior to installation of the pollution abatement system. The fish community was dominated by green sunfish (*Lepomis cyanellus*), eastern mosquitofish (*Gambusia holbrooki*), gizzard shad (*Dorosoma cepedianum*), and satinfin shiner (*Cyprinella analostana*). Their main observation was that satinfin shiner was a dominant cyprinid in the Se limited fish community prior to selenium reduction.

Barwick and Harrell (1997) evaluated fish population monitoring and tissue selenium concentration data to document the recovery of fish populations in Belews Lake for the ten years following installation of a dry fly ash pollution abatement system. Fish diversity and biomass data were collected from 1977 to 1994 (with the exception of 1978-1979 and 1982-1983) at two sites on the lake. In 1980 and 1981, fathead minnows (*Pimephales promelas*) dominated the fish community, representing 62 percent and 81 percent of the biomass, respectively (Barwick and Harrell 1997). By 1984, red shiner (*Cyprinella lutrensis*), common carp (*Cyprinius carpio*), and fathead minnows (*Pimephales promelas*) were the dominant cyprinids in the selenium limited fish community prior to selenium reduction. The authors noted that cyprinid abundance started to decrease as green sunfish, a more Se- tolerant sunfish recovered in 1989-1990, followed by further decreases in 1990-1994, as channel catfish, bluegill, and largemouth bass populations increased (Barwick and Harrell 1997).

Young et al. (2010), reviewing the studies of Belews Lake, NC, note that during the period of maximal selenium inputs, egg and ovary concentrations reached 40-159 mg Se/kg dw. Out of as many as 29 resident species prior to contamination, only catfish and the cyprinids common carp and fathead minnows remained during the period of maximum impact.

4. Presser, T.S., 2013, Selenium in ecosystems within the mountaintop coal mining and valley-fill region of southern West Virginia—assessment and ecosystem-scale modeling: U.S. Geological Survey Professional Paper 1803, 86 p. http://dx.doi.org/10.3133/pp1803.

USGS sampled southern West Virginia ecosystems affected by drainage from mountaintop coal mines and valleys filled with waste rock (valley fills) in the Coal, Gauley, and Lower Guyandotte watersheds during 2010 and 2011. Sampling data from earlier studies in these watersheds (for example, Upper Mud River Reservoir) and other mining-affected watersheds in WV are also are included to assess additional hydrologic settings and food webs for comparison.

1) Site-specific fish abundance and richness data documented the occurrence of various species of chub, shiner, dace, minnow, and central stoneroller (*Campostoma anomalum*) in the sampled watersheds.
2) Model species for streams were limited to creek chub (*Semotilus atromaculatus*) and central stoneroller. Creek chub was present at all sites during USGS sampling in 2010-2011. However, both of these species are considered to have high tolerance for environmental stressors based on results of traditional comparative fish community assessments. Concentrations of Se in water and whole body tissues of creek chub, blacknose dace, and stoneroller are shown in Table E-20.
3) The order of abundance for species with greater than 28 individuals was: creek chub, striped shiner, mottled sculpin, green sunfish, central stoneroller, blacknose dace, bluntnose minnow, and northern hog sucker. Shiners and darters were prevalent, but bluegill sunfish were absent during the 2010 survey.

Table E-20. Se in fish whole body tissue samples: Upper Mud River Basin and Tributaries.
(Compilations of data from different sources presented in (Presser et al. 2013).

Stream Segment	Year	[Se] in water Mean (Range) in ug/L	Creek Chub Mean (Range) in mg/kg dw	Blacknose Dace Mean (Range) in mg/kg dw	Stoneroller Mean (Range) in mg/kg dw
Upper Mud River	2011	10.5, 18.2	9.0 (6.4–11)	Not Sampled	Not Sampled
Upper Mud River 1	2010	Not Sampled	10.3 (9.4–10.9)	Not Sampled	Not Sampled
Lower Mud River	2008	7.9	10.3 (9.4-15.4)	Not Sampled	Not Sampled
	2011	5.2, 7	9 (6.4-11)	Not Sampled	Not Sampled
Upper Mud River 2 (above Upper Mud River 1)	2005	9.8 (4–22)[1]	2.9 (<1-8.7)	Not Sampled	Not Sampled
	2006	Not Sampled	5.6 (2.2-10)	Not Sampled	Not Sampled
	2007	Not Sampled	7.7 (3.7-10)	Not Sampled	Not Sampled
Berry Branch	2009-2010	8.3 (1.7–18)[2]	4.0 (3.3–5.0)	9.6 (7.8–13)	Not Sampled
Stanley Fork	2009-2010	6.0 (3.0–7.4)[3]	10.3 (7.2–13)	Not Sampled	Not Sampled
Lower Kanawha River Watershed					
Little Scary Creek	2006	20	Not Sampled	55	Not Sampled
	2009	31.4 (23-42)	28 (3-80)	Not Sampled	Not Sampled
Connor Run	2009	47.8 (4-90)	(21-36)	Not Sampled	Not Sampled
Upper Kanawha River Watershed					
Jack's Branch Mining Complex					
Bull push fork w/pond	2010	9.0-10.0	Not Sampled	66 (19–113)	Not Sampled
Bull push fork downstream	2010	9.1–10	8.6 (6.2–13)	10.7 (5.5–14)	6.9 (3.1–17)
Hughes Fork	2005 - 2007	5.3 (2–10)	7.8 (4.1–10.9) 2005 7.9 (2.7–12.9) 2007	Not Sampled	12.4 (0.5–34.5) 2005
Hughes Creek	2010-2011	2.1-13	9.9 (3.7–17)	16.9 (6.8–25)	9.0 (3.6–14)
Big Coal River Watershed					
Beech Creek	2005-2007	Not Sampled	(3-18)	Not Sampled	Not Sampled
Seng Creek	2005-2009	27.5 (15–42)	8.2 (4.8–14.7)	Not Sampled	Not Sampled
	2011	23.3	8.1 (5.4-10)	Not Sampled	Not Sampled
White Oak Creek	2005-2007	15.8 (8–27)	5.8 (<1-12.8)	Not Sampled	7.1 (2.5–12.8)

[1] Water samples collected between 2005 and 2008.
[2] Water samples collected in 2009 and 2010.
[3] Water samples collected in 2009 and 2010.

Study Summary:

Samples in various environmental media (water, sediment, algae, macroinvertebrates, fish) were collected by USGS (2010-2011), and others (e.g. WVDEP, Potesta) between 2005 and 2011. The stream segments presented here represent a subset of the stream segments with available data. Only streams with water [Se] > 5.0 ug/L are presented to facilitate comparison with other studies with Se-impacted streams. Overarching observations include:

1) [Se] in water averaged from 5.3 ug/L – 31.4 ug/L with a high of 90 ug/L (Connor Run, 2009).
2) [Se] in fish tissue: creek chub – averaged from 5.8 mg/kg wb to 28 mg/kg wb, with a maximum whole body concentration of 80 mg/kg wb (Little Scary Creek, 2009).
3) [Se] in fish tissue: blacknose dace – averaged from 10.7 mg/kg wb to 66 mg/kg wb, with a maximum whole body concentration of 113 mg/kg wb (Bull push fork w/pond, 2010)
4) [Se] in fish tissue: central stoneroller – averaged from 6.9 mg/kg wb to 12.4 mg/kg wb, with a maximum whole body concentration of 34.5 mg/kg wb (Hughes Fork, 2005). Note also, that central stoneroller, although common through stream segments samples, were not ubiquitous, as was observed in the study conducted by NAMC in the Arkansas River near Pueblo CO.

5. Selenium concentrations in fish tissue collected from the Gunnison River.
http://pubs.usgs.gov/of/2012/1235/of12-1235.pdf

Approach: In sampling conducted in summer 2010, muscle tissue plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and whole body tissue samples were collected from speckled dace (*Rhinichthys osculus)* inhabiting critical habitat in the Gunnison River in Western Colorado (Table E-21). Total selenium in fish muscle plugs (mg/kg dw) for roundtail chub, or in whole body (speckled dace) was calculated for all tissues. In follow-up sampling conducted in the summer of 2011, muscle plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and bonytail chub (*Gila elegans, listed*) inhabiting critical habitat in the Gunnison River in Western Colorado.

This study was intended to document any changes in selenium concentration in fish over the last 20 years based on remediation efforts that have been completed to date.

Table E-21. Fish tissue concentrations observed in Cyprinids.

Species	**Year**	**Mean (Range) [Se]**	**# > muscle = 11***	**# > whole body = 8**
Roundtail Chub	2010	9.7 mg/kg dw (5.2-32.4)	2/15	
	2011	7.33 mg/kg dw (5.6-11.2)	1/15	
Speckled Dace	2010	7.46 mg/kg dw (5.7-9.7)		6/15

* Muscle plugs were collected since this species is large enough for non-destructive sampling, and b) a listed species.

5.0 OTHER DATA – CHRONIC STUDIES WITH INVERTEBRATE SPECIES

A limited number of studies have evaluated the effects of selenite on invertebrate species, an important prey item for fish and birds as summarized by Debruyn and Chapman (2007). The following **studies** with a rotifer, and annelid, and an insect (mayfly) were found suitable for establishing species sensitivity.

5.1 Rotifers

Dobbs et al. (1996) exposed *Brachionus calyciflorus* to selenate in natural creek water for 25 days in a three-trophic level food chain test system. This is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In this particular study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but received additional selenium from their diet (i.e., the algae fed to rotifers and the rotifers fed to fish). The overall exposure lasted for 25 days. Rotifers did not grow well at concentrations exceeding 108.1 µg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 µg Se/L in the water (40 µg Se/g dw in the algae). Regression analysis of untransformed growth data (dry weight) determined 4 day post-test initiation resulted in a calculated EC_{10} of 37.84 µg Se/g dw tissue.

5.2 Aquatic Worms

Although not intended to be a definitive toxicity study for this invertebrate, Besser et al. (2006) evaluated the bioaccumulation and toxicity of selenized yeast to the oligochaete, *Lumbriculus variegatus,* which was intended to be used for dietary exposure in subsequent studies with the endangered desert pupfish, *Cyprinidon macularius*. Oligochaetes fed selenized-yeast yeast diets diluted with nutritional yeast (54 to 210 mg Se/kg) had stable or increasing biomass and accumulated Se concentrations as high as 140 mg/kg dw. The oligochaetes fed the undiluted selenized-yeast (826 µg/g Se dry wt.) showed reduced biomass. The effect level is considered >140 mg Se/kg dw.

5.3 Aquatic Insects (Plecoptera: Mayfly)

Conley, J.M., D.H. Funk and D.B. Buchwalter. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ. Sci. Technol. 43:7952-7957.

Conley, J.M., D.H. Funk, N.J. Cariello and D.B. Buchwalter. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicol. 20:1840-1851.

Conley, J.M., D.H. Funk, D.H. Hesterberg, L-C. Hsu, J. Kan, Y-T. Liu and D.B. Buchwalter. 2013. Bioconcentration and biotransformation of selenite versus selenite exposed to periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ. Sci. Technol. 47:7965-7973.

Conley et al. (2009) exposed mayfly larvae (*Centroptilum triangulifer*) to dietary selenium contained in natural periphyton biofilms to eclosion. The periphyton fed to the mayfly larvae were exposed to dissolved selenite (radiolabeled ^{75}Se) in November 2008 (12.6 and 13.9 µg/L) and in January 2009 (2.4, 2.4, 4.9, 10.3, and 10.7 µg/L). Periphyton bioconcentrated Se an average of 1113-fold over the different aqueous Se concentrations (Table E-22). Twenty 4 to 6-day old mayfly larvae were exposed for 4.5 to 6 weeks to each of the periphyton diets until the larvae eclosed to subimagos. The subimagos were allowed to emerge to the adult imago stage which deposited their egg masses in Petri dishes. Selenium was measured in postpartum adults along with their dry weights and clutch size.

Table E-22. Selenium concentrations in water exposed to periphyton, periphyton and mayfly adults.

Treatment	Dissolved [Se] exposed to periphyton, µg/L	[Se] in periphyton, mg/kg dw	[Se] in mayfly adult, mg/kg dw
5A	2.4	2.2	4.2
5B	2.4	2.0	5.7
10A	4.9	4.4	9.7
20C	10.3	8.7	16.2
20D	10.7	11.3	27.5
20A	12.6	25.5	56.7
20B	13.9	17.5	34.8

Selenium increased in concentration from periphyton to the adult mayflies (trophic transfer factor) an average of 2.2-fold (Table E-22). The authors observed a decrease in fecundity as maternal postpartum Se concentrations increased. Fecundity was also related to growth of the mayflies. The authors observed a reduction in fecundity for this mayfly when they were fed diets containing more than 11 mg Se/kg dw. This threshold is considered the effect value for this study. Using the trophic transfer factor of 2.2, the periphyton Se concentration of 11 mg/kg dw translates to an adult mayfly Se concentration of 24.2 mg/kg dw.

Conley et al. (2011) exposed larval *C. triangulifer* similar to Conley et al. (2009) to two different rations of periphyton (1x and 2x) to evaluate the effect of feeding ration on the bioaccumulation and life cycle performance of the mayfly. Periphyton (on plates) was initially exposed to low (1.1 to 3.4 µg/L), medium (5.9 – 8.9 µg/L) and high (19.2 – 23.1 µg/L) selenite. Fifteen 1-2 day-old mayfly larvae were then fed either 1 plate (1x ration) or 2 plates (2x ration) in bottles containing 1.8 L water to eclosion to subimagos (25-29 days). Subimagos were induced to emerge to adults in petri dishes and their clutch size measured through digital imaging. Selenium measurements from this study are given in Table E-23.

Table E-23. Selenium concentrations in water, periphyton and mayfly tissues for two feeding rations.
(Adapted from Table 1 in Conley et al. 2011)

Feeding ration – Se level	Mean dissolved Se exposed to periphyton, µg/L	Mean periphyton, mg Se/kg dw	Mean mayfly tissue, mg Se/kg dw
1x – low	1.1	4.2 ± 0.6 (4)	12.8 ± 3.6 (28)
1x – medium	5.9	11.9 ± 2.1 (4)	31.7 ± 7.5 (15)
1x - high	21.4	27.2 ± 4.2 (4)	68.4 ± 24.0 (9)
2x – low	2.7/3.4[a]	9.5 ± 0.9 (3)	14.1 ± 3.8 (19)
2x – medium	7.1/8.9[a]	19.9 ± 1.6 (3)	21.6 ± 2.8 (22)
2x - high	19.2/23.1[a]	40.9 ± 1.7 (3)	37.3 ± 6.7 (13)

[a] Two values represent two different loading exposures, September and October. The plates were combined for mayfly exposure.

Mayflies fed the 1x ration had 54% and 72% reductions in survival relative to controls in the medium and high Se treatment levels, respectively, both significant ($p<0.05$). The mayflies fed the 1x ration also had significant reductions in fecundity in the low (44% reduction), medium (63% reduction) and high (77% reduction) Se treatment levels. However, for the mayflies fed the 2x ration, there were no significant differences between the controls and any of the three Se treatment levels for any of the endpoints measured including survival and fecundity. The 2x ration mayflies had 60% more biomass than the 1x ration mayflies. This growth difference explains why the 1x ration mayflies had higher concentrations of Se in their tissues. The two different rations resulted in vastly different effect levels for Se, <12.8 mg/kg dw in the 1x ration test and >37.3 mg/kg dw in the 2x ration. It is apparent from this study that if the mayflies do not obtain sufficient nutrition, they are more sensitive to selenium. Although reduced feeding levels occur in nature, it is a confounding variable in this study that cannot be used to set a chronic effect level for selenium.

Conley et al. (2013) evaluated the accumulation of selenite and selenate into periphyton with subsequent feeding exposure to mayfly larvae. As in his previous studies, *C. triangulifer* larvae were fed periphyton previously exposed to different concentrations of selenium. In this study, periphyton plates were first exposed to low (10 µg/L) and high (30 µg/L) concentrations of either selenite or selenate and then fed to mayfly larvae to ecolsion to subimagos. The selenite and selenate treatment exposures resulted in similar levels of selenium in the subimagos. Since no differences in selenium accumulation was observed, the selenite and selenate treatments could be pooled for measuring the endpoints, survival and secondary production (total mayfly biomass produced). Mean selenium concentrations fed the mayflies were 2.2, 12.8 and 37 mg/kg Se dw in the control, low and high treatments, respectively. Mayfly tissue (subimago) concentrations (extrapolated from Figure 4a in Conley et al. 2013) were approximately 4-7, 20-35, and 45-75 mg/kg Se dw, in the control, low and high treatments, respectively. The authors reported significant reductions in survival from the control in the high Se treatment (both pooled data and individual selenite and selenate treatments) but no significant differences were observed in the low Se treatments. Secondary production was significantly reduced relative to the control in the high Se treatment for both selenium species. For the low Se exposure treatment, secondary production was not significantly different than the control for the selenite treated periphyton exposure, but was for the selenate and pooled data suggesting an effect level between 20 and 35 mg/kg Se dw. These results as well as those observed in 2x ration exposures in Conley et al. (2011) where no effects were observed at 37.3 mg/kg Se dw generally support the chronic value determined for Conley et al. (2009) of 24.2 mg/kg Se dw.

The following invertebrate studies were inconclusive for establishing species sensitivity because of limitations in the experimental designs, as explained for each.

5.4 Aquatic Insect (Midge: Chironimids)

Malchow et al. (1995) fed fourth instar *Chironomus decorus* midge larvae a diet of seleniferous algae under laboratory conditions for 96 hours. For algae cultured with selenite, a larval tissue concentration of 4.05 µg Se/g dry weight resulted in a 46% reduction in growth relative to the controls. At a larval tissue concentration of 8.6 µg Se/g dry weight, larval growth was reduced by only 39%. Since the study only reported two exposure concentrations, it is unclear if the tissue effect concentration at 4.05 µg Se/g dry weight is real or an anomaly. Additional exposure concentrations and subsequent effect levels are needed to resolve this issue.

Malchow et al. (1995) also fed fourth instar *Chrionomus decorus* midge larvae a diet of algae cultured with selenate, and the midge larvae were exposed under laboratory conditions for 96 hours. A dietary exposure of 2.11 µg Se/g dry weight significantly reduced larval growth (15% reduction) at tissue concentrations of 2.55 µg Se/g dry weight. At a larval tissue concentration of 6.62 µg Se/g dry weight, growth was reduced 20% relative to the controls. The 15-20% reduced growth at larval tissue concentrations 2.55 µg Se/g dry weight may be statistically significant, but not biologically meaningful. In addition, exposure to only two selenium concentrations precludes confirmation of a dose-response.

Alaimo et al. (1994) also exposed *2010* midge larvae to selenite diet, but the selenium source was from field contaminated widgeongrass (*Ruppia maritima*). *Ruppia* stems and leaves were collected from four selenium contaminated evaporation ponds located in the San Joaquin Valley of California. Three-day old larvae were exposed to each of the four treatment diets (*Ruppia* from each pond) plus a Cerophyll control for 14 days (egg to pupation), with the moderately hard reconstituted water renewed at day 7 and every three days thereafter. The growth (weight) of exposed larvae was significantly reduced in all of the selenium treatments when compared to the controls. The lowest effect level was observed for the Westlake pond (primarily selenite), where growth was reduced 40 percent relative to the controls at a larval tissue concentration below the detection level (1.0 ppm dry weight, or 1.0 µg Se/g dry weight). These results are suspect because the field collected *Ruppia* likely contained contaminants other than selenium, the control organisms were fed a different diet (Cerophyll), and the single concentration exposure is difficult to defend.

6.0 OTHER DATA – FIELD STUDY WEST VIRGINIA IMPOUNDMENTS

In response to the USEPA (2004) draft whole fish tissue criterion for selenium, the West Virginia Department of Environmental Protection (2010) initiated a study to assess selenium bioaccumulation among fishes residing in the State's lakes and streams. A focus of the study was the collection and evaluation of bluegill, *Lepomis macrochirus*, larvae (ichthyoplankton) from selected waterbodies since

2007, based on concerns regarding fish population health at locations subjected to elevated selenium inputs, particularly during the more sensitive developmental life stages of fishes (e.g. yolk-sac larvae). Also, in 2009, WVDEP began acquiring data about selenium concentrations within fish eggs of various species within reference and selenium-impacted waters. WVDEP also conducted deformity surveys of adult fishes in selenium enriched waters as well as at reference locations in 2008-2009.

WVDEP scientists found that larval deformity rates were variable throughout the study duration but were nonetheless correlated with waterborne selenium exposure. Reference locations produced age-based larval bluegill subsamples (24-168 hours) with low deformity rates (0 - 1.27%); whereas, locations with seleniferous inputs exhibited bluegill deformity rates ranging from 0% to 47.56% in developmental stages up to 312 hours. Maximum deformity rates among staged bluegill subsamples as determined through these evaluations were 19.28%, representing specimens collected from selenium-enriched waters. Concentrations of selenium within fish eggs also varied according to study location and ranged from <0.8 mg/kg dry weight among bluegill eggs at the control site to 64.62 mg/kg dry weight among largemouth bass, *Micropterus salmoides,* eggs collected from selenium-enriched waters. Searches for more mature, yet developmentally-deformed fishes revealed increased deformity rates (14%) among largemouth bass residing in a selenium impacted reservoir as compared to deformity rates among largemouth bass found in the reference lake (0%). The data on egg selenium concentrations are not adequate for constructing a concentration-response curve. Nevertheless, the overall deformity rate in the contaminated Upper Mud River Reservoir was 5% among 10,000 individual fish, average egg selenium concentration 9.8 mg/kg dw. The overall deformity rate in the reference Plum Orchard Lake was 0.5% among 13,000 individuals, average egg selenium concentration nondetectable or <0.8 mg/kg dw.

7.0 OTHER DATA - NUTRITIONAL DEFICIENCY/SUFFICIENCY STUDIES CONTAINING MEASURED SELENIUM IN THE DIET AND WHOLE BODY FISH TISSUE

Ingested dietary dose studies in fish designed to identify nutritionally deficient and/or nutritionally sufficient selenium doses in fish food or prey primarily describe selenium effects on growth, with survival reductions and effects on antioxidant enzyme activity also occasionally reported. A number of the dietary studies have measured a range of dietary doses that maximize fish growth, as opposed to a single dietary dose associated with nutritional sufficiency for growth. Regardless of whether nutritionally sufficient dietary doses are reported as a single concentration or as a range of concentrations, reduced growth or survival is observed at both lower dietary doses (nutritional deficiency) and at higher dietary doses (toxicity).

Although dietary doses are normally presented as selenium concentrations in food, expressed in terms of mg/kg Se in the diet, several studies have also concurrently presented nutritionally deficient/sufficient Se levels in terms of the whole body Se concentration in the fish. These studies permit a comparison of nutritionally deficient/sufficient whole body Se residues in fish to the national criterion for Se in whole bodies of fish. When combined with measured whole body fish tissue residues associated with toxicity, a complete picture of the range of Se residues in whole body fish tissue associated with nutritional deficiency, nutritional sufficiency and toxicity emerges.

Eight fish species have information on both nutritionally deficient dietary doses and whole body concentrations of selenium measured in the same study (Table E-24). Six of the eight species are native to North America. Nutritionally deficient dietary doses of Se range between 0.03 mg/kg dw in Atlantic salmon (*Salmo salar*, Poston et al. 1976) associated with reduced survival to 1.4 mg/kg dw in Atlantic cod (*Gadus morhua*, Hamre et al. 2008), also associated with reduced survival. Whole body Se residues identified as nutritionally deficient range between 0.64 mg/kg dw in Malabar grouper (*Epinephelus malabaricus*) associated with suboptimal weight gain and feed efficiency (Lin and Shiau 2005) and 4.72 mg/kg dw in North African catfish (*Clarias gariepinus*), also associated with suboptimal weight gain (Abdel-Tawwab et al. 2007). The whole body Se residues associated with growth and/or survival reductions due to nutritional deficiency of the six North American species (Prussian carp, Han et al. 2011; common carp, Gaber 2007; Atlantic cod, Hamre et al. 2008; Coho salmon, Felton et al. 1990; cobia, Lin et al. 2010; Atlantic salmon, Poston et al. 1976) all range between 1.0 and 2.7 mg/kg dw.

Ten fish species have information on both nutritionally sufficient dietary doses and whole body concentrations of selenium measured in the same study (Table D-23). Eight of the 10 species are native to North America. Nutritionally sufficient dietary doses of Se for the North American resident species, all but one of which are based on maximum growth of fish, range between 0.1 mg/kg dw in hybrid striped

bass (Jaramillo 2006) and 6.6 mg/kg dw in rainbow trout (Hilton and Hodson 1983). Several studies have identified a range of dietary doses and associated whole body residues that maximize growth and survival relative to that of fish fed lower dietary doses and which subsequently contain lower whole body selenium residues. Whole body Se residues associated with nutritional sufficiency based on maximal growth and/or survival of all North American species except for hybrid striped bass (Jaramillo 2006) range between 0.2 – 3.63 mg/kg dw (Table D-23). For hybrid striped bass, Jaramillo (2006) observed that maximum weight gain occurred in selenite supplemented diets containing 1.19 mg/kg dw Se, which resulted in whole body Se residues of 5.13 mg/kg dw. Jaramillo (2006) also exposed hybrid striped bass to seleno-DL-methionine supplemented diets containing 0.90 mg/kg dw, which resulted in the maximum weight gain of all seleno-DL-methionine supplemented diets tested, and a whole body Se residue of 7.2 mg/kg dw.

The nutritional sufficiency study of Rider et al. (2009) with rainbow trout is unique in that it determined dietary and whole body selenium requirements for both stressed and unstressed fish. Rider et al. (2009) observed that rainbow trout stressed by a combination of low water levels in holding tanks and twice daily handling of fish by 30 second aerial exposure in dip nets resulted in a higher nutritional requirement for selenium than was observed in fish not subjected to the stress routine. They concluded that trout exposed to physical stressors could benefit from an additional 0.3 – 2.0 mg/kg dw additional selenium supplementation over and above the Se content of nutritionally Se sufficient diets for fish not undergoing stress.

The fish with the highest known nutritional requirement for selenium is the non-North American resident North African catfish (*Clarias gariepinus*). Abdel-Tawwab et al. (2007) determined in a 12 week study with fingerlings that Se dietary doses of 1.04 mg/kg dw and 3.67 mg/kg dw were associated with suboptimal and maximum weight gains of the catfish, respectively. Catfish survival was 100% in both the Se-deficient and Se-sufficient dietary dose exposures during the 12 week study period. The respective whole body selenium tissue residues at the end of the 12 week study were 4.72 mg/kg dw in the Se-deficient fish and 15.43 mg/kg dw in the fish fed the nutritionally sufficient Se diet. North African catfish (Abdel-Tawwab et al. 2007) is the only known fish species with an identified whole body nutritional requirement for Se higher than the national aquatic life criterion for whole body Se in fish.

Table E-24. Studies with both empirically measured selenium dietary doses and whole body residues associated with nutritional deficiency and sufficiency in fish.

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.21	Basal diet	0.64	Deficiency	Suboptimal weight gain and feed efficiency	Lin and Shiau 2005
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	0.47	Seleno-methionine	1.0	Deficiency	Suboptimal growth, feeding rate and feed conversion rate	Han et al. 2011
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.04	Basal diet	1.04	Deficiency	Reduced growth and survival	Gaber 2007
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	1.4	Basal diet	1.1	Deficiency	Larval survival 32% lower compared to larvae fed selenium-enriched diet	Hamre et al. 2008
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.21 - 0.62	0.21 = Basal diet, 0.62 = seleno-DL-methionine	1.13 - 2.11	Deficiency	Statistically significantly reduced specific growth rate and survival	Liu et al. 2010
Coho salmon (*Oncorhynchus kisutch*)	Smolt 22.7 g	Hatchery reared	0.7 - 0.9	Not given	1.974	Deficiency	Survival of hatchery reared smolts 1.5 - 2.0x lower than wild smolts	Felton et al. 1990
Atlantic salmon (*Salmo salar*)	Fry 0.1 g	4 weeks	0.03 - 0.04	Basal diet	2.7	Deficiency	Decreased survival relative to fry fed diet supplemented with 0.1 µg/g Se and 0.5 IU/g vitamin E	Poston et al. 1976
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	1.04	Organic Se	4.72	Deficiency	Suboptimal weight gain and specific growth rate	Abdel-Tawwab et al. 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 0.6 g	16 weeks	0.6 - 6.6	Selenite $Na_2SeO_3 \cdot 5H_2O$	0.2 - 1.0	Sufficiency	No deficiency or toxicity signs on growth	Hilton and Hodson 1983

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	1.2	Basal diet	0.58 - 0.70	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	0.77	Basal diet	0.9	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.77	Seleno-methionine	0.92	Sufficiency	Maximal weight gain and feed efficiency	Lin and Shiau 2005
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.4	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.13	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.24 - 0.32	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.23 - 1.29	Sufficiency	Maximal growth and survival	Gaber 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.3 - 3.9	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.6 - 2.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	1.23 - 2.77	Seleno-methionine	1.7 - 3.4	Sufficiency	Maximal growth, no effect on survival, no increase in oxidative stress	Han et al. 2011
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	0.10	Basal diet	2.01	Sufficiency	Minimum dietary requirement for acceptable survival and growth	Jaramillo 2006
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.1	Seleno-methionine	2.06	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.85 - 1.36	Seleno-DL-methionine	2.58 - 2.62	Sufficiency	Maximal and statistically identical specific growth rate and survival	Liu et al. 2010

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.4 - 4.1	Organic Se - yeast	2.8 - 4.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	4.8	Selenite $Na_2SeO_3 \cdot 5H_2O$	3.5	Sufficiency	Larval survival increased 32%, growth essentially unchanged relative to survival of larvae fed basal diet	Hamre et al. 2008
Coho salmon (*Oncorhynchus kisutch*)	Smolt 14.28 g	Wild smolts	Se in natural diet unknown	Unknown	3.63	Sufficiency	Survival of wild smolts 1.5 - 2.0x higher than hatchery reared smolts	Felton et al. 1990
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	1.19	Selenite $Na_2SeO_3 \cdot 5H_2O$	5.13	Sufficiency	Highest weight gain of any selenite diet test, significantly higher than basal diet weight gain	Jaramillo 2006
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.92 g	12 weeks	0.90	Seleno-DL-methionine	7.2	Sufficiency	Highest survival and weight gain of any seleno-DL-methionine diet tested	Jaramillo 2006
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	3.67	Organic Se	15.43	Sufficiency	Maximal weight gain, specific growth rate and survival	Abdel-Tawwab et al. 2007

APPENDIX F: TOXICITY OF SELENIUM TO AQUATIC PLANTS

1.0 SELENITE

Data are available on the toxicity of selenite to 13 species of freshwater algae and plants (Table F-1). Results ranged from an LC_{50} of 70,000 µg/L for the green alga, *Chlorella ellipsoidea* (Shabana and El-Attar 1995) to 522 µg/L for incipient inhibition of the green alga, *Scenedesmus quadricauda* (Bringmann and Kuhn 1977a, 1978a,b, 1979, 1980b). Foe and Knight (Manuscript) found that 75 µg/L decreased the dry weight of *Selenastrum capricornutum* (Table F-1). Wehr and Brown (1985) reported that 320 µg/L increased the growth of the alga *Chrysochromulina breviturrita*.

The 96-hr EC_{50} for the saltwater diatom, *Skeletonema costatum*, is 7,930 µg/L, based on reduction in chlorophyll *a* (Table F-1). Growth of *Chlorella* sp., *Platymonas subcordiformis*, and *Fucus spiralis* increased at selenite concentrations from 2.6 to 10,000 µg/L (Table F-1). Other marine algae exposed to selenite from 14 to 60 days had no observed effect concentrations (NOAEC) that ranged from 1,076 to 107,606 µg/L. These data suggest that saltwater plants will not be adversely affected by concentrations of selenite that do not affect saltwater animals.

2.0 SELENATE

Growth of several species of green algae was affected by concentrations ranging from 100 to 40,000 µg/L (Table F-1). Blue-green algae appear to be more tolerant to selenate with 1,866 µg/L being the lowest concentration reported to affect growth (Kiffney and Knight 1990). Kumar (1964) found that a blue-green alga developed and lost resistance to selenate. The difference in the sensitivities of green and blue-green algae to selenate might be of ecological significance, particularly in bodies of water susceptible to nuisance algal blooms. For example, Patrick et al. (1975) reported that a concentration of 1,000 µg/L caused a natural assemblage of algae to shift to a community dominated by blue-green algae.

The saltwater coccolithophore, *Cricosphaera elongata*, had reduced growth when exposed to 41,800 µg/L selenate for 14 days (Boisson et al. 1995). Seven other saltwater algal species investigated by Wong and Oliveira (1991a) exhibited NOEC growth values that ranged from 1,043 to 104,328 µg/L. At 10,000 µg/L, selenate is lethal to four species of saltwater phytoplankton and lower concentrations increase or decrease growth (Table F-1). Wheeler et al. (1982) reported that concentrations as low as 10 µg/L reduced growth of *Porphyridium cruentum* (Table F-1).

Although selenite appears to be more acutely toxic than selenate to most aquatic animals, this does not seem to be true for aquatic plants. Selenite and selenate are about equally toxic to the freshwater algae *Anabaena cylindrica*, *Anabaena flos-aquae*, *Anabaena variabilis*, *Anacystis nidulans*, and *Scenedesmus dimorphus* (Kiffney and Knight 1990; Kumar and Prakash 1971; Moede et al. 1980) and the saltwater algae *Agemenellum quadroplicatum*, *Chaetoceros vixvisibilis* and *Amphidinium carterae* (Wong and Oliveira 1991a). The two oxidation states equally stimulated growth of *Chrysochromulina*

breviturrita (Wehr and Brown 1985). On the other hand, selenate is more toxic than selenite to the freshwater *Selenastrum capricornutum* (Richter 1982; Ibrahim and Spacie 1990) and the saltwater *Chorella* sp., *Platymonas subcordiformis* and *Nannochloropsis oculata* (Wheeler et al. 1982; Wong and Oliveira 1991a). In addition, Fries (1982) found that growth of thalli of the brown macroalga, *Fucus spiralis*, was stimulated more by exposure to selenite at 2.605 μg/L than to the same concentration of selenate.

A Final Plant Value, as defined in the Guidelines, cannot be obtained because no test in which the concentrations of selenite or selenate were measured and the endpoint was biologically relevant has been conducted with an important aquatic plant species.

Table F-1. Toxicity of selenium to aquatic plants.

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Chlorella vulgaris*	Sodium selenite	-	90-120	Reduced growth	5,480	De Jong 1965
Green alga, *Chlorella ellipsoidea*	Sodium selenite	-	7	EC50	70,000	Shabana and El-Attar 1995
Green alga, *Scenedesmus dimorphus*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	522	Bringmann and Kuhn 1977a; 1978a,b; 1979; 1980b
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	2,500	Bringmann and Kuhn 1959a
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	4	EC50	2,900	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	6	EC50	65,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena constricta*	Sodium selenite	-	7	EC50	67,000	Shabana and El-Attar 1995
Blue-green alga, *Anabaena cylindrica*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenite	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anabaena variabilis*	Sodium selenite	-	6-18	LC50	15,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anacystis nidulans*	Sodium selenite	-	10-18	LC50	30,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcystis aeruginisa*	Sodium selenite	-	8	Incipient inhibition	9,400 (9,300)	Bringmann and Kuhn 1976; 1978a,b
Alga, *Euglena gracilis*	-	-	15	Reduced growth	5,920	Bariaud and Mestre 1984
Duckweed, *Lemna minor*	-	-	4	EC50	2,400	Wang 1986
Duckweed, *Lemna minor*	Sodium selenite	-	14	EC50 (mult. rate)	3,500	Jenner and Janssen-Mommen 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Duckweed, *Lemna minor*	Sodium selenite	-	14	NOEC (mult. rate)	800	Jenner and Janssen-Mommen 1993
Selenium (VI)						
Green alga, *Ankistrodesmus falcatus*	Sodium selenate	-	14	Did not reduce growth	10	Vocke et al. 1980
Green alga, *Scenedesmus dimorphus*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Green alga, *Scenedesmus obliquus*	Sodium selenate	-	14	Reduced growth	100	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	14	Reduced growth	300	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	4	EC50	199	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	6	EC50	<40,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena cylindrica*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenate	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anacystis nidulans*	Sodium selenate	-	6-18	EC50	39,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anabaena viriabilis*	Sodium selenate	-	10-18	EC50	17,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcoleus vaginatus*	Sodium selenate	-	14	Reduced growth	10,000	Vocke et al. 1980
Duckweed, *Lemna minor*	Sodium selenate	-	14	EC50 (mult. rate)	11,500	Jenner and Janssen-Mommen 1993
Duckweed, *Lemna minor*	Sodium selenate	-	14	NOEC (mult. rate)	>2,400	Jenner and Janssen-Mommen 1993

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Green alga, *Dunaliella tertiolecta*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Diatom, *Skeletonema costatum*	Selenious acid[c]	-	4	EC50 (reduction in chlorophyll a)	7,930	U.S. EPA 1978
Coccolithophore, *Cricosphaera elongata*	Sodium selenite	-	14	Reduced growth	4,570	Boisson et al. 1995
Dinoflagellate, *Amphidinium carterae*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Dinoflagellate, *Peridinopsis borgei*	Selenium oxide	-	70-75	Maximum growth	0.01-0.05	Lindstrom 1985
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenite	-	60	NOEC growth	107,606	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodiun selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Selenium (VI)						
Green alga, *Dunaliella tertiolecta*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenate	-	60	NOEC growth	1,043	Wong and Oliveira 1991a
Coccolithophore, *Cricosphaera elongate*	Sodium selenate	-	14	Reduced growth	41,800	Boisson et al. 1995
Dinoflagellate,	Sodium	-	60	NOEC	10,433	Wong and

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Amphidinium carterae	selenate			growth		Oliveira 1991a
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a

[a] Concentration of selenium, not the chemical.
[b] Estimated from published graph.
[c] Reported by Barrows et al. (1980) in work performed under the same contract.

APPENDIX G: UNUSED DATA

Based on the requirements set forth in the guidelines (Stephen et al. 1985) the following studies are not acceptable for the following reasons and are classified as unused data. Note the acceptance of chronic toxicity data included diet and field exposures where selenium was the dominant toxicant.

Studies Were Conducted with Species That Are Not Resident in North America

Ahsanullah and Brand (1985)
Ahsanullah and Palmer (1980)
Baker and Davies (1997)
Barghigiani et al. (1993)
Chidambaram and Sastry (1991a,b)
Congiu et al. (1989)
Cuvin and Furness (1988)
Fowler and Benayoun (1976a,b)
Gaikwad (1989)
Gotsis (1982)
Hiraika et al. (1985)
Juhnke and Ludemann (1978)
Kitamura (1990)
Manoharan and Prabakaran (1994)
Minganti et al. (1994, 1995)
Niimi and LaHam (1975, 1976)
Regoli (1998)
Regoli and Principato (1995)
Rhodes et al. (1994)
Ringdal and Julshamn (1985)
Rouleau et al. (1992)
Sastry and Shukla (1994)
Savant and Nilkanth (1991)
Shultz and Ito (1979)
Srivastava and Tyagi (1985)
Takayanagi (2001)
Tomasik et al. (1995b)
Tian and Liu (1993)
Wrench (1978)

Deelstra et al. (1989), Forsythe and Klaine (1994), Okasako and Siegel (1980) and Petrucci et al. (1995) conducted tests with brine shrimp species that are too atypical to be used in derving national criteria.

These Studies or Reviews Contain Relevant Data That Have Been Published Elsewhere

Adams and Johnson (1981)
Biddinger and Gloss (1984)
Bowie et al. (1996)
Brandao et al. (1992)
Brooks (1984)
Burton and Stemmer (1988)
Chapman et al. (1986)
Davies (1978)
Debruyn and Chapman (2007)
Devillers et al. (1988)
Eisler (1985)
Hall and Burton (1982)
Hodson and Hilton (1983)
Hodson et al. (1984)
Jenkins (1980)
Kaiser et al. (1997)
Kay (1984)
LeBlanc (1984)
Lemly (1993c, 1996ab, 1997d)
Lemly and Smith (1987)
McKee and Wolf (1963)
National Research Council (1976) Neuhold (1987)
NCDNR&CD (1986)
Peterson and Nebeker (1992)
Phillips and Russo (1978)
Presser (1994)
Roux et al. (1996)
Swift (2002)
Thompson et al. (1972)
Versar (1975)

Authors Did Not Specify the Oxidation State of Selenium Used in Study

Greenberg and Kopec (1986)
Hutchinson and Stokes (1975)
Kapu and Schaeffer (1991)
Kramer et al. (1989)
Mahan et al. (1989)
Rauscher (1988)
Snell et al. (1991b)

Not Useful Because of No Effects Observed at Exposure Concentrations or Insufficient Number of Treatments

Muscatello and Janz (2009)
Pyle et al. (2005)
Schlenk et al (2003)

Chronic Study with no Dietary Exposure

Hopkins et al. (2002)
Oti (2005)
Rowe (2003)
Teh et al. (2002)

Selenium Was a Component of an Effluent, Fly Ash, Formulation, Mixture, Sediment or Sludge

Apte et al. (1987)
Baer et al. (1995)
Baker et al. (1991)
Berg et al. (1995)
Besser et al. (1989)
Biedlingmaier and Schmidt (1989)
Bjoernberg (1989)
Bjoernberg et al. (1988)
Bleckmann et al. (1995)
Boisson et al. (1989)
Bondavalli et al. (1996)
Bowmer et al. (1994)
Brieger et al. (1992)
Burton and Pinkney (1984)
Burton et al. (1983, 1987a)
Cherry et al. (1987)
Cieminski and Flake (1995)
Clark et al. (1989)
Cooke and Lee (1993)
Cossu et al. (1997)
Coyle et al. (1993)
Crane et al. (1992)
Crock et al. (1992)
Cushman et al. (1977)
Davies and Russell (1988)
de Peyster et al. (1993)
Dickman and Rygiel (1996)
Dierenfeld et al. (1993)
Doebel et al. (2004)
Drndarski et al. (1990)
Eriksson and Forsberg (1992)
Eriksson and Pedros-Alio (1990)
Fairbrother et al. (1994)
Fava et al. (1985a,b)
Feroci et al. (1997)
Finger and Bulak (1988)
Finley (1985)
Fisher and Wente (1993)
Fjeld and Rognerud (1993)
Fletcher et al. (1994)
Follett (1991)
Gerhardt (1990)
Gerhardt et al. (1991)
Gibbs and Miskiewicz (1995)
Graham et al. (1992)

Gunderson et al. (1997)
Hall (1988)
Hall et al. (1984, 1987, 1988,1992)
Hamilton et al. (1986, 2000)
Harrison et al. (1990)
Hartwell et al. (1987ab, 1988, 1997)
Hatcher et al. (1992)
Haynes et al. (1997)
Hayward et al. (1996)
Hellou et al. (1996b)
Henebry and Ross (1989)
Henny et al. (1989, 1990, 1995)
Hildebrand et al. (1976)
Hjeltnes and Julshman (1992)
Hockett and Mount (1996)
Hodson (1990)
Hoffman et al. (1988, 1991)
Homziak et al. (1993)
Hopkins et al. (2000)
Hopkins et al. (2004)
Hothem and Welsh (1994a)
Jackson (1988)
Jackson et al. (1990)
Jacquez et al. (1987)
Jay and Muncy (1979)
Jayasekera (1994)
Jayasekera and Rossbach (1996)
Jenner and Bowmer (1990) (1992)
Jin et al. (1997)
Jorgensen and Heisinger (1987)
Karlson and Frankenberger (1990)
Kemble et al. (1994)
Kennedy (1986)
Kersten et al. (1991)
King and Cromartie (1986)
King et al. (1991, 1994)
Klusek et al. (1993)
Koh and Harper (1988)
Koike et al. (1993)
Krishnaja et al. (1987)
Kruuk and Conroy (1991)
Kuehl and Haebler (1995)
Kuehl et al. (1994)
Kuss et al. (1995)
Landau et al. (1985)
Livingston et al. (1991)
Lobel et al. (1990)
Luoma and Phillips (1988)
Lundquist et al. (1994)
Lyle (1986)
MacFarlane et al. (1986)
Mann and Fyfe (1988)
Marcogliese et al. (1987)
Marvin and Howell. (1997)Maurer et al (1999)
McCloskey and Newman (1995)
McCloskey et al. (1995)
McCrea and Fischer (1986)
McLean et al. (1991)
Mehrle et al. (1987)
Metcalf-Smith (1994)
Micallef and Tyler (1989)
Mikac et al. (1985)
Miles and Tome (1997)
Miller et al. (1996)
Misitano and Schiewe (1990)
Moore (1988)
Munawar and Legner (1993)
Muskett et al. (1985)
Naddy et al. (1995)
Nielsen and Bjerregaard (1991)
Norman et al. (1992)
Nuutinen & Kukkonen (1998)
Oberbach and Hartfiel (1987, 1988)
Oberbach et al. (1989)
Ohlendorf et al. (1989, 1990, 1991)
Olson and Welsh (1993)
Peters et al.(1999)
Phillips and Gregory (1980)
Pratt and Bowers (1990)
Presser and Ohlendorf (1987)
Prevot and Sayer-Gobillard (1986)
Pritchard (1997)
Pyle et al. (2001)
Reash et al. (1988, in press)
Rhodes and Burke (1996)

Ribeyre et al. (1995)
Rice et al. (1995)
Riggs and Esch (1987)
Riggs et al. (1987)
Robertson et al. (1991)
Roper et al. (1997)
Rowe et al. (1996)
Russell et al. (1994)
Ryther et al. (1979)
Saiki and Jenings (1992)
Saiki and Ogle (1995)
Saleh et al. (1988)
Seelye et al. (1982)
Sevareid and Ichikawa (1983)
Skinner (1985)
Somerville et al. (1987)
Sorenson and Bauer (1983)
Specht et al. (1984)
Steele et al. (1992)
Stemmer et al. (1990)
Summers et al. (1995)
Thomas et al. (1980b)
Timothy et al. (2001)
Trieff et al. (1995)
Turgeon and O=Conner (1991)
Twerdok et al. (1997)
Unsal (1987)
Van Metre and Gray (1992)
Wahl et al. (1994)
Wandan and Zabik (1996)
Wang et al. (1992, 1995)
Welsh (1992)
Weres et al. (1990)
White and Geitner (1996)
Wiemeyer et al. (1986)
Wildhaber and Schmitt (1996)
Williams et al. (1989)
Wolfe et al. (1996)
Wolfenberger (1987)
Wong and Chau (1988)
Wong et al. (1982)
Wu et al. (1997)
Yamaoka et al. (1994)
Zagatto et al. (1987)
Zaidi et al. (1995)
Zhang et al. (1996)

Exposed enzymes, excised tissue or tissue extractor

Tripathi and Pandey (1985) and Heinz (1993b) used test organisms that had been previously exposed to pollutants in food or water.

Albers et al. (1996)
Al-Sabti (1994, 1995)
Arvy et al. (1995)
Augier et al. (1993a, b)
Avery et al. (1996)
Baatrup (1989)
Baatrup and Dansher (1987)
Baatrup et al. (1986)
Babich et al. (1986, 1989)
Barrington et al. (1997)
Becker et al. (1995a,b)
Bell et al. (1984, 1985, 1986a,b, 1987ab)
Berges and Harrison (1995)
Blondin et al. (1988)
Boisson et al. (1996)
Bottino et al. (1984)
Braddon (1982)
Braddon-Galloway and Balthrop (1985)
Bradford et al. (1994a,b)
Brandt et al. (1990)
Byl et al. (1994)
Chandy and Patel (1985)
Chen et al. (1997)
Cheng et al. (1993)
Christensen and Tucker (1976)
Dabbert and Powell (1993)
DeQuiroga et al. (1989)
Dierickx (1993)
Dietrich et al. (1987)
DiIlio et al. (1986)

Doyotte et al. (1997)
Drotar et al. (1987)
Dubois and Callard (1993)
Ebringer et al. (1996)
Engberg and Borsting (1994)
Engberg et al. (1993)
Eun et al. (1993)
Foltinova and Gajdosova (1993)
Foltinova et al. (1994)
Freeman and Sanglang (1977)
Grubor-Lajsic et al. (1995)
Hait and Sinha (1987)
Hanson (1997)
Heisinger and Scott (1985)
Heisinger and Wail (1989)
Henderson et al. (1987)
Henny and Bennett (1990)
Hoffman and Heinz (1988, 1998)
Hoffman et al. (1989, 1998)
Hoglund (1991)
Hontela et al. (1995)
Hsu et al. (1995)
Hsu and Goetz (1992)
Ishikawa et al. (1987)
James et al. (1993)
Jovanovic et al. (1995, 1997)
Kai et al. (1995)
Kedziroski et al. (1996)
Kelly et al. (1987)
Kralj and Stunja (1994)
Lalitha and Rani (1995)
Lan et al. (1995)
Lemaire et al. (1993)
Livingstone et al. (1992)
Low and Sin (1995, 1996)
Micallef and Tyler (1990)
Montagnese et al. (1993)
Murata et al. (1996)
Nakonieczny (1993)
Neuhierl and Boeck (1996)
Nigro (1994)
Nigro et al (1992)
Norheim and Borch-Iohnsen (1990)
Norheim et al. (1991)
O=Brien et al. (1995)
Olson and Christensen (1980)
Overbaugh and Fall (1985)
Palmisano et al. (1995)
Patel et al. (1990)
Patel and Chandy (1987)
Perez Campo et al. (1990)
Perez-Trigo et al. (1995)
Phadnis et al. (1988)
Price and Harrison (1988)
Rady et al. (1992)
Rani and Lalitha (1996)
Regoli et al. (1997)
Schmidt et al. (1985)
Schmitt et al. (1993)
Segner et al. (1994)
Sen et al. (1995)
Shigeoka et al. (1990, 1991)
Siwicki et al. (1994)
Srivastava and Srivastava (1995)
Sun et al. (1995)
Takeda et al. (1992a,b,(1993, 1997)
Treuthardt (1992)
Vazquez et al. (1994)
Veena et al. (1997)
Wise et al. (1993a,b)
Wong and Oliveira (1991b)
Yokota et al. (1988)

Test procedures test material or results were not adequately described by Botsford (1997), Botsford et al. (1997, 1998), Bovee (1978), Gissel-Nielsen and Gissel-Nielsen (1973, 1978), Greenberg and Kopec (1986), Mauk (2001), and Nassos et al. (1980) or when the test media contained an excessive amount (>200 μg/L) of EDTA (Riedel and Sanders (1996).

Some data obtained from tests conducted with just one exposure concentration to evaluate acute or chronic toxicity were not used (e.g., Bennett 1988; Heinz and Hoffman 1998; Munawar et al. 1987; Pagano et al. 1986; Wolfenberger 1986).

Kaiser (1980) calculated the toxicities of selenium(IV) and selenium(VI) to *Daphnia magna* based on physiochemical parameters. Kumar (1964) did not include a control treatment in the toxicity tests. The daphnids were probably stressed by crowding in the tests reported be Schultz et al. (1980). Siebers and Ehlers (1979) exposed too few test organisms as did Owsley (1984) in some tests.

Selenium Concentrations Reported in Wild Aquatic Organisms Were Insufficient to Calculate BAF

Abdel-Moati and Atta (1991)
Adeloju and Young (1994)
Aguirre et al. (1994)
Akesson and Srikumar (1994)
Aksnes et al. (1983)
Allen and Wilson (1990)
Ambulkar et al. (1995)
Amiard et al. (1991, 1993)
Andersen and Depledge (1997)
Andreev and Simeonov (1992)
Angulo (1996)
Arrula et al. (1996)
Arway (1988)
Ashton (1991)
Augier et al. (1991, 1993, 1995a,b)
Augspurger et al. (1998)
Avery et al. (1996)
Badsha and Goldspink (1988)
Baines and Fisher (2001)
Baldwin and Maher (1997)
Baldwin et al. (1996)
Barghigiani (1993)
Barghigiani et al. (1991)
Baron et al. (1997)
Batley (1987)
Baumann and Gillespie (1986)
Baumann and May (1984)
Beal (1974)
Beck et al. (1997)
Beland et al. (1993)
Beliaeff et al. (1997)
Bell and Cowey (1989)
Benemariya et al. (1991)
Berry et al. (1997)
Bertram et al. (1986)
Besser et al. (1994, 1993)
Birkner (1978)
Boisson and Romeo (1996)
Bowerman et al. (1994)
Braune et a. (1991)
Brezina and Arnold (1977)
Brugmann and Hennings (1994)
Brugmann and Lange (1988)
Brumbaugh and Walther (1991)
Burger (1992, 1994, 1995, 1996, 1997a,b)
Burger and Gochfeld (1992a,b, 1993, 1995 ab, 1996, 1997)
Burger et al. (1992a,b,c,1993, 1994a,b)
Byrne and DeLeon (1986)
Byrne et al. (1985)
Cantillo et al. (1997)
Capar and Yess (1996)
Capelli et al. (1987, 1991)
Cappon (1984)
Cappon and Smith (1981) (1982a,b)
Cardellicchio (1995)
Carell et al. (1987)
Carter and Porter (1997)
Caurant et al. (1994, 1996)
Chau and Riley (1965)
Chiang et al. (1994)

Chou and Uthe (1991)
Chvojka (1988)
Chvojka et al. (1990)
Clifford and Harrison (1988)
Collins (1992)
Combs et al. (1996)
Cosson et al. (1988)
Courtney et al. (1994)
Cruwys et al. (1994)
Crutchfield (2000)
Cumbie and Van Horn (1978)
Currey et al. (1992)
Custer and Hohman (1994)
Custer and Mitchell (1991, 1993)
Custer et al. (1997)
Dabeka and McKenzie (1991)
Davoren (1986)
Deaker and Maher (1997)
Demon et al. (1988)
Dietz et al. (1995, 1996)
Doherty et al. (1993)
Elliott and Scheuhammer (1997)
Eriksson et al. (1989)
Evans et al. (1993)
Felton rt al. (1990)
Felton et al. (1994)
Fitzsimons et al. (1995)
Focardi et al. (1985, 1988)
Fowler (1986)
Fowler et al. (1975, 1985)
France (1987)
Friberg (1988)
Froslie et al. (1985, 1987)
Gabrashanske and Daskalova (1985)
Gabrashanska and Nedeva (1994)
Galgan and Frank (1995)
Garcia - Hermandez et al. (2000)
Giardina et al. (1997)
Gillespie and Baumann (1986)
Gochfeld (1997)
Goede (1985, 1991, 1993a,b)
Goede et al. (1989, 1993)
Goede and DeBruin (1984, 1985)
Goede and Wolterbeek (1993, 1994a,b)
Gras et al. (1992)
Greig and Jones (1976)
Gutenmann et al. (1988)
Gutierrez-Galindo et al. (1994)
Guven et al. (1992)
Halbrook et al. (1996)
Hall and Fisher (1985)
Hamilton and Waddell (1994)
Hamilton and Wiedmeyer (1990)
Hansen et al. (1990)
Hardiman and Pearson (1995)
Hargrave et al. (1992)
Harrison and Klaverkamp (1990)
Hasunuma et al. (1993)
Haynes et al. (1995)
Hein et al. (1994)
Heiny and Tate (1997)
Heinz (1993a)
Heinz and Fitzgerald (1993a,b)
Heit (1985)
Heit and Klusek (1985)
Heit et al. (1980, 1989)
Hellou et al. (1992a,b) (1996a,b)
Henny and Herron (1989)
Hodge et al. (1996)
Hilton et al. (1982)
Honda et al. (1986)
Hothem and Ohlendorf (1989)
Hothem and Welsh (1994b)
Hothem and Zador (1995)
Hothem et al. (1995)
Houpt et al. (1988)
Hunter et al. (1995, 1997)
Ibrahim and Farrag (1992)
Ibrahim and Mat (1995)
Ishikawa et al. (1993)
Itano et al. (1984, 1985a,b)
Jarman et al. (1996)
Johns et al. (1988)
Johnson (1987)
Jop et al. (1997)

Jorhem et al. (1994)
Julshamn et al. (1987)
Kai et al. (1986a,b, 1988, 1992a,b, 1996)
Kaiser et al. (1979)
Kalas et al. (1995)
Kidwell et al. (1995)
Koeman et al. (1973)
Kovacs et al. (1984)
Krogh and Scanes (1997)
Krushevska et al. (1996)
Lakshmanan and Stephen (1994)
Lalitha et al. (1994)
LamLeung et al. (1991)
Lan et al. (1994a,b)
Langlois and Langis (1995)
Larsen and Stuerup (1994)
Larsen et al. (1997)
Lauchli (1993)
Law et al. (1996)
Lee and Fisher (1992a,b, 1993)
Leighton and Wobeser (1994)
Leland and Scudder (1990)
Lemly (1985a, 1994)
Leonzio et al. (1986, 1989, 1992)
Leskinen et al. (1986)
Li et al. (1996)
Lie et al. (1994)
Liu et al. (1987)
Lizama et al. (1989)
Lobel et al. (1989, 1991, 1992a,b)
Lonzarich et al. (1992)
Lourdes et al. (1990)
Lowe et al. (1985)
Lucas et al. (1970)
Lytle and Lytle (1982)
Mackey et al. (1996)
Maher (1987)
Maher et al. (1992, 1997)
Mann et al. (1988)
Mason et al. (2000)
Masuzawa et al. (1988)
Matsumoto (1991)
Maven et al. (1995)
May and McKinney (1981)
Mcdowell et al. (1995)
McKenzie-Parnell et al. (1988)
Meador et al. (1993)
Mehrle et al. (1982)
Meltzer et al. (1993)
Metcalfe-Smith et al. (1992, 1996)
Michot et al. (1994)
Mills et al. (1993)
Moharram et al. (1987)
Moller (1996)
Mora and Anderson (1995)
Morera et al. (1997)
Muir et al. (1988)
Mutanen et al. (1986)
Nadkarni and Primack (1993)
Nakamoto and Hassler (1992)
Narasaki and Cao (1996)
Navarrete et al. (1990)
Nettleton et al. (1990)
Nicola et al. (1987)
Nielsen and Dietz (1990)
Norheim (1987)
Norheim et al. (1992)
Norrgren et al. (1993)
Norstrom et al. (1986)
O=Conner (1996)
O=Shea et al. (1984)
Ober et al. (1987)
Oehlenschlager (1997)
Ohlendorf (1986)
Ohlendorf and Harrison (1986)
Ohlendorf and Marois (1990)
Ohlendorf et al. (1986a,b, 1987, 1988a,b)
Okazaki and Panietz (1981)
Ostapczuk et al. (1997)
Pakkala et al. (1972)
Pal et al. (1997) Palawski et al. (1991)
Palmer-Locarnini and Presley (1995)
Paludan-Miller et al. (1993)
Papadopoulou and Andreotis (1985)
Park and Presley (1997)
Park et al. (1994)

Paveglio et al. (1994)
Payer and Runkel (1978)
Payer et al. (1976)
Pennington et al. (1982)
Presley et al. (1990)
Quevauviller et al. (1993a,b)
Ramos et al. (1992)
Rao et al. (1996)
Reinfelder and Fisher (1991)
Reinfelder et al. (1993, 1998)
Renzoni et al. (1986)
Riget et al. (1996)
Risenhoover (1989)
Roditi (2000)
Roux et al. (1994)
Ruelle and Keenlyne (1993)
Sager and Cofield (1984)
Saiki (1986 ab, 1987, 1990)
Saiki and Lowe (1987)
Saiki and May (1988)
Saiki and Palawski (1990)
Saiki et al. (1992, 1993)
Sanders and Gilmour (1994)
Scanes (1997)
Scheuhammer et al. (1988)
Schantz et al. (1997)
Schmitt and Brumbaugh (1990)
Schramel and Xu (1991)
Schuler et al. (1990)
Scott and Latshaw (1993)
Secor et al. (1993)
Seelye et al. (1982)
Sharif et al. (1993)
Shen et al. (1997)
Shirasaki et al. (1996)
Shultz and Ito (1979)
Simopoulos (1997)
Skaare et al. (1990, 1994)
Smith and Flegal (1989)
Smith et al. (1992)
Sorensen (1988)
Sorensen and Bauer (1984a,b) Sorensen and Bjerregaard (1991)
Sorensen et al. (1982, 1983, 1984)
Southworth et al. (2000)
Sparling and Lowe (1996)
Speyer (1980)
Steimle et al. (1994)
Stoeppler et al. (1988)
Stone et al. (1988)
Stripp et al. (1990)
Sundarrao et al. (1991) (1992)
Svensson et al. (1992)
Tabaka et al. (1996)
Talbot and Chang (1987)
Tallandini et al. (1996)
Tan and Marshall (1997)
Tang et al. (1997)
Tao et al. (1993)
Teherani (1987)
Teigen et al. (1993)
Thomas et al. (1999)
Tilbury et al. (1997)
Topcuoglu et al. (1990)
TranVan and Teherani (1988)
Trocine and Trefry (1996)
Uthe and Bigh (1971)
Vanderstoep et al. (1990)
Varanasi et al. (1993, 1994)
Vitaliano and Zdanowicz (1992)
Vlieg (1990)
Vlieg et al. (1993)
Vos et al. (1986)
Waddell and May (1995)
Wagemann (1988)
Wagemann and Stewart (1994)
Wagemann et al. (1988) (1996) Walsh et al. (1977)
Wang (1996)
Ward and Flick (1990)
Warren et al. (1990)
Weber (1985)
Welsh and Maughan (1994)
Wen et al. (1997)
Wenzel and Gabrielsen (1995)
Whyte and Boutillier (1991)
Williams et al. (1994)
Wilson et al. (1992, 1997)
Winger and Andreasen (1985)
Winger et al. (1984, 1990)
Woock and Summers (1984)
Wren et al. (1987)
Wu and Huang (1991)
Yamaoka et al. (1996)

Yamazaki et al. (1996)
Yoshida and Yasumoto (1987)
Zatta et al. (1985)
Zeisler et al. (1988, 1993)
Zhou and Liu (1997)

APPENDIX H: CALCULATION OF EF VALUES

EPA calculated EF values by searching its database of selenium measurements and identifying all the selenium measurements from algae, detritus, or sediment. EPA then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water measurement was available for any given particulate measurement, the median was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median ratio. The geometric mean of the algae, detritus, and sediment ratios was used as the site *EF*. Because there were at most only 3 possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric mean in order to reduce the potential for one of the values to have excessive influence on the final site *EF* value. Sites with insufficient data to fulfill these criteria are left blank.

EPA evaluated differences in bioaccumulation between different categories of aquatic systems by analyzing EF values for different categories. EPA sequentially consolidated categories and examined differences in the distribution of EF values between categories. See text for a complete description of this analysis.

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Birkner 1978	East Allen Reservoir, Medicine Bow WY	20	Reservoir	Lentic	3.00		41.00	11.09	4.80	2.31
Birkner 1978	Galett Lake, Laramie WY	7	Lake	Lentic	0.18		2.80	0.70	0.80	0.88
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	30	Pond	Lentic	15.50		47.30	27.08	15.90	1.70
Birkner 1978	Meeboer Lake, Laramie WY	3	Lake	Lentic	0.10		0.30	0.17	0.30	0.58
Birkner 1978	Miller's Lake, Wellington CO	22	Lake	Lentic	4.60		44.00	14.23	6.00	2.37
Birkner 1978	Sweitzer Lake, Delta CO	27	Lake	Lentic	10.35		6.50	8.20	9.40	0.87
Birkner 1978	Twin Buttes Reservoir, Laramie WY	23	Reservoir	Lentic	7.80		10.80	9.18	7.60	1.21

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Bowie et al. 1996	Hyco Reservoir		Reservoir	Lentic	27.00			27.00	11.50	2.35
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	N2	Reservoir	Lentic	2.65		0.60	1.26	1.00	1.26
Butler et al. 1997	Large pond on Dove Creek	DCP1	Pond	Lentic	1.00		2.10	1.45	2.00	0.72
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	MNP2	Pond	Lentic	5.40		6.70	6.01	3.00	2.00
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	MNP3	Pond	Lentic	4.50		5.90	5.15	1.00	5.15
Butler et al. 1997	Pond on Cahone Canyon, west of 1 5 Road	CHP	Pond	Lentic	4.00		2.10	2.90	5.00	0.58
Butler et al. 1997	Pond on Woods Canyon at 15 Road	WCP	Pond	Lentic	2.30		3.20	2.71	3.00	0.90
Butler et al. 1997	West pond at CC Road	PVP1	Pond	Lentic	1.50		1.40	1.45	2.00	0.72
Grasso et al. 1995	Arapahoe Wetlands Pond	17	Pond	Lentic	1.87		0.40	0.86	1.00	0.86
Lemly 1985	Badin Lake		Lake	Lentic	7.70		2.07	3.99	0.32	12.48
Lemly 1985	Belews Lake		Lake	Lentic	44.10		8.27	19.10	10.91	1.75
Lemly 1985	High Rock Lake		Lake	Lentic	6.20		1.80	3.34	0.67	4.99
Muscatello and Janz 2009	Vulture Lake		Lake	Lentic	0.35		0.54	0.43	0.43	1.01
Orr et al. 2006	Barns Lake Wetland	BLW	Lake	Lentic	4.40		2.00	2.97	0.50	5.93

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Orr et al. 2006	Fording River Oxbow	FRO	Oxbow	Lentic	5.55		7.90	6.62	5.04	1.37
Orr et al. 2006	Fording Settling Pond (Clode Pond)	FSP	Pond	Lentic	5.49		2.80	3.92	42.99	0.09
Orr et al. 2006	Goddard Marsh	GM	Marsh	Lentic	3.21		26.00	9.14	90.95	0.10
Orr et al. 2012	Clode Pond 11	CL11	Pond	Lentic	25.80			25.80	36.10	0.71
Orr et al. 2012	Elk Lakes 14	EL14	Lake	Lentic	0.66			0.66	0.40	1.64
Orr et al. 2012	Flathead Wetland 17	FL17	Marsh	Lentic	1.42			1.42	0.20	7.10
Orr et al. 2012	Fording River Oxbow 10	FO10	Oxbow	Lentic	67.31			67.31	50.10	1.34
Orr et al. 2012	Goddard Marsh 13	GO13	Marsh	Lentic	18.15			18.15	16.30	1.11
Orr et al. 2012	Henretta Lake 27	HE27	Lake	Lentic	4.30			4.30	8.60	0.50
Saiki and Lowe 1987	Kesterson Pond 11		Pond	Lentic	18.15	47.95	8.56	19.53	38.60	0.51
Saiki and Lowe 1987	Kesterson Pond 2		Pond	Lentic	152.70	44.65	34.82	61.92	195.85	0.32
Saiki and Lowe 1987	Kesterson Pond 8		Pond	Lentic	136.50	92.00	6.05	42.34	70.35	0.60
Saiki and Lowe 1987	Volta Pond 26		Pond	Lentic	0.42	1.01	0.29	0.50	0.53	0.93
Saiki and Lowe 1987	Volta Pond 7		Pond	Lentic		1.39	0.39	0.74	0.63	1.17
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 7	Pond	Lentic	87.10		5.90	22.67	100.00	0.23

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 2	Pond	Lentic	52.50		9.30	22.10	90.00	0.25
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 11	Pond	Lentic	53.70		11.50	24.85	40.00	0.62
Stephens et al. 1988	Marsh 4720	*	Marsh	Lentic	2.10		4.20	2.97	31.00	0.10
Butler et al. 1991	Uncompahgre River at Colona	4	River	Lotic	0.95			0.95	1.50	0.63
Butler et al. 1993	Spring Cr. at La Boca	SP2	Creek	Lotic	1.60		0.50	0.89	5.00	0.18
Butler et al. 1995	Cahone Canyon at Highway 666	CH	Creek	Lotic	2.50		4.30	3.28	12.00	0.27
Butler et al. 1995	Hartman Draw near mouth, at Cortez	HD2	Draw	Lotic	0.45		0.20	0.30	2.00	0.15
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	ME1	Creek	Lotic	1.80			1.80	2.00	0.90
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	ME2	Creek	Lotic	1.11		1.10	1.10	3.00	0.37
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	ME4	Creek	Lotic	1.04		0.50	0.72	6.00	0.12
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	ME3	Creek	Lotic	0.82		0.40	0.57	6.00	0.10
Butler et al. 1995	Navajo Wash near Towaoc	NW	Wash	Lotic	3.45		1.60	2.35	12.00	0.20
Butler et al. 1995	San Juan River at Four Comers	SJ1	River	Lotic	0.52		0.30	0.39	1.50	0.26
Butler et al. 1995	San Juan River at Mexican Hat Utah	SJ3	River	Lotic	0.94		0.20	0.43	1.50	0.29
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	WC	Creek	Lotic	3.30		1.50	2.22	5.50	0.40

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Butler et al. 1997	Cahone Canyon at Highway 666	CH1	Creek	Lotic	2.05			2.05	10.50	0.20
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	MUD2	Creek	Lotic	1.30			1.30	18.50	0.07
Butler et al. 1997	Tributary of Cahone Canyon at 13 Road	CH2	Creek	Lotic	1.75			1.75	5.50	0.32
Butler et al. 1997	Tributary of Yellow Jacket Canyon at Highway 666	YJ1	Creek	Lotic	1.85			1.85	7.00	0.26
Butler et al. 1997	Unnamed tributary of Cow Canyon at 8 Road	COW	Creek	Lotic	1.45			1.45	3.50	0.41
Butler et al. 1997	Unnamed tributary of Cross Canyon upstream from Alkali Canyon	CCTR	Creek	Lotic	1.75			1.75	4.50	0.39
Casey 2005	Deerlick Creek		Creek	Lotic		1.00	0.20	0.45	0.20	2.24
Casey 2005	Luscar Creek		Creek	Lotic	5.50	3.20	2.40	3.48	10.70	0.33
Formation 2012	Crow Creek - 1A	CC-1A	Creek	Lotic	3.64		1.20	2.09	2.45	0.80
Formation 2012	Crow Creek - 3A	CC-3A	Creek	Lotic	3.10		0.83	1.60	2.20	0.81
Formation 2012	Crow Creek - CC150	CC-150	Creek	Lotic	1.20		0.63	0.87	0.80	1.04
Formation 2012	Crow Creek - CC350	CC-350	Creek	Lotic	1.50		0.70	1.02	0.86	1.16
Formation 2012	Crow Creek - CC75	CC-75	Creek	Lotic	1.01		0.54	0.74	0.52	1.19
Formation 2012	Deer Creek	DC-600	Creek	Lotic	4.55		1.40	2.52	1.62	1.55

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Formation 2012	Hoopes Spring - HS	HS	Spring	Lotic	12.00		2.30	5.25	20.95	0.24
Formation 2012	Hoopes Spring - HS3	HS-3	Spring	Lotic	12.00		7.00	9.17	17.05	0.54
Formation 2012	Sage Creek - LSV2C	LSV-2C	Creek	Lotic	8.09		4.60	6.10	13.80	0.45
Formation 2012	Sage Creek - LSV4	LSV-4	Creek	Lotic	9.56		3.60	5.87	8.45	0.69
Formation 2012	South Fork Tincup Cr.	SFTC-1	Creek	Lotic	0.73		0.31	0.47	0.44	1.32
Golder 2011; Teck Coal 2013	McLeod River below Cheviot Creek	MR-2	River	Lotic	1.47			1.47	2.38	0.62
Golder 2011; Teck Coal 2013	McLeod River below Luscar Dreek	MR-6	River	Lotic	0.86			0.86	4.29	0.20
Golder 2011; Teck Coal 2013	McLeod River below Whitehorse Creek	MR-4	River	Lotic	0.68			0.68	1.07	0.64
Golder 2011; Teck Coal 2013	McLeod River reference	MR-1	River	Lotic	0.75			0.75	0.30	2.50
Golder 2011; Teck Coal 2013	Prospect Creek far field	PC-3	Creek	Lotic	0.37			0.37	0.63	0.59
Golder 2011; Teck Coal 2013	Prospect Creek reference	PC-1	Creek	Lotic	0.86			0.86	0.40	2.15
Hamilton and Buhl 2004	lower East Mill Creek	LEMC	Creek	Lotic	25.70		38.90	31.62	24.00	1.32

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	ER 745	River	Lotic	0.31		1.28	0.63	0.10	6.30
McDonald and Strosher 1998	Elk R. above Fording R.	ER 750	River	Lotic	0.78		0.70	0.74	0.40	1.85
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	ER 746	River	Lotic	1.56		2.41	1.94	8.60	0.23
McDonald and Strosher 1998	Michel Cr. at Highway 3	ER 751	Creek	Lotic	1.26		2.32	1.71	7.10	0.24
Orr et al. 2006	Alexander Creek	AC	Creek	Lotic	4.49		0.90	2.01	0.90	2.23
Orr et al. 2006	Fording River	FR	River	Lotic	3.27		2.10	2.62	20.10	0.13
Orr et al. 2006	Line Creek	LC	Creek	Lotic	2.19		2.10	2.14	20.90	0.10
Orr et al. 2012	Elk River 1	EL1	River	Lotic	2.30			2.30	4.20	0.55
Orr et al. 2012	Elk River 12	EL12	River	Lotic	2.00			2.00	0.75	2.67
Orr et al. 2012	Fording River 23	FO23	River	Lotic	6.35			6.35	30.60	0.21
Orr et al. 2012	Michel Creek 2	MI2	Creek	Lotic	2.10			2.10	7.40	0.28
Presser and Luoma 2009	Upper Peters canyon (dry)	U PCW dry	Wash	Lotic	1.20		0.60	0.85	3.20	0.27
Saiki and Lowe 1987	San Luis Drain		Drain	Lotic	67.00	275.00	79.90	113.76	316.50	0.36
Saiki and Lowe 1987	Volta Wasteway		Wasteway	Lotic	0.87	2.03	0.24	0.76	0.74	1.03
Saiki et al. 1993	Mud Slough at Gun Club Road	GT5	Slough	Lotic	4.50	14.95		8.20	6.00	1.37

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	GT4	Slough	Lotic	1.39	8.40		3.42	8.00	0.43
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	SJR2	River	Lotic	1.25	5.00		2.50	7.00	0.36
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	SJR3	River	Lotic	0.45	1.25		0.75	1.00	0.75
Stephens et al. 1988	Drain J3	*	Drain	Lotic	24.00		48.00	33.94	110.00	0.31

APPENDIX I: OBSERVED VERSUS PREDICTED EGG-OVARY CONCENTRATIONS

The following table includes data for 317 individual fish tissue selenium measurements from the 64 sites where EFs could be calculated. Observed egg-ovary fish tissue measurements were compared to predicted egg-ovary fish tissue measurements calculated using equation 22 of the main text, also shown here for convenience.

$$C_{egg-ovary} = C_{water} \times TTF^{composite} \times EF \times CF \quad \text{(Equation 22)}$$

These data were used to generate the observed to predicted egg-ovary concentration Figure 6.3 of the main text. When the measured tissue type was either muscle or whole body, it was converted to egg-ovary using taxa specific conversion factors. The predicted and measured concentrations are highly correlated ($r = 0.82$, $t_{(315)} = 25.30$, $P < 0.001$).

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Birkner 1978	East Allen Reservoir, Medicine Bow WY	Iowa darter	4.80	2.31	2.87	1.45	46.14	52.68	WB
Birkner 1978	Galett Lake, Laramie WY	Iowa darter	0.80	0.88	2.87	1.45	2.91	3.05	WB
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	northern plains killifish	15.90	1.70	2.44	1.20	79.04	68.71	WB
Birkner 1978	Meeboer Lake, Laramie WY	northern plains killifish	0.30	0.58	2.44	1.20	0.51	9.22	WB
Birkner 1978	Miller's Lake, Wellington CO	fathead minnow	6.00	2.37	2.78	1.40	55.31	15.37	WB
Birkner 1978	Miller's Lake, Wellington CO	Iowa darter	6.00	2.37	2.87	1.45	59.18	33.38	WB
Birkner 1978	Sweitzer Lake, Delta CO	northern plains killifish	9.40	0.87	2.44	1.20	23.94	38.18	WB
Birkner 1978	Sweitzer Lake, Delta CO	fathead minnow	9.40	0.87	2.78	1.40	31.89	110.38	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	northern plains killifish	7.60	1.21	2.44	1.20	26.79	27.65	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	fathead minnow	7.60	1.21	2.78	1.40	35.69	48.20	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	Iowa darter	7.60	1.21	2.87	1.45	38.18	60.81	WB
Bowie et al. 1996	Hyco Reservoir	bluegill	11.50	2.35	2.00	2.13	114.97	87.47	WB
Butler et al. 1991	Uncompahgre River at Colona	flannelmouth sucker	1.50	0.63	1.52	1.41	2.03	2.40	WB
Butler et al. 1991	Uncompahgre River at Colona	white sucker	1.50	0.63	1.58	1.38	2.07	7.32	WB
Butler et al. 1991	Uncompahgre River at Colona	bluehead sucker	1.50	0.63	1.24	1.82	2.13	3.27	WB
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	3.77	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	6.39	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	4.77	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	5.06	WB
Butler et al. 1991	Uncompahgre River at Colona	rainbow trout	1.50	0.63	2.33	2.44	5.39	6.88	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	brown trout	1.00	1.26	2.78	1.45	5.08	6.20	E-O
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	channel catfish	1.00	1.26	1.35	1.45	2.47	2.32	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	2.03	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	3.05	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	5.19	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Spring Cr. at La Boca	white sucker	5.00	0.18	1.58	1.38	1.96	4.83	WB
Butler et al. 1993	Spring Cr. at La Boca	bluehead sucker	5.50	0.18	1.24	1.82	2.22	12.91	WB
Butler et al. 1993	Spring Cr. at La Boca	speckled dace	5.00	0.18	1.36	1.95	2.37	23.45	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.00	0.18	2.78	1.40	3.48	11.46	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.00	0.18	2.78	1.45	3.60	1.74	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.50	0.18	2.78	1.40	3.83	8.38	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.50	0.18	2.78	1.45	3.96	4.92	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	1.07	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	0.96	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.69	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.76	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.87	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	1.35	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.10	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.24	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	speckled dace	2.00	0.90	1.36	1.95	4.77	12.51	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	fathead minnow	2.00	0.90	2.78	1.40	7.00	7.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.25	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	1.97	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	3.10	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	1.51	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	2.36	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	speckled dace	3.00	0.37	1.36	1.95	2.92	11.92	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	fathead minnow	3.00	0.37	2.78	1.40	4.29	6.71	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.11	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	1.83	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.68	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	3.38	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	4.23	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.49	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.11	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.30	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	1.96	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	8.24	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	red shiner	6.00	0.12	2.27	1.95	3.20	9.97	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.96	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	3.38	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	5.07	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.27	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.09	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bullhead	6.00	0.10	1.62	1.45	1.34	4.35	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	5.47	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	13.68	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	10.75	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	8.45	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	9.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	green sunfish	6.00	0.10	2.29	1.45	1.91	7.26	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.01	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	7.41	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.15	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.21	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	16.91	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	13.09	WB
Butler et al. 1995	Navajo Wash near Towaoc	speckled dace	12.00	0.20	1.36	1.95	6.23	17.00	WB
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	2.98	M
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.70	M
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	5.95	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.11	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	3.10	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	0.86	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	1.55	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	5.92	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	1.71	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	8.40	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	9.97	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	5.67	WB
Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	10.18	WB

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Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	6.53	WB
Butler et al. 1995	San Juan River at Four Comers	red shiner	1.50	0.26	2.27	1.95	1.75	6.84	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	channel catfish	1.50	0.29	1.35	1.45	0.85	10.88	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.68	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	1.97	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.18	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.36	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.91	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	common carp	1.50	0.29	1.58	1.92	1.31	7.49	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	25.71	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	32.00	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	36.89	WB
Butler et al. 1997	Cahone Canyon at Highway 666	green sunfish	10.50	0.20	2.29	1.45	6.83	13.79	WB
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	fathead minnow	3.00	2.00	2.78	1.40	23.39	15.37	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	4.55	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	9.45	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	10.18	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	11.03	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	10.16	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	10.76	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	16.77	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	9.08	WB
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	smallmouth bass	1.00	5.15	1.93	1.42	14.09	17.03	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	13.97	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	20.96	WB
Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	3.14	M

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Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	8.16	E-O
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	16.79	M
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	33.48	E-O
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	14.43	WB
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	12.10	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	15.20	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	13.49	WB
Formation 2012	Crow Creek - 1A	sculpin	2.45	0.80	2.78	1.45	7.89	11.29	WB
Formation 2012	Crow Creek - 1A	brown trout	2.45	0.80	2.96	1.45	8.37	14.39	WB
Formation 2012	Crow Creek - 1A	sculpin	2.90	0.80	2.78	1.45	9.34	25.35	WB
Formation 2012	Crow Creek - 1A	brown trout	2.90	0.80	2.96	1.45	9.91	24.36	WB
Formation 2012	Crow Creek - 1A	sculpin	4.80	0.80	2.78	1.45	15.45	18.33	WB
Formation 2012	Crow Creek - 1A	brown trout	4.80	0.80	2.96	1.45	16.40	20.29	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	20.97	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	16.91	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	15.09	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	13.30	WB
Formation 2012	Crow Creek - 3A	sculpin	2.20	0.81	2.78	1.45	7.17	16.65	WB
Formation 2012	Crow Creek - 3A	brown trout	2.20	0.81	2.97	1.45	7.60	16.27	WB
Formation 2012	Crow Creek - 3A	brown trout	2.60	0.81	2.97	1.45	8.99	22.24	WB
Formation 2012	Crow Creek - 3A	sculpin	4.20	0.81	2.78	1.45	13.68	29.32	WB
Formation 2012	Crow Creek - 3A	brown trout	4.20	0.81	2.97	1.45	14.52	28.45	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	8.72	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	7.31	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	8.43	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	12.54	WB
Formation 2012	Crow Creek - CC150	sculpin	0.80	1.04	2.74	1.45	3.31	7.46	WB
Formation 2012	Crow Creek - CC150	brown trout	0.80	1.04	2.91	1.45	3.51	7.52	WB
Formation 2012	Crow Creek - CC150	sculpin	1.40	1.04	2.74	1.45	5.79	15.57	WB
Formation 2012	Crow Creek - CC150	brown trout	1.40	1.04	2.91	1.45	6.14	14.66	WB
Formation 2012	Crow Creek - CC150	sculpin	1.50	1.04	2.74	1.45	6.20	10.67	WB
Formation 2012	Crow Creek - CC150	brown trout	1.50	1.04	2.91	1.45	6.58	11.32	WB

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Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	9.39	WB
Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	10.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.86	1.16	2.79	1.45	4.02	7.66	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	9.08	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	12.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.89	1.16	2.79	1.45	4.19	14.56	WB
Formation 2012	Crow Creek - CC350	brown trout	0.86	1.16	2.97	1.45	4.27	8.36	WB
Formation 2012	Crow Creek - CC350	brown trout	0.89	1.16	2.97	1.45	4.44	16.63	WB
Formation 2012	Crow Creek - CC350	sculpin	1.10	1.16	2.79	1.45	5.15	13.83	WB
Formation 2012	Crow Creek - CC350	brown trout	1.10	1.16	2.97	1.45	5.47	11.49	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	8.10	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	7.30	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	5.86	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	7.74	WB
Formation 2012	Crow Creek - CC75	sculpin	0.52	1.19	2.69	1.45	2.39	5.47	WB
Formation 2012	Crow Creek - CC75	brown trout	0.52	1.19	2.87	1.45	2.54	4.60	WB
Formation 2012	Crow Creek - CC75	sculpin	0.85	1.19	2.69	1.45	3.94	10.43	WB
Formation 2012	Crow Creek - CC75	brown trout	0.85	1.19	2.87	1.45	4.18	14.92	WB
Formation 2012	Crow Creek - CC75	sculpin	1.00	1.19	2.69	1.45	4.64	10.28	WB
Formation 2012	Crow Creek - CC75	brown trout	1.00	1.19	2.87	1.45	4.92	9.54	WB
Formation 2012	Deer Creek	sculpin	1.45	1.55	2.81	1.45	9.17	11.07	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	12.34	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	11.42	WB
Formation 2012	Deer Creek	brown trout	1.45	1.55	3.00	1.45	9.73	8.46	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	12.35	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	8.96	WB
Formation 2012	Deer Creek	sculpin	2.00	1.55	2.81	1.45	12.65	11.55	WB
Formation 2012	Deer Creek	brown trout	2.00	1.55	3.00	1.45	13.43	18.55	WB
Formation 2012	Deer Creek	sculpin	2.40	1.55	2.81	1.45	15.18	12.51	WB
Formation 2012	Deer Creek	brown trout	2.40	1.55	3.00	1.45	16.11	15.24	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.71	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.74	WB

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Formation 2012	Hoopes Spring - HS	sculpin	20.95	0.24	3.63	1.45	26.96	15.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	23.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	36.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.95	0.24	3.86	1.45	28.61	36.00	WB
Formation 2012	Hoopes Spring - HS	sculpin	27.30	0.24	3.63	1.45	35.13	52.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	27.30	0.24	3.86	1.45	37.28	47.18	WB
Formation 2012	Hoopes Spring - HS	sculpin	40.45	0.24	3.63	1.45	52.05	59.94	WB
Formation 2012	Hoopes Spring - HS	brown trout	40.45	0.24	3.86	1.45	55.23	32.97	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	31.71	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	26.95	WB
Formation 2012	Hoopes Spring - HS3	sculpin	17.05	0.54	2.47	1.45	32.79	38.65	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	29.78	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	27.23	WB
Formation 2012	Hoopes Spring - HS3	brown trout	17.05	0.54	2.63	1.45	34.79	25.87	WB
Formation 2012	Hoopes Spring - HS3	sculpin	26.00	0.54	2.47	1.45	49.99	34.73	WB
Formation 2012	Hoopes Spring - HS3	brown trout	26.00	0.54	2.63	1.45	53.05	34.24	WB
Formation 2012	Hoopes Spring - HS3	sculpin	31.75	0.54	2.47	1.45	61.05	34.37	WB
Formation 2012	Hoopes Spring - HS3	brown trout	31.75	0.54	2.63	1.45	64.78	41.89	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	25.35	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	16.52	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.80	0.45	2.83	1.45	25.31	27.36	WB
Formation 2012	Sage Creek - LSV2C	sculpin	14.30	0.45	2.83	1.45	26.23	37.66	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	28.12	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	18.48	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.80	0.45	3.01	1.45	26.86	32.78	WB
Formation 2012	Sage Creek - LSV2C	brown trout	14.30	0.45	3.01	1.45	27.83	28.24	WB
Formation 2012	Sage Creek - LSV2C	sculpin	18.75	0.45	2.83	1.45	34.39	29.49	WB
Formation 2012	Sage Creek - LSV2C	brown trout	18.75	0.45	3.01	1.45	36.49	30.30	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	29.04	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	26.53	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	23.42	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	21.95	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Formation 2012	South Fork Tincup Cr.	sculpin	0.32	1.32	2.86	1.45	1.73	8.24	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.32	1.32	3.05	1.45	1.84	5.32	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.43	1.32	2.86	1.45	2.37	5.44	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.44	1.32	2.86	1.45	2.42	13.51	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.43	1.32	3.05	1.45	2.51	3.25	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.44	1.32	3.05	1.45	2.57	9.69	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.56	1.32	2.86	1.45	3.06	8.52	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.56	1.32	3.05	1.45	3.24	3.82	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	3.92	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.41	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.75	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.03	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.52	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.54	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.21	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.22	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	10.20	WB
Hamilton and Buhl 2004	lower East Mill Creek	cutthroat trout	24.00	1.32	2.29	1.96	142.01	102.73	WB
Lemly 1985	Badin Lake	black bullhead	0.32	12.48	1.72	1.45	9.99	4.44	M
Lemly 1985	Badin Lake	western mosquitofish	0.32	12.48	2.37	1.20	11.33	5.77	M
Lemly 1985	Badin Lake	common carp	0.32	12.48	1.58	1.92	12.10	5.81	M
Lemly 1985	Badin Lake	green sunfish	0.32	12.48	2.29	1.45	13.30	3.25	M
Lemly 1985	Badin Lake	fathead minnow	0.32	12.48	2.78	1.40	15.52	3.17	M
Lemly 1985	Badin Lake	red shiner	0.32	12.48	2.27	1.95	17.74	4.45	M
Lemly 1985	Belews Lake	black bullhead	10.91	1.75	1.72	1.45	47.79	29.84	M
Lemly 1985	Belews Lake	western mosquitofish	10.91	1.75	2.37	1.20	54.18	46.86	M
Lemly 1985	Belews Lake	common carp	10.91	1.75	1.58	1.92	57.86	38.97	M
Lemly 1985	Belews Lake	green sunfish	10.91	1.75	2.29	1.45	63.60	20.84	M
Lemly 1985	Belews Lake	fathead minnow	10.91	1.75	2.78	1.40	74.25	28.75	M
Lemly 1985	Belews Lake	red shiner	10.91	1.75	2.27	1.95	84.87	38.59	M
Lemly 1985	High Rock Lake	black bullhead	0.67	4.99	1.72	1.45	8.36	5.58	M

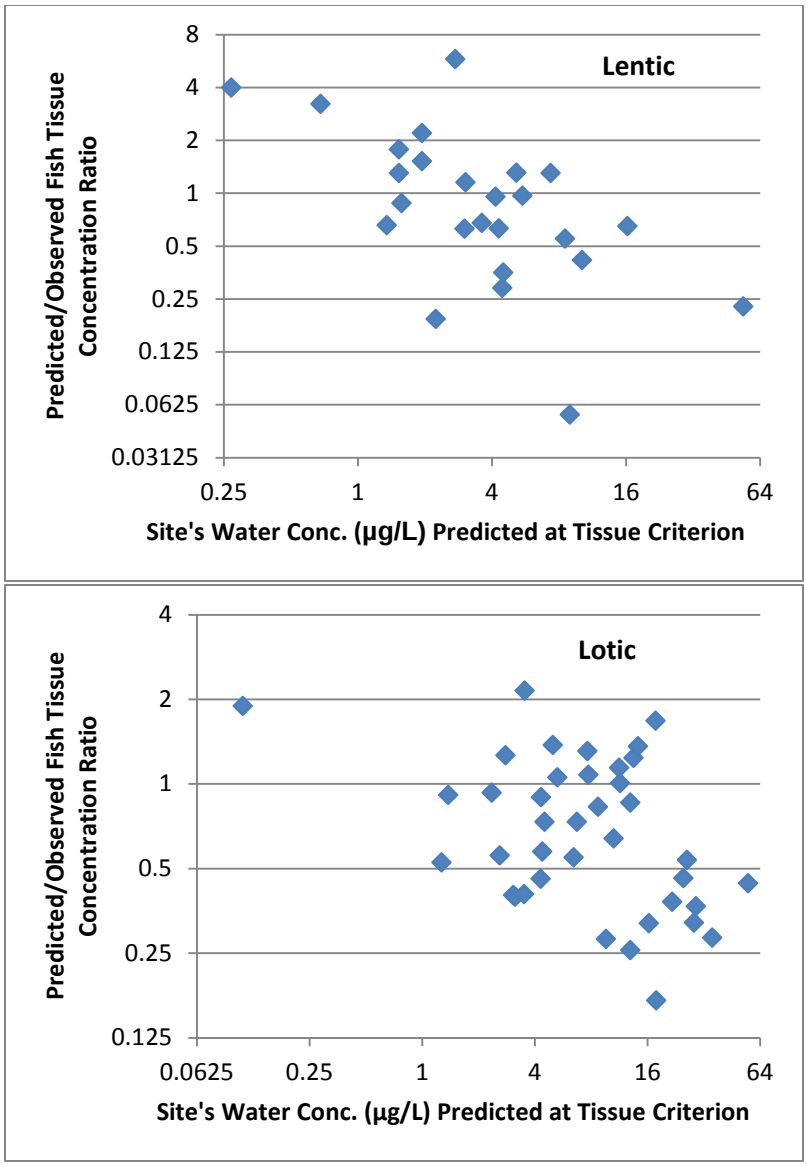
Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Lemly 1985	High Rock Lake	western mosquitofish	0.67	4.99	2.37	1.20	9.48	6.10	M
Lemly 1985	High Rock Lake	common carp	0.67	4.99	1.58	1.92	10.12	4.49	M
Lemly 1985	High Rock Lake	green sunfish	0.67	4.99	2.29	1.45	11.13	3.13	M
Lemly 1985	High Rock Lake	fathead minnow	0.67	4.99	2.78	1.40	12.99	4.00	M
Lemly 1985	High Rock Lake	red shiner	0.67	4.99	2.27	1.95	14.85	4.62	M
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	cutthroat trout	0.10	6.30	2.29	1.96	2.83	10.61	WB
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	mountain whitefish	0.10	6.30	2.97	7.39	13.83	7.11	WB
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	cutthroat trout	8.60	0.23	2.29	1.96	8.71	24.96	WB
Muscatello and Janz 2009	Vulture Lake	white sucker	0.43	1.01	1.58	1.38	0.95	4.65	WB
Muscatello and Janz 2009	Vulture Lake	burbot	0.43	1.01	2.45	1.45	1.54	15.91	WB
Muscatello and Janz 2009	Vulture Lake	ninespine stickleback	0.43	1.01	3.22	1.45	2.03	6.02	WB
Muscatello and Janz 2009	Vulture Lake	northern pike	0.43	1.01	4.02	2.39	4.17	1.83	WB
Orr et al. 2012	Clode Pond 11	cutthroat trout	36.10	0.71	2.29	1.96	115.88	81.06	E-O
Orr et al. 2012	Elk Lakes 14	cutthroat trout	0.40	1.64	2.29	1.96	2.95	14.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	11.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	15.60	E-O
Orr et al. 2012	Elk River 12	cutthroat trout	0.75	2.67	2.29	1.96	8.98	9.00	E-O
Orr et al. 2012	Fording River 23	cutthroat trout	30.60	0.21	2.29	1.96	28.52	15.56	E-O
Orr et al. 2012	Fording River Oxbow 10	cutthroat trout	50.10	1.34	2.29	1.96	302.30	47.81	E-O
Orr et al. 2012	Henretta Lake 27	cutthroat trout	8.60	0.50	2.29	1.96	19.33	13.56	E-O
Orr et al. 2012	Michel Creek 2	cutthroat trout	7.40	0.28	2.29	1.96	9.43	10.07	E-O
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	155.61	WB
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	124.49	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	268.13	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	295.66	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	196.31	WB
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	266.93	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	178.36	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	397.41	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.53	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.48	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.62	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.63	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	10.67	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	13.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.79	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	13.17	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	19.15	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.17	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.60	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	5.68	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	6.67	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.39	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.87	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	5.76	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	7.04	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.12	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.41	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	2.63	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	5.39	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.05	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.27	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.41	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.55	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.03	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.39	WB
Stephens et al. 1988	Marsh 4720	black bullhead	31.00	0.10	1.72	1.45	7.43	10.16	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	36.49	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	40.33	WB

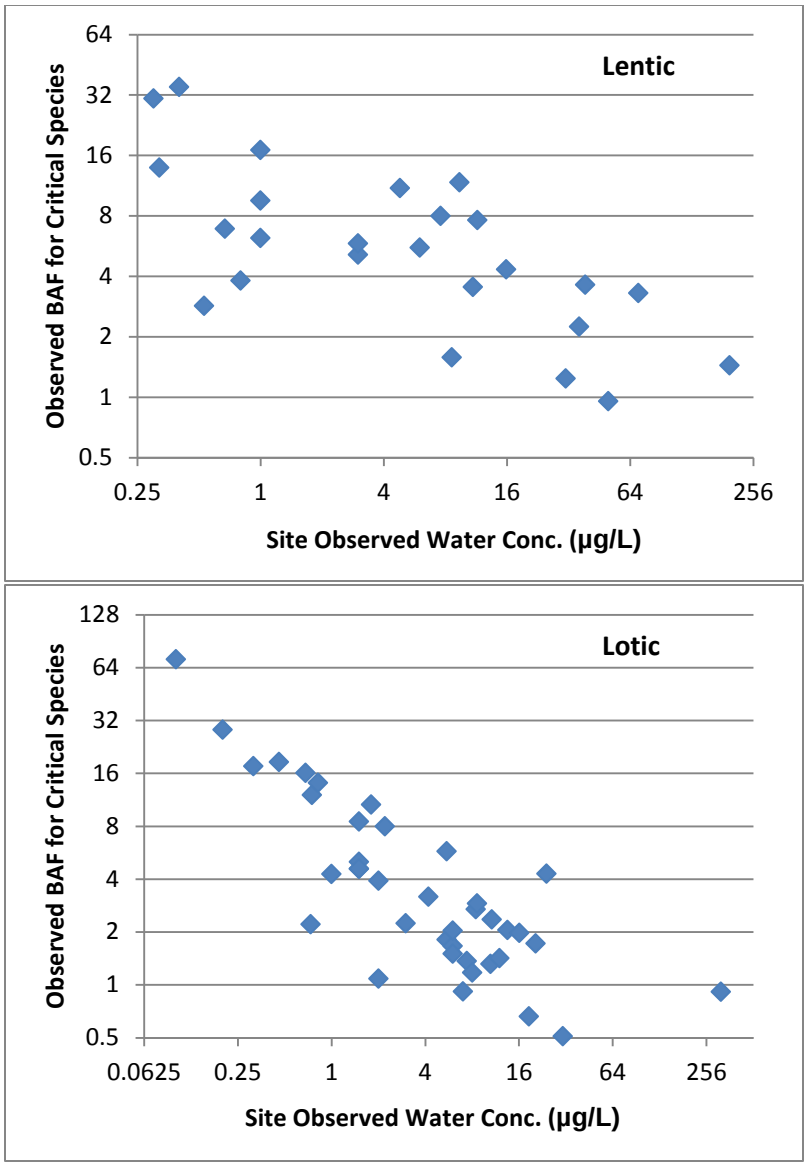
The ratio of predicted versus observed tissue concentrations in the above table can be compared against the main text Table 3.13 water concentrations that would be predicted to occur if each site's egg-ovary tissue concentration were at the criterion level. Figure I-1 shows the results. It can be seen that for those sites (in the left portion of each graph) where tissue concentrations equal to the egg-ovary criterion would be predicted to yield water concentrations not far on either side of the water criteria values, the predicted-to-observed tissue concentration ratios are not particularly biased relative to a ratio of 1.0. This indicates that the model is performing reasonably well for those sites strongly influencing the derived values of the water criteria.

The derivation of the water criteria concentrations involves an assumption of linearity in projecting the water concentration that would correspond to a tissue concentration equal to the tissue criterion. Figure I-2 suggests that high BAFs tend to be associated with low water concentrations, and low BAFs with high water concentrations. At the low concentrations associated with the 20^{th} percentile model-predicted BAF, the linearity assumption would appear to be environmentally conservative. At high concentrations, the opposite situation would occur, but overall, because the criterion is based on the 20^{th} percentile, the linearity assumption appears to be protective.



Figures I-1. For lentic (left panel) and lotic (right panel) waters, predicted-to-observed fish-tissue concentration ratio for each of the 65 sites, plotted versus each site's Table 3.13 water concentration that would be predicted to occur if its tissue levels were at the egg-ovary tissue criterion level.

Corresponding to how the water criteria concentrations were derived, for sites with multiple fish species, the plotted ratio is for the species having the highest predicted tissue-to-water ratio (i.e., highest predicted BAF). For sites having multiple samples of that species, the plotted value is the average predicted-to-observed ratio for that species.



Figures I-2. For lentic (left panel) and lotic (right panel) waters, observed BAFs (egg-ovary tissue-to-water concentration ratios) versus observed water concentration (both from the above table), for each site's fish species used in Table 3.13 (that is, for the species used in the water criteria calculations).

For sites having multiple samples of such species, tissue concentrations were averaged. Because nearly all samples were either whole body or muscle, the graphed BAFs include application of the CF, to normalize all samples to egg-ovary tissue. Since the CFs have been are assumed to be independent of concentration, the graphs do not reflect any potential CF nonlinearities, if they exist.

APPENDIX J: SUPPLEMENTARY INFORMATION ON SELENIUM BIOACCUMULATION IN AQUATIC ANIMALS

1.0 EFFECTS OF GROWTH RATE ON THE ACCUMULATION OF SELENIUM IN FISH

EPA analyzed the effect of the growth rate parameter *g* when estimating selenium bioaccumulation using the mechanistic bioaccumulation modeling described in Equation 1 of the main text. Because the addition of tissue associated with growth could have a dilution effect on the chemicals present in tissue, a parameter representing growth rate is present in the denominator of Equation 1. Indeed, growth can be an important factor in the bioaccumulation of very hydrophobic chemicals with low excretion rates such as polychlorinated biphenyls, (Connolly and Pedersen 1988). However, the effect of growth may not be as important for selenium because of its unique biogeochemical characteristics, route of exposure, and role as a micronutrient.

EPA tested the effect of the growth rate parameter *g* on estimates of selenium bioaccumulation using Equation 1 with different food web scenarios. Increasing growth rates from 0 (no growth) to 0.2/day (a relatively high rate of growth) reduced selenium concentrations in trophic level 2 and 3 organisms by as much as a factor of 10 to 20. Thus incorporating growth rate in Equation 1 could result in significant dilution of selenium and lower estimates of selenium bioaccumulation.

Although increasing the value of the growth parameter g in Equation 1 reduces estimates of selenium bioaccumulation, this simple analysis neglects an important physiological linkage between growth and food consumption. Organisms must consume enough food to support growth and meet their energy requirements for respiration, specific dynamic action, waste loss, and reproduction. These physiological requirements suggest that higher growth rates are associated with greater rates of food consumption. Because food consumption is the primary route of selenium exposure in aquatic organisms, increased selenium exposure associated with higher food consumption could counterbalance the dilution of selenium in tissue associated with higher growth rates.

EPA tested the effects of growth on estimates of selenium bioaccumulation using Equation 1 when increased food consumption was associated with higher growth rates. EPA modified Equation 1 to incorporate a simple relationship for bioenergetics (Thomann et al. 1992) and applied the model to reexamine the sensitivity of selenium bioaccumulation to growth rates in trophic level 2 and 3 organisms. The results of this analysis showed that increasing growth rates over two orders of magnitude increased selenium concentrations in trophic level 2 by a factor of 2, and decreased selenium concentrations in trophic level 3 by 10%. When growth rates were increased simultaneously in trophic levels 2 and 3, the selenium concentrations increased by less than a factor of 2. This analysis suggests that when bioenergetics is considered, selenium bioaccumulation is generally insensitive to organism growth rates. EPA believes that uncertainties in the toxicokinetic parameters of selenium far outweigh the effects on growth rate on selenium bioaccumulation. Thus, the growth rate parameter *g* was removed from Equation

1 for the purpose of deriving a translation equation that could be used to implement a tissue-based selenium water quality criterion.

2.0 Analysis of the Relative Contribution of Aqueous and Dietary Uptake on the Bioaccumulation of Selenium

EPA analyzed the relative contributions of direct aqueous uptake versus ingestion of selenium in consideration of removing the uptake rate constant k_u from Equation 1 in Section 3.2 of the main text. Because an important exposure route for some chemicals is direct contact with water, an uptake rate constant k_u is present in the numerator of Equation 1. However, fish and invertebrate organisms absorb selenium primarily through the consumption of food rather than from direct aqueous uptake (Forester 2007; Lemly 1985; Luoma et al. 1992). Thus, removing the uptake rate constant k_u could simplify Equation 1 while maintaining the key determinants of selenium bioaccumulation.

EPA tested the relative contribution of aqueous versus dietary uptake of selenium using a version of Equation 1 that incorporates both exposure pathways (Thomann et. al. 1992). For trophic level 2, selenium bioaccumulation was estimated for a range of uptake rates that varied according to the respiration rate and aqueous transfer efficiency of selenium relative to dissolved oxygen. For trophic level 3, uptake rates were varied within a range of values reported in Besser et al. (1993) and Bertram and Brooks (1986).

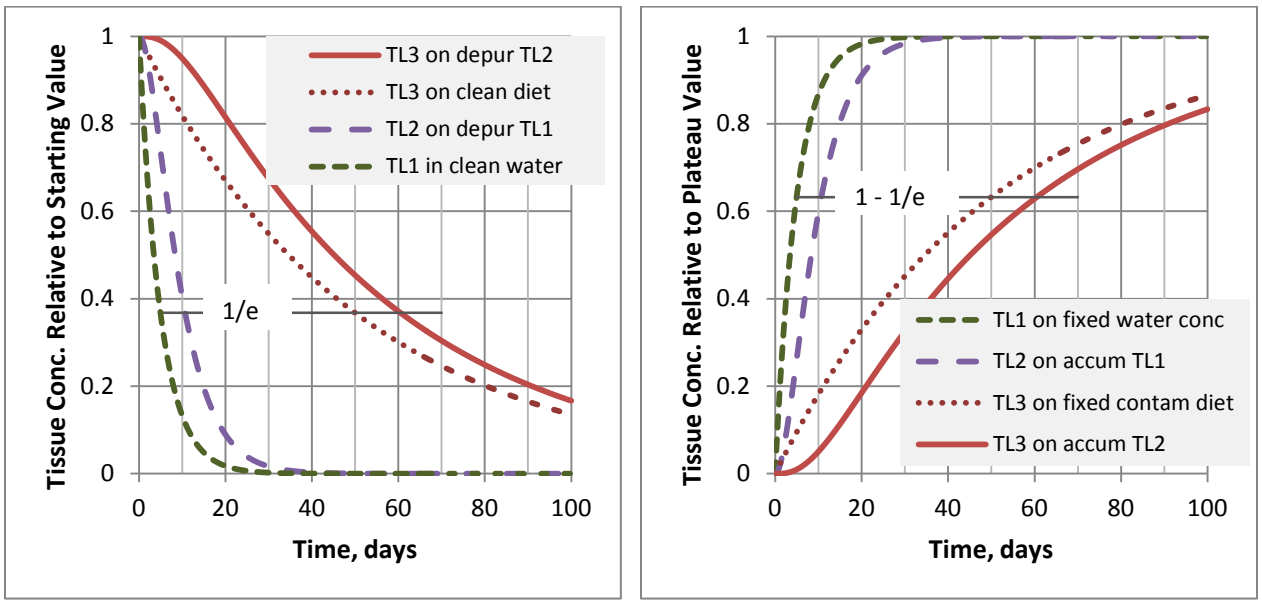
EPA's analysis showed that diet accounted for 34% - 92% of selenium bioaccumulation at trophic level 2, with a median of 74%. At trophic level 3, diet accounted for 62% - 100% of tissue selenium, with a median of 95%. Thus, disregarding aqueous uptake of selenium only resulted in a small (~5%) reduction in estimated selenium bioaccumulation in trophic level 3 organisms. These results are consistent with previous studies indicating that diet is the primary exposure route of selenium, and suggests that the uptake rate constant for selenium can be removed from Equation 1 with negligible effect for higher trophic levels organisms.

3.0 Kinetics of Accumulation and Depuration: Averaging Period

3.1 Background

For setting averaging periods for aquatic life criteria, U.S. EPA (1995b) used the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action. In the context of the water-borne direct toxicity of metals, characteristic time = 1/k, where k is the first-order kinetic coefficient in a toxico-kinetic model fitted to the relationship between LC50 and exposure duration.

In the context of selenium bioaccumulation in a single trophic level, k would the first-order depuration coefficient, and 1/k would equal the time needed to depurate to a concentration of 1/e times the initial concentration (where e=2.718). For depuration of multiple trophic levels sequentially, the characteristic time is likewise the time needed for c/c_o to reach a value of 1/e, as shown in Figure J-1a. The accumulation curve is the inverted depuration curve, as shown in Figure J-1b.



Figures J-1 a & b. Depuration and accumulation behavior for algae-detritus-sediment k=0.2/day, invertebrate k=0.2/day and fish k=0.02/day, calculated with time step = 0.1 day.
Concentration is expressed as a dimensionless ratio: concentration at time t divided by either starting concentration (J1a) or plateau concentration (J1b).

In the Figures J-1 a & b examples, the characteristic time for algae-detritus-sediment is 5 days, the characteristic time for invertebrates on an invariant diet is 5 days, the characteristic time for fish on an invariant diet is 50 days, and the characteristic time for fish on an invertebrate diet that is itself depurating or accumulating is the approximate sum of the individual characteristic times, or ~60 days.

In contrast to the model depuration rate, k, the model uptake rate (AE, assimilation efficiency, multiplied by IR, intake rate) does not affect the characteristic response time. Rather it affects the magnitude of the accumulation plateau. Uptake rate thus affects the TTF value itself but is not relevant to setting an averaging period.

Because short averaging periods are more environmentally conservative than long averaging periods, selecting parameter values for fast kinetics is more environmentally conservative. Figure J1 reflects environmentally conservative choices for k values.

3.2 Approach for Modeling Effects of Time-Variable Se Concentrations

Expression of concentrations. None of the concentrations in this analysis are expressed in ordinary units of concentration. All concentrations are modeled as values normalized to their allowable benchmark concentration – that is, concentration = 1 for a particular medium (water, algae-detritus-sediment, invertebrates, or fish) means that the medium is at its criterion concentration or corresponding benchmark. It is assumed that the benchmarks correctly align – water held at its benchmark concentration will ultimately yield Trophic Levels 1, 2, and 3 at their respective benchmark concentrations. The Trophic Level 3 benchmark is the reproductive EC10 for the 5th percentile taxon: i.e., the fish tissue criterion.

Formulation of the bioaccumulation model for kinetic analysis. For algae-detritus-sediment, for invertebrates, and for fish, accumulation at time t equals accumulation at time t-1 plus intake minus depuration, as follows:

Algae-detritus-sediment:

$$C_{TL1}[t] = C_{TL1}[t\text{-}1] + k_{uptake}\, C[t\text{-}1]water - k_{TL1}\, C_{TL1}[t\text{-}1]$$

Invertebrates:

$$C_{TL2}[t] = C_{TL2}[t\text{-}1] + AE_{TL2}\, IR_{TL2}\, C_{TL1}[t\text{-}1] - k_{TL2}\, C_{TL2}[t\text{-}1]$$

Fish:

$$C_{TL3}[t] = C_{TL3}[t\text{-}1] + AE_{TL3}\, IR_{TL3}\, C_{TL2}[t\text{-}1] - k_{TL3}\, C_{TL3}[t\text{-}1]$$

For algae-detritus-sediment, the depuration rate k is assigned a value of 0.2/day, similar to the sum of depuration and growth-dilution rate coefficients used by Brix and DeForest (2008). Because a lentic system would involve the slower kinetics of sediment exchange, the rapid rate used here implies a lotic system.

For invertebrates, a value of 0.2/day was assigned, considerably higher than those for *Lumbriculus*, Asian clam, zebra mussel, but close to those of mayfly and copepods, which are very small in size. As previously mentioned, higher k (more rapid kinetics) is an environmentally conservative assumption in this context.

For fish, the median depuration coefficient measured by Bertram and Brooks (1986) for 6-9 month-old (early adult) fathead minnows was used, providing a k_{TL3} value of 0.02/day. Because of the small size of adults of this species, this represents faster kinetics than would likely be applicable the salmonids and centrarchids of greatest concern for selenium toxicity. The striped bass k value of Baines et al. (2002) is inapplicable here because it was measured in the early juvenile life stage, a size that is too small to be relevant to reproductive impairment stemming from exposure of adult females. The concentration in fish could be equivalently viewed as either whole body or egg-ovary, relative to their

respective benchmarks. That is, partitioning within body of the fish is assumed not to involve a time delay.

The value of a TTF is given by AE x IR/k (or k_{uptake}/k for algae-detritus-sediment). Concentrations in TL1, TL2, and TL3 are normalized to their benchmarks, meaning that all benchmark concentrations have a value of 1.0. In this normalized context, the TTFs must also equal 1.0, since upon reaching steady state, TL1 at its benchmark will yield TL2 at its benchmark, which in turn will yield TL3 at its benchmark. Again, the analysis is not intended to reflect actual concentrations, merely portray temporal behavior. Since 1 = TTF = AE x IR/k, it follows that AE x IR = k within this normalized framework. Although only the product AE x IR is relevant, they are retained as distinct parameters to maintain parallelism with remainder of the criterion document. AE was assigned a value of 0.5 for fish and invertebrates, and IR = k/AE in the normalized framework.

Time step durations of 0.1-1.0 day were considered. Short time steps increase accuracy by decreasing the numerical dispersion inherent in expressing C[t] = f(C[t-1]). A time step of 0.5 day was found to yield sufficient accuracy, as measured by predicted values at the characteristic time for depuration or accumulation (per Figure J-1).

Prediction of Effects. The effect level associated with the tissue concentration at any time t is calculated via the log probit concentration-response curve, one of the commonly used sigmoid curves. It assumes that the sensitivities in the underlying population are log-normally distributed such that the concentration yielding effects on k percentage of the population is given by:

$$EC_k = EC50 \exp(\sigma z)$$

where σ is the inverse of the concentration-response curve slope and z is the normal deviate corresponding to k percent (e.g., for k=10%, z=NORMSINV(0.1)=−1.28155). Among the reproductive impairment studies presented in Appendix C, an approximate median ratio for EC50/EC10 is 1.5. This translates to σ=0.3164.

Since the fish tissue criterion concentration equals 1.0 in this normalized framework, at any time t, the fractional level of effect corresponding to any value of C_{TL3} is given by:

Fractional Effect[t] = NORMSDIST(z[t])

where z[t] is given by:

$z[t] = LN(C_{TL3}[t]/1.5)/0.3164$

Exposure Scenarios. Three exposure scenarios were evaluated under which the water criterion was just barely attained. The first two are absolute worst case scenarios, in which the 30-day average water concentration remains continuously at the criterion concentration at all times. The third is a realistic scemario.

1. Steady concentrations at the criterion: this is worst-case continuous exposure. In the real world this could not occur because water concentrations vary substantially over time. For the 30-day average concentration not to exceed more than once in three years, the realistically varying daily concentrations must remain well below the criterion concentration a large majority of the time.

2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals (i.e., separated by 29 days of zero concentration) such that the 30-day average always equals the criterion. This is the worst-case intermittent scenario, attaining the criterion through a time series that continually maximizes the 30-day average exposure at the water criterion concentration while also imposing the highest variability possible from spikes of 1-day duration. In the real world intermittent runoff sources do not occur at uniform intervals: merely averaging 30-days between discharges would yield an exceedance each time the discharge occurred with less than 30-days spacing. Further, the once-per-month peak concentrations could never be controlled at exactly 30X the chronic water criterion per the above discussion of the first scenario.

   It is because they lack real-world random variability that the above two scenarios are not realistic. They are used as absolute worst cases for purposes of comparison. The following third scenario represents a realistic and indeed typical situation for continuous exposure:

3. Log-normally distributed, smoothly variable concentrations with the 30-day average exceeding the criterion once in three years when counted using the procedure of EPA (1986). The log

standard deviation of 0.5 applied here represents typical real-world time variability for continuously flowing waters. The log serial correlation coefficient $\rho = 0.8$ represents that typical of smaller streams.

With respect to maximizing toxic effects while attaining the criterion, Scenarios #1 and #2 are absolute worst cases. In contrast, Scenario #3 represents typical time variability in ambient waters. This third scenario requires randomly generated concentrations (having specified target statistical characteristics). Multiple runs of long series are therefore needed to assure some reasonable degree of accuracy. A minimum of 20 runs of random series of 3000 days were used. The concentrations at each half-day time step were generated by the following formula:

*C[t]water = C[t-1]water^(ρ′) * GM^(1-ρ′) * EXP{σ * SQRT(1-ρ′^2)*NORMSINV(RAND)}*

where ρ′ (rho prime) is the desired serial correlation coefficient between half-day time steps: ρ′=SQRT(ρ) [approximation], where ρ (rho) is the desired serial correlation coefficient between daily values; GM is the desired geometric mean or median, and σ is the desired log standard deviation. The above formula allows a time series with the desired statistical characteristics to be generated.

3.2.1 Model Results

3.2.1.1 Steady concentrations at the water criterion concentration.

No graphic is needed to explain this scenario. With water steady at its criterion, algae-detritus-sediment and invertebrates are likewise steady at their benchmark concentrations, and fish tissue is at its criterion concentration. For the 5th percentile taxon, the effect would thus be 10% since the concentration is steady at the EC10.

3.2.1.2 Uniformly spaced spikes at maximum concentrations

Figure J-2. Scenario 2, uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion. Read invertebrate and fish tissue concentrations on left scale, water concentrations on right scale. Time=0 does not represent the beginning of exposure; prior to Time=0 the same exposure pattern had been going on for a long time (e.g., 10,000 days).

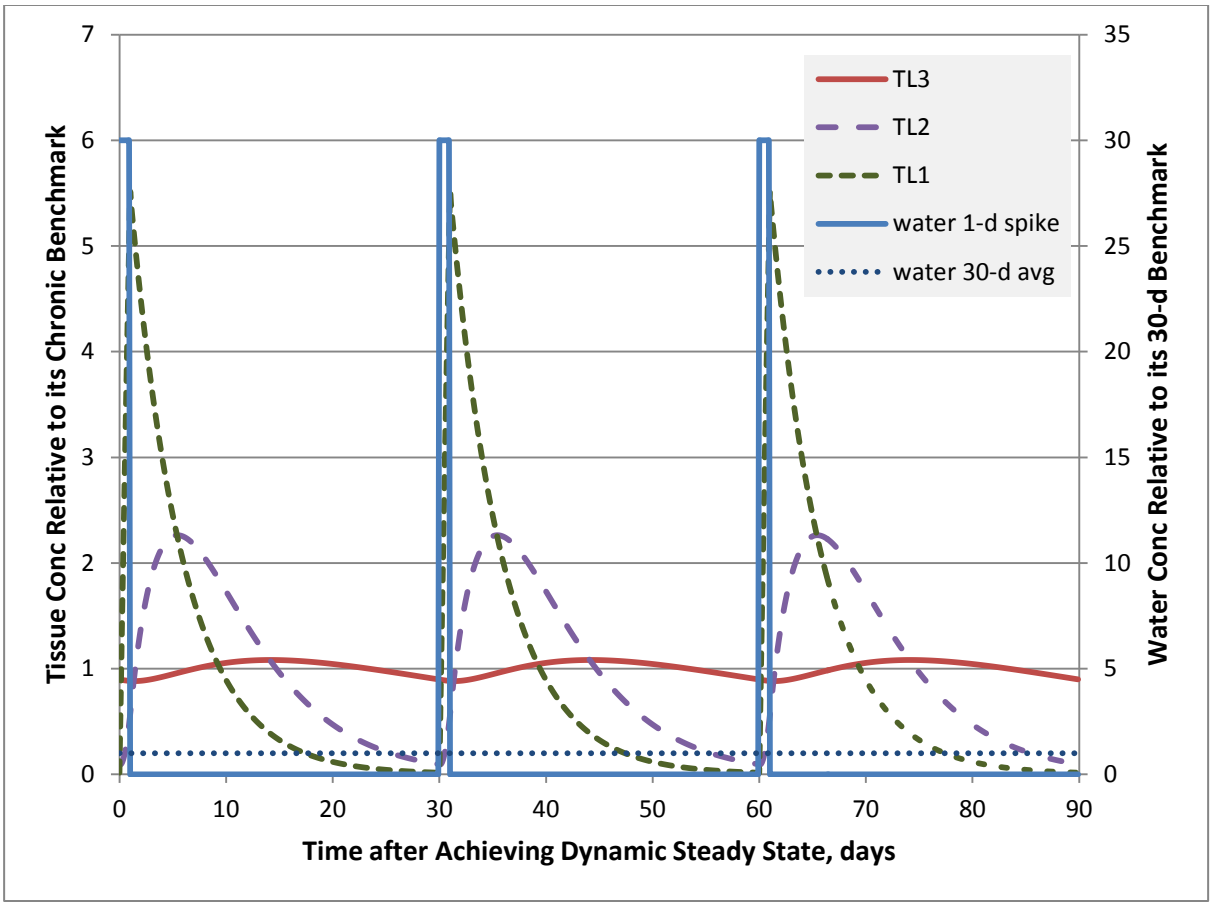


Figure J-2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion.
Tissue and water concentrations are expressed as dimensionless ratios relative to their respective criteria or benchmarks, as explained in the text.

With their more rapid kinetics, TL1 and TL2 tissue concentration swings are much more drastic than TL3 (fish) tissue concentration swings, but were the spike to continue as a steady exposure 30-fold above the water benchmark, TL1, TL2, and TL3 would all ultimately plateau at 30-fold above their respective benchmarks.

The key point here is that attaining the 30-day average via 1-day spikes spaced 30 days apart generates a small oscillation in fish tissue concentrations. Averaged over the 30-days, the fish tissue concentrations exactly attain their criterion and the predicted effect is 10%.

3.2.1.3 Log-normally distributed, smoothly varying concentrations

This is the most realistic scenarios, corresponding to typical variability observed in streams.

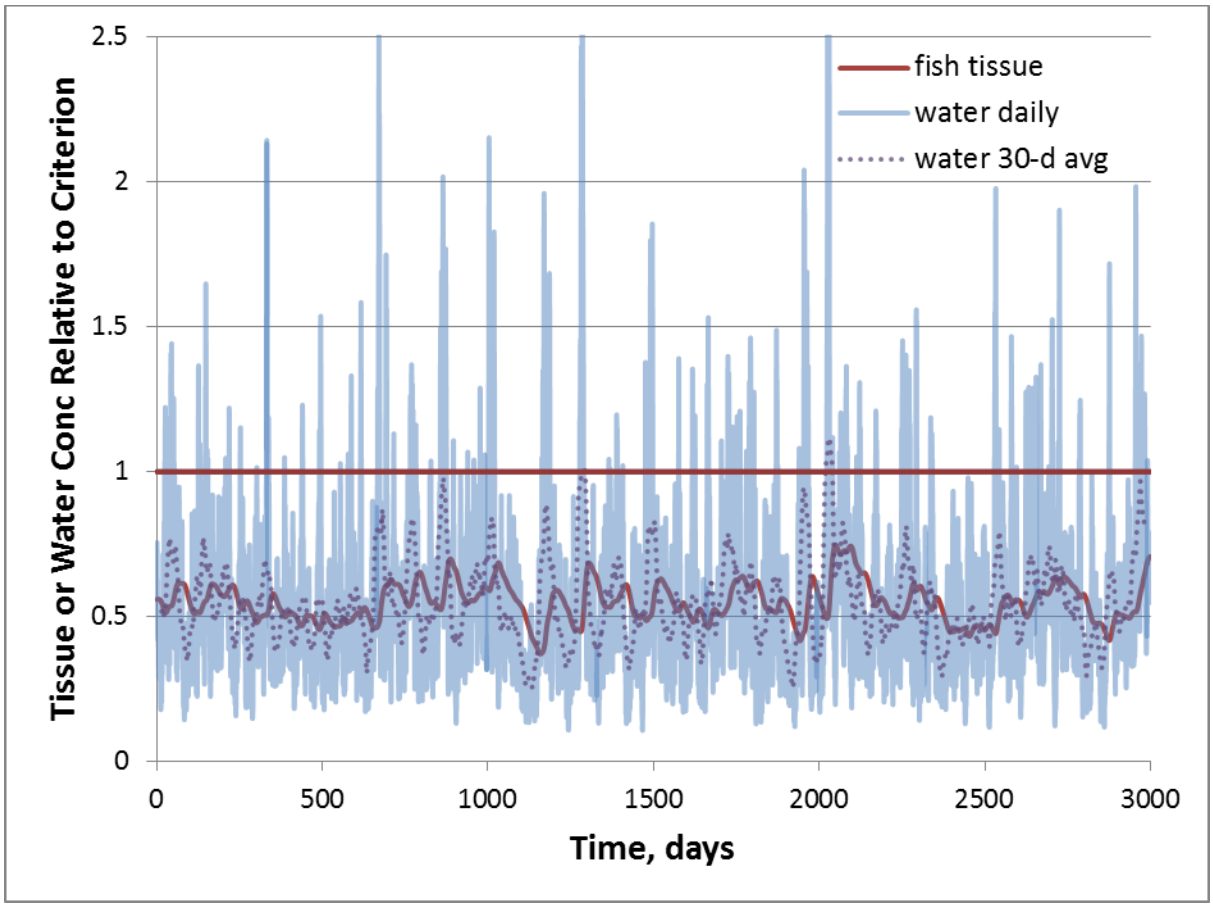


Figure J-3. A typical example of log-normally distributed, smoothly variable concentrations. The standard deviation of natural logs is 0.5 and the serial correlation coefficient of logs is 0.8 for daily values, both typical real-world situations. (The compression of 3000 days into the graph might make it difficult to recognize that the time series is smoothly varying – it has serial correlation.) At time=0, TL1, TL2, and TL3 begin at their average concentrations.

In the Figure J-3 example run, instantaneous water concentrations exceed the 30-day average criterion 7% of the time. The 30-day average concentrations exceed the criterion 1.05 times per 3 year period, counted per the EPA (1986) counting method. Tissue concentrations do not exceed their criterion at any time, and the aggregate effect is 0.12%.

In contrast to the previous scenario, the elevated concentrations here are random in their magnitude, duration, and spacing. This randomness reduces the average exposure (and aggregate effect) compatible with attainment of the 30-day average water target.

3.2.2 Summary of Scenario Results

Because Scenario 3 involves generation of random concentrations, the above graphs show just one run (3000 days) for each. Full results for the 20 runs of that scenario are shown below.

Scenario	Water: # 30-day avg. exceedances / 3-yr 1	Water: % of time exceeding	Tissue: % of time exceeding	Mean effect for 5th %ile Taxon	Comment
1. Steady	0.00	0.00	0.00	10.0	Steady at water and tissue benchmarks
2. Uniform spikes	0.00	3.33	56.7	10.0	30-d avg water conc. remains steady at benchmark (Fig. J2)
3. Smooth variable	1.01	7.8	0.00	0.18	Median=0.49 x benchmark, log stdev=0.5, rho(daily)=0.8 (e.g., Fig. 5) 2
1. Counting procedure for 30-d avg. exceedances is that of U.S. EPA (1986). 2. Results for Scenario 3 are average of 20 runs of 3000 days, each run with 0.6-1.4 exceedances / 3 yr. Runs not yielding exceedances within these bounds were not used. Among the 20 runs used, the effect CV=0.35.					

It can be concluded that the kinetics of selenium accumulation and depuration are sufficiently slow that applying a 30-day averaging period to the water criterion concentration affords protection even under unrealistic worst case conditions.

3.2.3 Example Responses to Increases in Water Concentrations

The previous Figures J-2 and J-3 illustrate situations after achievement of a dynamic steady state, where daily water concentrations change but longer-term mean water concentrations do not change. Given the same kinetic parameters as used above (i.e., yielding a 60-day characteristic time), this section addresses the rate at which tissue concentrations respond to increases in mean water concentrations, for example as would result from a new source. This is similar to the rising curve previously shown in Figure J-1b. The rapid kinetics used here for the water-TL1 step imply a small lotic system having little involvement of the bed sediments.

3.2.3.1 Step-function example

This example addresses the question: If water concentrations are increased to a level that is slightly too high, ultimately (at Time=∞) yielding fish-tissue concentrations at the EC20 instead of the EC10, how long would it take for those tissue concentrations to rise to a level that exceeds the (EC10-based) criterion?

Prior to Time=0 in this example the concentrations in TL3 had been at a moderate background concentration of 0.406 times the criterion, corresponding to the median West Virginia reference-site egg concentrations tabulated by West Virginia Department of Environmental Protection (2010). The concentrations in TL1 and TL2 are likewise assumed to have been at 0.406 normalized to their corresponding benchmarks. At Time=0 the water concentrations increase such that ultimately they will

produce an effect 10% higher than the target, thus at the EC20 of the hypothetical 5th percentile sensitive species. For typical selenium concentration-response slopes, this is 1.15-fold above the EC10. Figure J4 illustrates this scenario, which shows that 90 days are needed for TL3 concentrations to rise above the criterion.

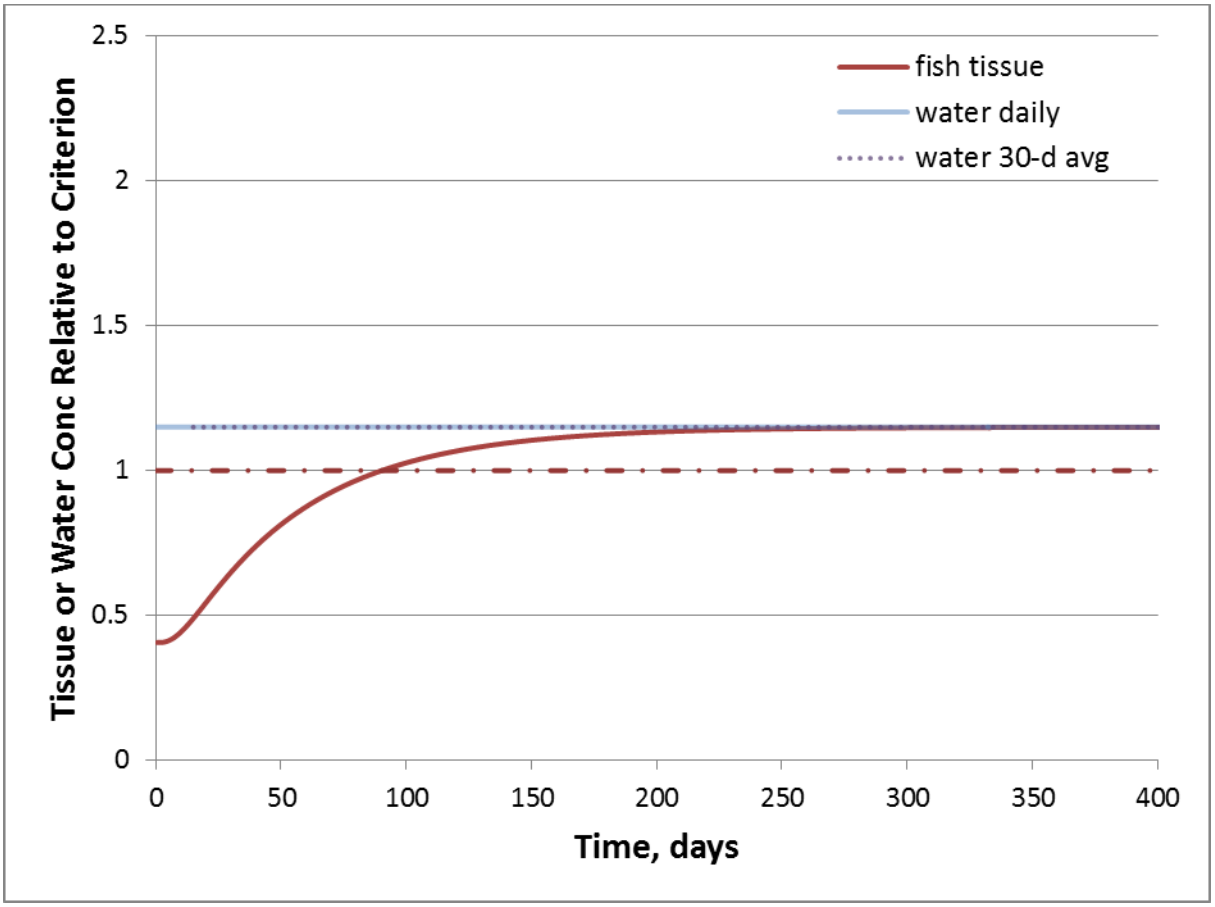


Figure J-4. TL3 concentration responding to a Time=0 step-function increase in water concentration that remains time-invariant thereafter.
Given that the water concentration is too high, ultimately yielding tissue concentrations at the hypothetical sensitive species EC20, 1.15-fold above the criterion, and given the previously presented kinetic parameters, it is calculated to take 90 days for TL3 concentrations to rise above the criterion.

3.2.3.2 Continuously time-variable example for flowing waters

To provide more realism, this example considers typical time variability, following up on Figure J3. In this example, prior to Time=0, TL1, TL2, and TL3 concentrations were at a low background concentration, 0.1 normalized to their criterion or respective benchmark. At Time=0 begin water concentrations having median = geometric mean = 0.49 normalized as a dimensionless ratio, concentration/criterion. Because the water concentrations are log-normally distributed, with log standard deviation = 0.5, the arithmetic mean is higher than the median and has the normalized value 0.56. If the simulation went on for a very long time, this time series (designed to have geometric mean 0.49 times the criterion, log standard deviation 0.5, and log serial correlation coefficient 0.8) would average one exceedance every three years, when exceedances are counted using the EPA (1986) approach. Figure J-5 shows a typical short series of 400 days.

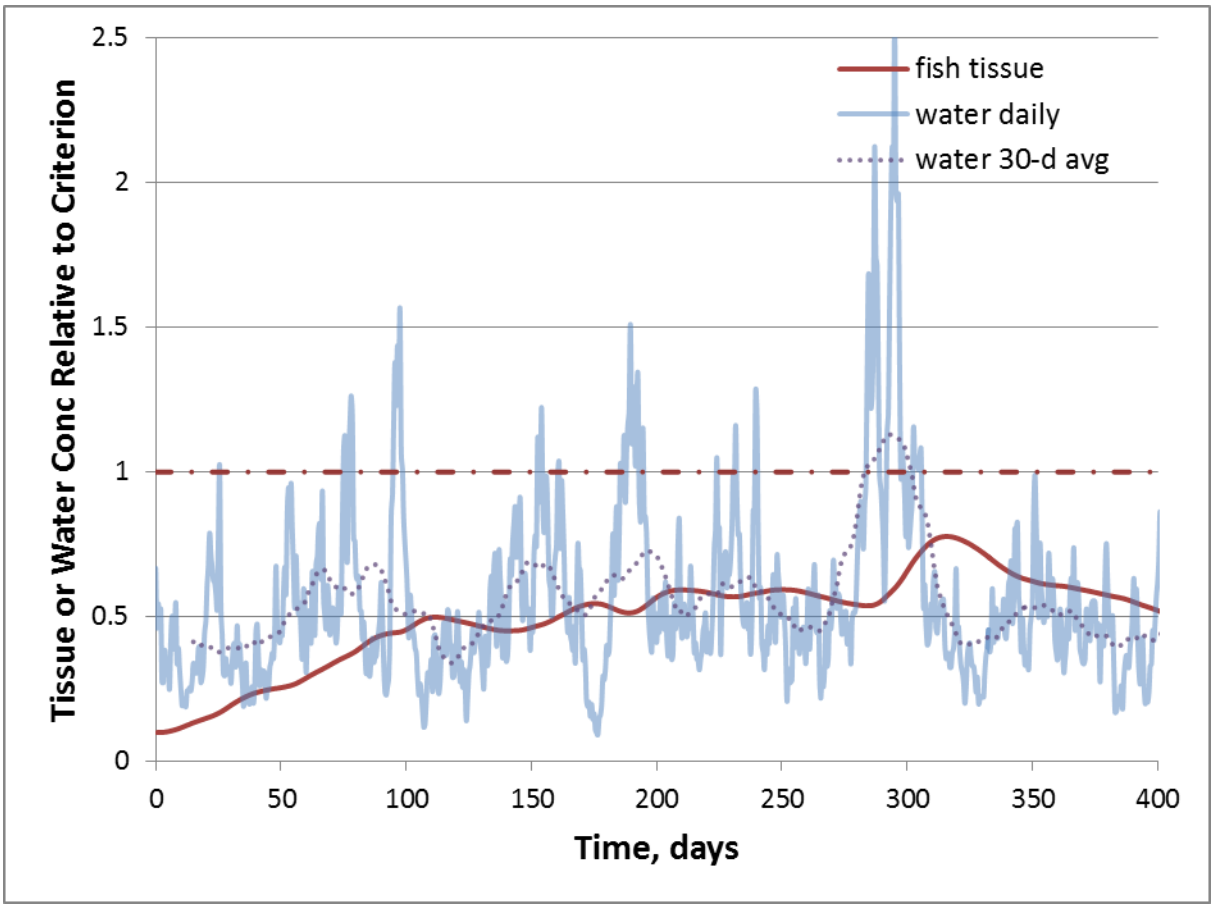


Figure J-5. Flowing water example of TL3 concentration starting at a concentration of 0.1 normalized to the criterion, and responding to randomly varying log-normally distributed water concentrations having median 0.49 (expressed as a dimensionless ratio: concentration/criterion), log standard deviation 0.5, and log serial correlation coefficient 0.8.
Again, all concentrations are as dimensionless ratios relative to the criteria concentrations.

Several points are worth noting. Because the water concentrations happen (by chance) to be below average for the first 50 days, the TL3 concentrations rise somewhat slowly during that period. Were they to be above average during that period, the TL3 concentrations would more rapidly approach their dynamically varying plateau. In such a short time series it is not graphically apparent what the long-term average TL3 concentration will be; however, because the long-term arithmetic mean water concentration would be 0.56 (normalized the its criterion), the TL3 concentration would likewise end up averaging 0.56 normalized to its criterion, if tracked for many years.

It is also worth noting that most 400-day series of the type shown in Figure J-5 would not have occurrences of 30-day average concentrations above the criterion (as suggested by Figure J-3). This particular random series does have a period of 30-day average exceedances, near Day 300, but it does not persist long enough to cause the TL3 concentration to approach its criterion.

Lastly, it should be noted that when concentrations are randomly varying as in Figure J-5, the water concentrations that one observes are highly dependent on when the samples are taken. The TL3 concentrations observed are far less dependent on when the samples are taken (after the plateau is approached), but time variations, although muted, are still present.

The example scenarios depicted here show lotic time to steady state of approximately 3 months to less than 1 year under different discharge scenarios including both continuous and intermittent discharges. The scenarios also assume that the new selenium input is from one source; multiple new sources particularly with varying discharge patterns, might have a different response time and pattern for various trophic levels.

The example is likely not appropriate for lentic systems, because they would not be expected to have the rapidly varying water concentrations of Figure J-5. In addition, the water-to-TL1 kinetics would likely be slower in lentic systems with new or time-varying sources because of the role of bottom sediments acting as a reservoir in recycling selenium. Ultimately this should yield slower rising and smoother TL3 concentrations compared to those in Figure J-5.

APPENDIX K: TRANSLATION OF A SELENIUM FISH TISSUE CRITERION ELEMENT TO A SITE-SPECIFIC WATER COLUMN VALUE

1.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING MECHANISTIC BIOACCUMULATION MODELING

Introduction:

EPA recommends fish tissue elements of the selenium criterion supersede water column elements under steady state conditions because the selenium concentration in fish tissue is a more sensitive and reliable indicator of the negative effects of selenium in aquatic life. However, implementation of a fish tissue criterion element can be challenging because many state and tribal Clean Water Act (CWA) programs prefer the expression of water quality criteria as an ambient concentration in the water-column. Therefore, EPA also recommends two monthly average water-column criterion elements, one for lotic (flowing) waters, and the other for lentic (still) waters. EPA derived all water column criterion elements from the egg/ovary criterion element representing a protective selenium concentration for fish species populations. Thus the water column criterion elements also represent protective selenium concentrations for fish species populations. If threatened or endangered fish species are present, states and tribes may need to derive alternative water column elements with a refined protection goal that account for site-specific bioaccumulation characteristics.

EPA derived water-column criterion elements by modeling selenium bioaccumulation in aquatic systems. The EPA worked with the United States Geological Survey to derive a translation equation utilizing a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al., 1992; Wang et. al., 1996; Luoma and Fisher, 1997; Wang, 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006; Presser and Luoma 2010; Presser 2013). EPA translated the selenium egg-ovary criterion element into two set(s) of site-specific water concentration values (lentic and lotic), and used the distribution(s) of those water column values to derive the respective water-column criterion elements. This appendix describes approaches that states and tribes may choose to use regarding application of this same mechanistic modeling approach (or alternatively an empirical bioaccumulation factor (BAF) approach) to translate a fish tissue criterion element (egg-ovary, whole body, or muscle) into site-specific water-column concentrations to more precisely manage selenium in specific aquatic systems.

The relationship between the concentration of selenium in the tissues of fish and the concentration of selenium in the water column can vary substantially among aquatic systems. The species of fish, the species and proportion of prey, and a variety of site-specific biogeochemical factors affect selenium bioaccumulation and thus determine the allowable concentration of selenium in ambient water protective of aquatic life. States and tribes may choose to adopt the results of site-specific water column translations as site-specific criteria (SSC) or adopt a translation procedure into state or tribal water quality

standards. Under both options, the water quality standards revisions must be approved by EPA under Section 303(c) of the Clean Water Act. If a state or tribe adopts a translation procedure that will be implemented by other CWA programs, it must be scientifically defensible, produce repeatable, predictable outcomes, and result in criteria that protect the applicable designated use. Examples of such approaches include the mechanistic modeling approach and the empirical BAF approach described within this Appendix.

EPA considered both mechanistic and empirical modeling approaches to translate the selenium egg-ovary criterion element into water column concentration elements. A mechanistic modeling approach uses scientific knowledge of the physical and chemical processes underlying bioaccumulation to establish a relationship between the concentrations of selenium in the water column and the concentration of selenium in the tissue of aquatic organisms. The mechanistic modeling approach enables formulation of site-specific models of trophic transfer of selenium through aquatic food webs and translation of the egg-ovary criterion element into an equivalent site-specific water concentration. The empirical modeling approach establishes a relationship between concentrations of selenium in fish tissue and ambient water directly by measuring selenium concentrations in both media and calculating the ratio of the two concentrations. The ratio (BAF) can then be used to estimate the target concentration of selenium in the water column as related to the adopted fish tissue element.

Both the mechanistic and empirical modeling approaches have advantages and disadvantages that should be considered before deciding which approach to use. On the one hand, the mechanistic modeling approach has the advantage of not requiring extensive fish tissue sampling and analysis by using knowledge of aquatic system food webs. However, uncertainty in the selection of model parameters increases uncertainty in the outcome leading to a reduction in defensibility. Of particular concern with respect to the mechanistic model EPA developed is the selection of the value for the enrichment factor parameter *EF* (discussed in more detail below). On the other hand, the empirical BAF approach is conceptually and computationally simpler because it relies only on field measurements and does not require extensive knowledge of the physical, chemical, or biological characteristics of the aquatic system. However, obtaining a sufficient number of measurements in fish tissue and water may be logistically difficult and/or more expensive.

The appropriate modeling approach to use when translating the selenium egg-ovary criterion element to a site-specific water-column concentration depends on individual circumstances and site-specific characteristics. The mechanistic modeling approach may be a useful method in situations where there is little or no data on the amount of selenium in an aquatic system, the empirical BAF approach may be desirable in circumstances where in fish tissue and water data are available. Below is a description of

methodology than can be used to translate the egg-ovary criterion element to a site-specific water-column concentration for site-specific management of selenium.

1.1 Relating the Concentration of Selenium in Fish Tissue and Water using the Mechanistic Modeling Approach

The relationship between the concentration of selenium in the eggs or ovaries of fish and the concentration of selenium in the water column is given in Equation K-1 (Equation 18 from the main text):

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \textit{(Equation K-1)}$$

Where:

C_{water} = the concentration of selenium in water (µg/L),

$C_{egg\text{-}ovary}$ = the concentration of selenium in the eggs or ovaries of fish (µg/g),

$TTF^{composite}$ = the product of the trophic transfer factor (*TTF*) values of the fish species that is the target of the egg-ovary criterion element and the *TTF* values of all lower trophic levels in its food web (no units of measurement, see explanation below).

EF = the steady state proportional bioconcentration of dissolved selenium at the base of the aquatic food web (L/g),

CF = the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues (no units of measurement).

The basic principles expressed in Equation K-1 are illustrated in the conceptual model shown in Figure K-1.

Selenium dissolved in surface water enters aquatic food webs by becoming associated with trophic level 1 primary producer organisms (e.g., algae) and other biotic (e.g., detritus) and abiotic (e.g., sediment) particulate material. An enrichment function (*EF*) quantifies the bioconcentration of selenium in particulate material and thus its bioavailability in the aquatic system. The parameter *EF* is a single value that represents the steady state proportional concentration of selenium in particulate material relative to the concentration of selenium dissolved in water.

Organic particulate material is consumed by trophic level 2 organisms (usually aquatic invertebrates, but also some fish species that are herbivores/detritivores) resulting in the accumulation of selenium in the tissues of those organisms. Trophic level 2 invertebrates are consumed by trophic level 3 fishes resulting in further accumulation of selenium in the tissues of those fish. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer factor (*TTF).* A *TTF* is a single value that represents the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. Different species of organisms metabolize selenium in different ways. Thus each species is associated with a specific *TTF* value. Because the trophic transfer of selenium through all trophic levels is mathematically equal to the product of the individual *TTF* values, all consumer-resource interactions in a particular aquatic ecosystem are simplified in Equation K-1 by representing the product of all the individual *TTF* values as the single parameter $TTF^{composite}$.

Fish accumulate selenium in different tissues of the body in differing amounts. Species physiology, age, diet, sex, and spawning status are some of the factors that affect selenium partitioning in body tissues. Because the primary selenium criterion element is expressed as a concentration in the eggs and/or ovaries, a conversion factor (*CF*) quantifies the relationship between the concentration of selenium in the eggs and/or ovaries and the average concentration of selenium in the whole body or muscle tissues. The parameter *CF* in Equation K-1 is a single value that represents the steady state proportional concentration of selenium in the eggs and/or ovaries relative to the average concentration of selenium in all body tissues. Different species of fish accumulate selenium in their eggs and ovaries to different degrees. Thus each species of fish is associated with a specific *CF* value.

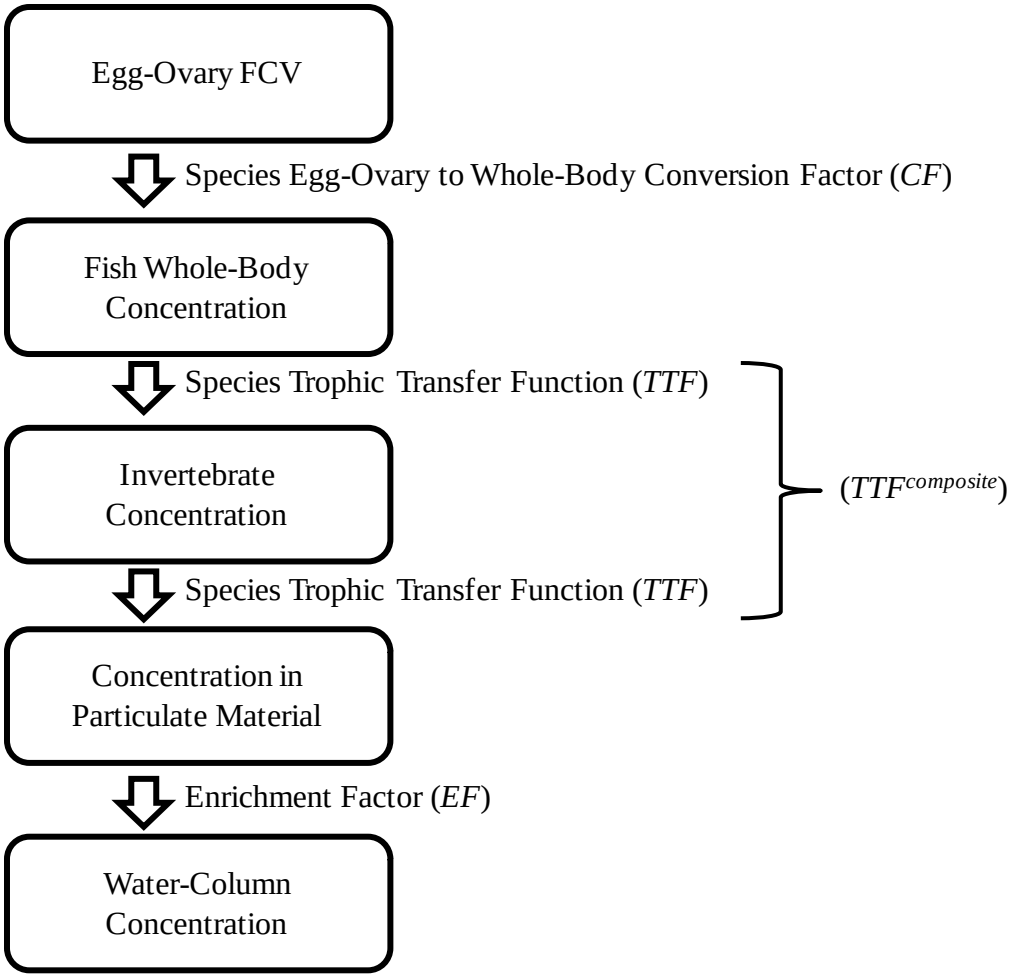


Figure K-1. Conceptual model for translating the egg-ovary FCV to a water-column concentration.
Note: States may want to use the whole body or muscle criterion elements as the starting point for site specific translation to a water column concentration.

Once the parameters that quantify the transfer of selenium through each step in this pathway are identified, they can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific concentration of selenium in the water column (i.e., target water column concentration).

Because each *TTF* value is species-specific, it is possible to differentiate bioaccumulation in different aquatic systems by modeling the food web of the target fish species. For example, where the food web contains more than 3 trophic levels, $TTF^{composite}$ can be represented as the product of all *TTF* values for each trophic level given in Equation K-2, which is a generalization of Equation 10 from the main text:

$$TTF^{composite} = TTF^{TL2} \times TTF^{TL3} \times \ldots \times TTF^{TLn} \qquad \textit{(Equation K-2)}$$

Where:

$TTF^{composite}$ = the product of all *TTF* values at all trophic levels.

TTF^{TLn} = the *TTF* value of the highest trophic level.

The consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* value at a particular trophic level as the average *TTF* values of all species at that trophic level weighted by the proportion of species consumed given as Equation K-3 (Equation 11 in the main text):

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \textit{(Equation K-3)}$$

Where:

TTF_i^{TLx} = the trophic transfer factor of the i^{th} species at a particular trophic level

w_i = the proportion of the i^{th} species consumed.

These concepts can be used to formulate a mathematical expression of $TTF^{composite}$ that models selenium bioaccumulation in a variety of aquatic ecosystems. Figure K-2 illustrates five hypothetical food web scenarios and the formulation of $TTF^{composite}$ for each of them. For each scenario, the value of $TTF^{composite}$, the *CF* value associated with the targeted fish species, and the site-specific *EF* value can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific water concentration value. The hypothetical food web models in Figure K-2 are a few possible examples of food web models for illustrative purposes. It is desirable to derive and use of a food web model that best represents the aquatic system for which the water column translation will apply. The general steps for deriving a site-specific translation of the egg-ovary criterion element to a water concentration value are described below.

A) Three trophic levels (simple):

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$



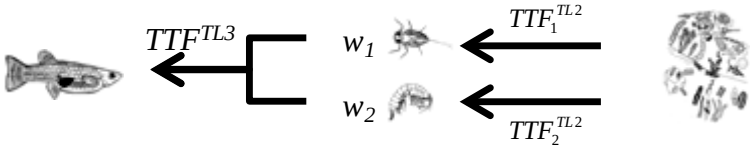
B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$



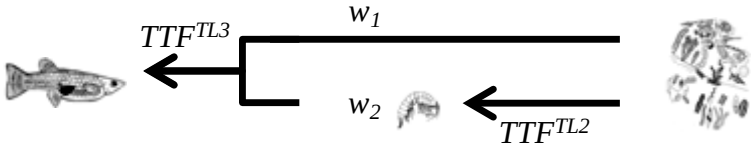
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

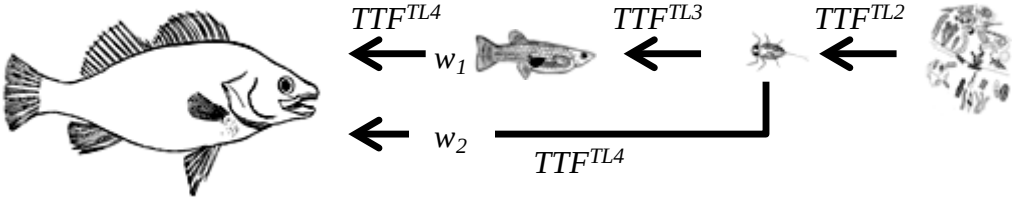


Figure K-2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios.

$TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food web model is used to calculate a value for $TTF^{composite}$ using appropriate species-specific *TTF* values and the proportions of each species consumed at each trophic level. See text for further explanation.

1.2 Steps for Deriving a Site-Specific Water Concentration Value from the Egg-Ovary Criterion Element

Below are the steps for deriving a site-specific water concentration value from the selenium egg-ovary criterion element using EPA's mechanistic model approach:

1) Identify the appropriate target fish species.
2) Model the food web of the targeted fish species.
3) Identify appropriate *TTF* values by either:
    a. selecting the appropriate *TTF* values from a list of EPA-derived values, or
    b. deriving *TTF* values from existing data, or
    c. deriving *TTF* values by conducting additional studies, or
    d. extrapolating *TTF* values from existing values.
4) Determine the appropriate value of *EF* by either
    a. deriving a site-specific *EF* value from field measurements, or
    b. deriving an appropriate *EF* value from existing data, or
    c. extrapolating from *EF* values of similar waters.
5) Determine the appropriate *CF* value by either,
    a. selecting the appropriate *CF* value from a list of EPA-derived values, or
    b. deriving a *CF* value from existing data, or
    c. deriving a *CF* value by conducting additional studies, or
    d. extrapolating a *CF* value from existing values.
6) Translate the selenium egg-ovary criterion element into a site-specific water concentration value using Equation K-1.

Below are detailed descriptions of each step followed by example calculations using a variety of hypothetical scenarios. EPA is providing this information to support help states and tribes that choose to develop selenium water column values from the egg-ovary criterion element or develop translation procedures. Successful application of the mechanistic approach described here requires use of particular food web models and parameter values that are appropriate for particular aquatic systems.

1.2.1 Identify the Appropriate Target Fish Species

1.2.1.1 When fish are present

In developing a site-specific translation of the egg-ovary criterion element, the user wshould select whether to use a mechanistic model or empricial (BAF) approach. This decision will in large part

determine the data and information requirements. A mechanistic model approach will likely require information on the spatial and temporal distribution of aquatic organisms, and may require measurements of selenium in ambient water and particulate material. An empirical model approach will use measurements of selenium is fish tissue and ambient water.

Developing a site-specific translation of the egg-ovary criterion element will also entail selection of which species of fish to target. The concentration of selenium in eggs and ovaries is the most sensitive and consistent indicator of toxicity. However, toxicity and bioaccumulation potential can vary among species. Species in the families Acipenseridae, Centrarchidae, and Salmonidae are particularly sensitive to selenium (Table 3.3 in the main document), whereas species such as stoneroller species, creek chub, blackside dace, and white sucker have documented tolerance to selenium and can be found in selenium contaminated systems (NAMC 2008, Presser 2012). Green sunfish accumulate less selenium than other species with comparable exposures in the same aquatic system (Hitt and Smith 2015). Selection of the fish species in the aquatic system with the greatest selenium sensitivity and bioaccumulation potential is recommended.

Several additional factors should also be considered in deciding which species to target when developing a site-specific translation of the egg-ovary criterion element. Anadromous species (species that migrate from salt water to spawn in fresh water) should generally avoided because selenium exposure and bioaccumulation occurs over a relatively long period through the consumption of locally contaminated aquatic organisms. Additionally considerations include whether the fish species selected typically consume organisms known or suspected to readily bioaccumulate selenium (e.g., mollusks). For example, high concentrations of selenium in San Francisco Bay white sturgeon are associated with their consumption of *Potamocorbula amurensis*, a bivalve in close proximity to selenium-contaminated sediments that rapidly and efficiently accumulates selenium (Stewart et al. 2004). In contrast, striped bass from the same aquatic system have substantially lower concentration of selenium in their tissues because their zooplankton-based food web has substantially lower selenium bioaccumulation characteristics (Schlekat et al. 2004; Stewart et al. 2004). The 2016 selenium criterion was developed for freshwater, but if considering other ecosystems, it may be worth noting that salinity may also affect bioaccumulation of selenium. Freshwater mollusks tend to have relatively higher *TTF* values when compared to other freshwater invertebrate taxa (e.g., aquatic insects), but they are lower than mollusks in marine or brackish systems (and particularly *P. amurensis*, an invasive clam in the San Francisco Bay). In aquatic systems with resident fish species of unknown selenium sensitivity and bioaccumulation potential, other factors such as ecological significance could be considered when choosing a target species.

Data from fisheries or biological surveys or other biological assessments could be considered to determine the fish species that reside in specific surface waters. State and tribal resource agency personnel

familiar with fish sampling activities could also be a source of information on resident fish species. General information on the fish species present in state and tribal surface waters may also be found at:

- State Fish and Game agencies
- U.S. Fish and Wildlife Service (http://www.fws.gov)
- U.S. Geological Survey (http://www.usgs.gov)
- NatureServe.org (http://www.natureserve.org)
- Fishbase (http://www.fishbase.org)
- State or local sources of biological information (e.g. Biota Information System of New Mexico at http://www.bison-m.org)

Measurements of selenium in fish tissue would most reflect the ecosystem if adult (reproductively mature) fish are sampled. Selenium measurements in fish tissue will likely be more stable in adult fish because they are more likely to have a stable prey base. Reproductively mature (ripe or gravid) females would be needed for measures selenium in eggs and/or ovary tissue for comparison to the the egg-ovary tissue criterion element. It would be prudent to avoid sampling ovary tissue "post-spawn" due to a potential decrease in selenium concentration presumably due to the loss of selenium through spawning and release of eggs with relatively high concentrations of selenium. Consideration of closely related taxonomic surrogates (same genus or family) for threatened or endangered species may be useful.

Figure K-3 shows an example decision tree that may help in selection of the appropriate fish species for deriving a site-specific water concentration value from the selenium egg-ovary, whole-body, or muscle FCV. The use of taxonomic hierarchies for anlysis utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993). Additional information on fish tissue sampling (e.g., species selection, temporal and spatial considerations) is under development and will be published in the form of a technical support document (TSD) by the EPA in the near future.

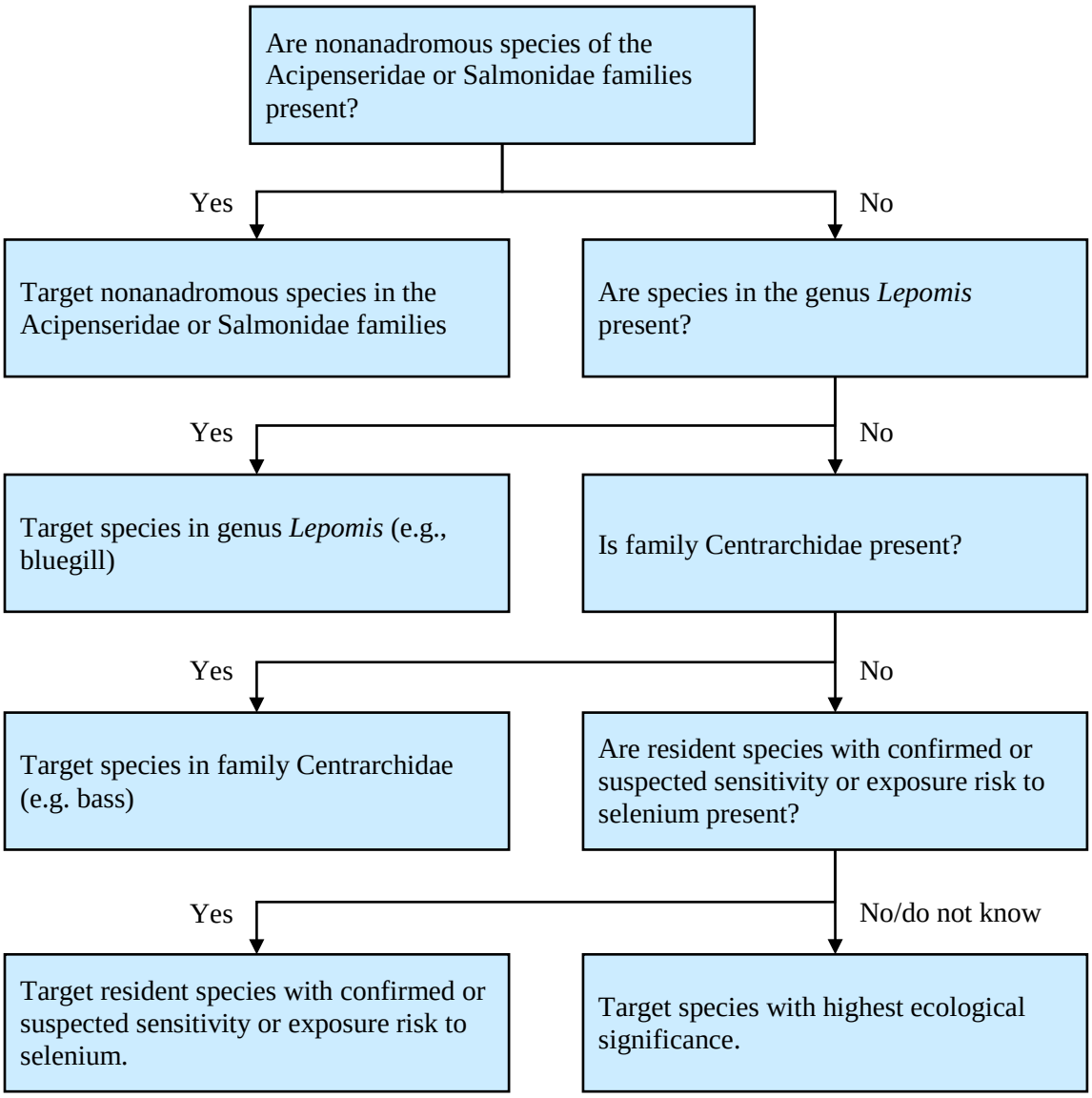


Figure K-3. Recommendeed decision process for selection of the fish species to use when deriving a water concentration from the selenium egg-ovary FCV.
This decision tree is also generally applicable when using the whole body or muscle tissue as the starting point for development of SSC, particularly when using the BAF approach.

1.2.1.2 When fish are absent from a site

Some aquatic systems do not contain resident fish. Fish may be absent from a waterbody because of intermittent or persistent low flows, physical impediments such as waterfalls or impoundments, lack of adequate habitat for feeding and/or spawning, or intolerable aquatic conditions related to pH, turbidity, temperature, salinity, total dissolved solids, chemical contaminants, or pathogens. These conditions could be due to natural or anthropogenic causes. Some streams may be naturally intermittent or ephemeral, or

they might exhibit low or intermittent flows because of impoundments or water draw-down for agricultural irrigation, industrial uses, drinking water supply, or other uses.

When fish are absent from a waterbody, consideration of sampling the most sensitive fish species inhabiting nearby, most proximate downstream waters may be useful in order to understand selenium bioaccumulation potential in such systems. Although the upper reaches of some aquatic systems may not support fish communities, the invertebrate organisms that reside there may tolerate high concentrations of selenium and pose a selenium risk to predator fish if transported downstream. Users may choose to evaluate upstream waters without fish by measuring the selenium concentration in water, biotic and/or abiotic particulate material, and/or the tissues of invertebrate aquatic organisms that reside there. Because selenium associated with particulate material and invertebrate organisms can be transported downstream during intermittent high flows, elevated concentrations of selenium in the tissues of downstream fish could indicate upstream sources of selenium that require a more detailed evaluation of upstream conditions.

1.2.2 Model the Food-Web of the Targeted Fish Species

After selecting the target fish species, model users should formulate a mathematical expression of the target species food-web that will be used to calculate the value of $TTF^{composite}$. As discussed previously, $TTF^{composite}$ is the product of the *TTF* values across trophic levels of the target fish species food-web. The complexity of the food-web model will depend on the species of fish that is targeted, the diversity of prey species in the aquatic system, and the amount of information that is available. Many of the same information sources used to identify the targeted fish species in a waterbody could also be used to obtain information about its food web. The types and proportions of food organisms the targeted fish species consumes can be directly assessed through studies that examine stomach contents or from information gathered through biological assessments. If site-specific information is not available, model users could estimate the target fish species food-web using publicly available databases such as NatureServe (http://www.natureserve.org). For example, the NatureServe database record for fathead minnow in the HUC watershed #5040004 in Ohio indicates under the heading: “Ecology and Life History - Food Comments,” the fathead minnow “feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990).”

Additional sources of information include:

- FishBase (http://www.fishbase.org). FishBase is a relational database developed at the World Fish Center in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and many other partners.

- Carlander, K.D. Handbook of Freshwater Fishery Biology, volumes 1, 2 and 3. Iowa state University Press, Ames, Iowa. 1969-1997.

1.2.3 Identify Appropriate *TTF* Values

The food-web model uses appropriately selected species-specific *TTF* values (and, if appropriate, proportions within the same trophic level). Model users identify the appropriate *TTF* values by using one of the following four procedures, or by using other scientifically defensible methods.

1.2.3.1 Select the appropriate *TTF* values from the provided list of EPA-derived values

Species-specific TTF values represent the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. EPA-derived *TTF* values for aquatic invertebrates and fish are provided in Tables K-1 and K-2 (Tables 3.10 and 3.11 in main text; see also main text for a complete explanation of the procedure EPA used to derive these values).

Table K-1. EPA-derived Trophic Transfer Factor (TTF) values for freshwater aquatic invertebrates.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Crustaceans					
amphipod	*Hyalella azteca*	-	-	-	1.22
copepod	copepods	0.520	0.420	0.155	1.41
crayfish	*Astacidae*	-	-	-	1.46
water flea	*Daphnia magna*	0.406	0.210	0.116	0.74
Insects					
dragonfly	*Anisoptera*	-	-	-	1.97
damselfly	*Coenagrionidae*	-	-	-	2.88
mayfly	*Centroptilum triangulifer*	-	-	-	2.38
midge	*Chironimidae*	-	-	-	1.90
water boatman	*Corixidae*	-	-	-	1.48
Mollusks					
asian clam[a]	*Corbicula fluminea*	0.550	0.050	0.006	4.58
zebra mussel	*Dreissena polymorpha*	0.260	0.400	0.026	4.00
Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	zooplankton	-	-	-	1.89

[a] Not to be confused with *Potamocorbula amurensis*

Table K-2. EPA-derived Trophic Transfer Factor (*TTF*) values for freshwater fish.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79

Common name	Scientific name	AE	IR	k_e	TTF
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

The *TTF* values from these lists could be used exclusively, or in conjunction with *TTF* values obtained from other sources (see below). Note that these tables do not represent an exhaustive list of all *TTF* values that may be required to calculate a site-specific water concentration value. If this list does not include a required *TTF* value, another approach could be considered to obtain an appropriate value.

1.2.3.2 Deriving *TTF* values from existing data

If model users cannot obtain one or more required *TTF* values from Tables K-1 and/or K-2, species-specific *TTF* values could be derived using existing data. One approach for deriving species-specific *TTF* values is to use the physiological coefficients representing food ingestion rate (*IR)*, selenium efflux rate (k_e), and selenium assimilation efficiency (*AE*) to calculate a *TTF* value using Equation K-4 (Equation 3 from the main text, Reinfelder et al. 1998**)** given as:

$$TTF = \frac{AE \times IR}{k_e} \qquad \textit{(Equation K-4)}$$

Where:

TTF = species-specific trophic transfer factor

AE = species-specific assimilation efficiency (%)

IR = species-specific ingestion rate (g/g-d)

k_e = species-specific efflux rate constant (/d)

The physiological coefficients *IR*, *AE* and are species-specific values. Values for AE and k_e can only be derived from laboratory studies. Values for IR may be derived from laboratory studies or obtained from published literature. After the three physiological coefficients are obtained, a *TTF* value can be calculated using Equation K-4.

Another way to derive species-specific *TTF* values is to empirically assess the relationship between the selenium concentration in the tissue of organisms and the selenium concentration in the food they consume using paired measurements from field studies. Species-specific *TTF* values can be derived from such measurements by calculating ratios, using regression techniques, or other scientifically defensible methods.

Model users could choose to use the same approach EPA used to calculate species-specific *TTF* values. EPA derived *TTF* values using a combination median and regression approach. EPA defined the *TTF* value for any trophic level as:

$$TTF^{TLn} = \frac{C_{tissue}^{TLn}}{C_{food}^{TLn}} \qquad \textit{(Equation K-5)}$$

Where:

TTF^{TLn} = The trophic transfer factor of a given trophic level,

C_{tissue}^{TLn} = The selenium concentration (mg/kg dw) in the tissues of the consumer organism,

C_{food}^{TLn} = The selenium concentration (mg/kg dw) in the consumer organism's food.

EPA used the median of the ratios given in Equation K-5 as the species-specific *TTF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear

regression analysis. EPA considered the relationship acceptable if a linear regression of tissue selenium concentration on food selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope (i.e., selenium concentrations in the consumer increases with increasing selenium in food).

1.2.3.3 Deriving *TTF* values by conducting additional studies

Additional studies could be conducted to obtain the data needed to derive *TTF* values for specific needs, or to revise existing *TTF* values, if the existing *TTF* values do not appear to be appropriate for a particular aquatic system.

1.2.3.4 Extrapolating *TTF* values from existing values

If one or more necessary *TTF* values are not available, and the information needed to derive a species-specific *TTF* value is not available or impractical to obtain, model users could consider extrapolating a new *TTF* value from other known *TTF* values. One possible method to extrapolate a *TTF* value is to sequentially consider higher taxonomic classifications until one or more of the organisms with a known *TTF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *TTF* values, the average *TTF* from the matching table entries could be used. The use of taxonomic hierarchies in this way utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993).

EPA used such an extrapolation approach to derive some of the *TTF* values necessary to develop the water column criterion elements. For example, the *TTF* value for *Chrosomus eos* (northern redbelly dace) was not available. *TTF* values were also not available for other species in the genus *Chrosomus*, but *TTF* values were available for species in the family Cyprinidae, including *Rhinichthys atratulus* (blacknose dace), *Cyprinus carpio* (common carp), *Semotilus atromaculatus* (creek chub), *Pimephales promelas* (fathead minnow), *Cyprinella lutrensis* (red shiner), *Richardsonius balteatus* (redside shiner), and *Notropis stramineus* (sand shiner). Because Cyprinidae is the lowest taxonomic classification where *Chrosomus eos* matches one or more species with an available *TTF* value, EPA used the median *TTF* value of blacknose dace, common carp, creek chub, fathead minnow, red shiner, redside shiner, and sand shiner as the *TTF* value for northern redbelly dace.

1.2.4 Determine the Appropriate *EF* Value

The selenium enrichment function (*EF*) value represents the bioavailability of selenium at the base of the aquatic food web. The base of the aquatic food web includes phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma, 2010). EPA refers to this mixture of living and non-living entities as particulate material. The parameter *EF* varies more widely across aquatic systems than any other parameter, and is influenced by

the source and form of selenium, water residence time, the biogeochemical characteristics of the waterbody, and the type of particulate matter collected. Because *EF* can vary greatly across waterbodies, this parameter has the greatest potential to introduce uncertainty in the translation from an egg-ovary selenium concentration to a water column concentration. For this reason, use *EF* values derived from site-specific data is recommended whenever possible in applying the model. One of the following four procedures could be used to derive *EF* values, or other scientifically defensible methods could be used.

1.2.4.1 Deriving a site-specific *EF* value from field measurements

Equation 12 from the main text defines the parameter *EF* as the ratio of the concentration of selenium in particulate material to the concentration of selenium dissolved in water given as:

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \textit{(Equation K-6)}$$

Where:

$C_{particulate}$ = Concentration of selenium in particulate material (µg/g)

C_{water} = Concentration of selenium dissolved in water (µg/L)

EF = Enrichment Function (L/g)

To calculate a site-specific *EF* value, EPA first calculates the ratio of each individual particulate measurement and its associated water measurement (if more than one water measurement is available for any given particulate measurement, the median water measurement is used). If more than one ratio for any given category of particulate material is available (e.g., more than one ratio of algae to water), EPA takes the median of the ratios. EPA then calculates the geometric mean of the median ratios for each category of particular material as the site *EF* value. EPA only uses sediment measurements if there are at least one measurement from either algae or detritus.

Deriving a site-specific *EF* value in this manner is a relatively straightforward procedure. However, consideration of data that appropriately accounts for the spatial and temporal variability of an aquatic system would be useful in the development of any sampling plan. Aquatic system characteristics such as dimension, volume, shape, residence time, velocity, and growing season are a few important factors that should be considered in designing a sampling plan that will adequately account for variability. State and Federal agencies (USGS, ACOE) as well as watershed groups may be useful sources of information that can help characterize the temporal and spatial variability at a particular aquatic system. When developing the selenium criterion, EPA observed a relatively lower correlation between the selenium concentration in water and abiotic (benthic sediments) particulate samples compared to the same analysis between water and biotic (algae and detritus) particulate samples, resulting in EPA's decision

that calculation of any site-specific *EF* values include information from at least one type of biotic particulate indeveloping its criteiron. Prioritization of sampling of biotic particulate material over abiotic samples should be considered. Regaridng selenium measurements from abiotic particulate material, consideration of utilizing at least one type of biotic particulate material when deriving the *EF* value of an aquatic system is recommended.

Site-specific *EF* values using particulate and water samples that are as spatially and temporally coincident as possible would be considered the most robust. Although EPA's analysis of particulate and water samples from a sample population of aquatic systems found that samples taken within one year of each other, based on data availability, were appropriate in deriving the national criterion (Figure 3.5 in the main document), a site-specific EF value would ideally involve collecting particulate and water samples at the same location and time to ensure their representativeness of sirte-specific conditions. One simple and effective sampling and analysis scenario would be to collect water samples or a combination of particulate and water samples, separate the particulate material from the water in each sample by filtering, measure the concentration of selenium in the separated water and particulate material, compute the ratio of the two measurements from each sample, and then calculate the mean or median of all the ratios.

Selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as lakes, reservoirs, oxbows, and wetlands) and with fine particulate sediments high in organic carbon. A well-planned sampling protocol was developed in association with the development of a site-specific water-column criterion for selenium in the San Francisco Bay Delta[2]. States and tribes may also want to consult Doblin et al. (2006) for specific particulate sampling methods. EPA's National Rivers and Streams Assessment[3] also provides methods for quantitative periphyton sampling that commonly represents the base of many aquatic food webs. Analytical methods to measure selenium in particulate material and in water are discussed in Appendix L.

1.2.4.2 Deriving an appropriate *EF* value from existing data

If suitable and sufficient site-specific measurements of selenium in particulate material and water are already available, the model user may be able to use that data to derive an appropriate *EF* value. However, it would be important to ensure that the data represents current conditions, were collected and analyzed using scientifically sound sampling and analytical techniques, and proper quality assurance and quality control protocols were implemented.

1.2.4.3 Extrapolating from *EF* values of similar waters

[2] https://www3.epa.gov/region9/water/ctr/selenium-modeling_admin-report.pdf

[3] https://www.nemi.gov/methods/method_summary/12558/ (EPA-841-B-07-009) and https://www.nemi.gov/methods/method_summary/12565/ (EPA-841-B-12-009)

In circumstances where a site-specific, field-derived *EF* value is not available or practical to develop, an *EF* value from one or more aquatic systems with similar hydrological, geochemical, and biological characteristics could be used to estimate *EF*. However, there is a possibility of introducing significant uncertainty when using *EF* values extrapolated from other aquatic systems. More information on this topic is contained in Appendix H of this document.

1.2.5 Determine the Appropriate *CF* Value

1.2.5.1 Selecting the appropriate *CF* value from the list of values that were used to derive EPA's recommended water criteria concentration values

The parameter *CF* represents the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues. EPA derived species-specific *CF* values for 20 species of fish from studies that measured selenium concentrations in both eggs and/or ovaries and in whole body and/or muscle. These *CF* values can be found in Appendix B and are reproduced below (Table K-3).

Table K-3. Selenium Whole Body to Egg-Ovary Conversion Factors (*CF*).

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Species				
Bluegill	2.13			2.13
Bluehead sucker	1.82			1.82
Brook trout		1.09	1.27	1.38
Brown trout	1.45			1.45
Creek chub	1.99			1.99
Common carp	1.92			1.92
Cutthroat trout	1.96			1.96
Desert pupfish	1.20			1.20
Dolly Varden		1.26	1.27	1.61
Fathead minnow	1.40			1.40
Flannelmouth sucker	1.41			1.41
Green sunfish	1.45			1.45
Mountain whitefish		5.80	1.27	7.39
Northern pike		1.88	1.27	2.39
Rainbow trout		1.92	1.27	2.44

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Razorback sucker		2.31	1.34	3.11
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sturgeon		1.33	1.27	1.69
White sucker	1.38			1.38
Genus				
Catostomus				1.41
Gila				2.07
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				1.96
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				1.95
Salmonidae				1.71
Order				
Cyprinodontiformes				1.20
Perciformes				1.45
Class				
Actinopterygii				1.45

The data and methods used to derive the *CF* in this table are described in Appendix B.

1.2.5.2 Deriving a *CF* value from existing data

The parameter *CF* is mathematically expressed as Equation K-7 (Equation 16 in the main text):

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \textit{(Equation K-7)}$$

Where:

CF = Whole-body to egg-ovary conversion factor (dimensionless ratio).

$C_{egg\text{-}ovary}$ = Selenium concentration in the eggs or ovaries of fish (µg/g)

$C_{whole\text{-}body}$ = Selenium concentration in the whole body of fish (mg/kg).

If suitable and sufficient data are available, a model user could derive a species-specific *CF* value using the same numerical methods described above to calculate the parameter *EF*. The median of the ratios given in Equation K-7 could be used as the species-specific *CF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear regression analysis. IN deriving the national criterion, EPA considered it to be acceptable if a linear regression of egg-ovary selenium concentration on whole body selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope. Other scientifically defensible methods could be used. Regardless of the method used, the user should ensure that the data used to derive *CF* values were collected using adequate quality assurance and quality control protocols.

1.2.5.3 Deriving a *CF* value by conducting additional studies

Additional studies could be performed to obtain data needed to derive *CF* values for specific needs or to revise existing *CF* values if there is reason to believe doing so may increase the accuracy of the resulting water concentration value. Analytical methods to measure selenium in tissue are discussed in Appendix L. Where appropriate, additional data could be obtained as part of a NPDES permit application by invoking authority under CWA section 308 (or comparable state or tribal authority) to require NPDES-regulated facilities to collect information necessary to develop permit limits**.**

1.2.5.4 Extrapolating the CF value from the list of values that were used to derive EPA's recommended water criteria concentration values

If one or more necessary *CF* values are not available, and the information needed to derive a species-specific *CF* value is not available or impractical to obtain, a model user could could consider extrapolating a new *CF* value from other known *CF* values. One possible method to extrapolate a *CF* value is to use the same taxonomic approach EPA uses for *TTF* values that are not available for specific

species (Section 1.2.3.4). Sequentially consider higher taxonomic classifications could be considered until one or more of the fish species with a known *CF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *CF* values, the average *CF* value from the matching table entries could be used.

1.2.6 Translate the Selenium Egg-Ovary Criterion Element into a Site-Specific Water Concentration Value using Equation K-1

Model users could derive a site-specific water concentration value from the egg-ovary criterion element value using Equation K-1 with appropriate values of *CF*, $TTF^{composite}$ (derived from the product of the individual *TTF* values from each trophic level) and *EF*. Note that NPDES permitting regulations at 40 CFR § 122.45(c) requires that a Water Quality-Based Effluent Limit (WQBEL) for metals be expressed as total recoverable metal, unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Equation K-1 assumes selenium concentrations dissolved in water. While states and tribes may express ambient water quality criteria in water quality standards as dissolved selenium, an additional step would be necessary to convert the dissolved selenium concentration to a total recoverable selenium concentration for the purpose of NPDES permitting. Guidance for converting expression of metal concentrations in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996).

1.3 Managing Uncertainty using the Mechanistic Modeling Approach

Uncertainty in the translation of the egg-ovary criterion element to a water column value using the mechanistic bioaccumulation modeling approach (Equation K-1) can arise from several sources. These include:

- Measurement error when deriving input parameters,
- Inaccurate food web models due to misidentification and/or incorrect proportions of prey organisms,
- Inaccurate or inappropriate *EF*, *TTF*, and/or *CF* values,
- Biological variability,
- Unaccounted factors affecting bioaccumulation (e.g. selenium speciation), and
- Other unknown factors.

The most influential step in selenium bioaccumulation occurs at the base of aquatic food webs (Chapman et al. 2010). The parameter *EF* characterizes this step by quantifying the partitioning of

selenium between the dissolved and particulate state. *EF* can vary by at least two orders of magnitude across aquatic systems (Presser and Luoma 2010). The greatest reduction in uncertainty could be achieved when translating a fish tissue concentration of selenium to a water column concentration using Equation K-1 by using temporally and spatially coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity to accurately characterize *EF*.

Presser (2013) provides several recommendation to reduce uncertainty in an ecosystem scale approach to deriving a site-specific selenium water column criterion in a coal mining impacted area of West Virginia. Suggested actions to reduce uncertainty include:

- Obtaining temporally matched pairs of selenium measurements in dissolved and particulate material across a broad range of sites to ensure the samples accurately characterize the aquatic system and to assess sample variability;
- Characterizing particulate material across seasons to better define the base of the food web;
- Evaluating aquatic systems variables such as residence time, watershed dilution, and physical habitat attributes on as fine a scale as possible;
- Refining model assumptions to accurately characterize dietary preferences and composition of fish, and develop additional *TTF* values if necessary;
- Identify and target fish species particularly sensitive to selenium;
- Consider temporal changes in the bioaccumulation potential of the aquatic system and changes in selenium sensitivity over the life cycle of fish; and
- Consider variability in hydrology and selenium discharges.

The suitability of selected equation parameters could be determined by obtaining fish tissue and water column measurements of selenium from small-scale field studies, use of equation K-1 to estimate one measurements using the other, and comparison of the estimated concentration with the actual concentration (see Section 6.2.1 of the main document for a description of EPA's validation approach).

1.4 Example Calculations

Below are six hypothetical examples that demonstrate how to translate the egg-ovary FCV to a site-specific water concentration criterion using Equation K-1. These examples encompass a variety of hypothetical aquatic systems with various fish species and food webs. For these hypothetical examples, species-specific *TTF* values were taken from Tables K-1 and K-2, and *CF* values were taken from Table K-3. To calculate EF in these examples, the EPA used a hypothetical water concentration of 5 µg/L and the hypothetical particulate concentrations of 4.25 µg/g and 8.75 µg/g in lotic and lentic aquatic systems, respectively.

1.4.1 Example 1

Bluegill (*Lepomis macrochirus*) in a river that consume mostly amphipods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for amphipods (TTF^{TL2})	1.22
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$\mathrm{EF} = \frac{\mathrm{C_{particulate}}}{\mathrm{C_{water}}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$
$$= 1.03 \times 1.22$$
$$= 1.26$$

$$C_{water} = \frac{15.1}{1.26 \times 0.85 \times 2.13}$$

= 6.62 µg/L

1.4.2 Example 2

Fathead minnow (*Pimephales promelas*) in a river that consume mostly copepods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods (TTF^{TL2})	1.41
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$

$= 1.57 \times 1.41$

$= 2.21$

$$C_{water} = \frac{15.1}{2.21 \times 0.85 \times 1.40}$$

= 5.74 µg/L

1.4.3 Example 3

Bluegill (*Lepomis macrochirus*) in a lake that consume mostly aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	8.75
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for aquatic insects (median of Odonates, Water boatman, Midges, and Mayflies) (TTF^{TL2})	2.14
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{8.75}{5.00}$$

= 1.75 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$TTF^{composite}$ = $TTF^{TL3} \times TTF^{TL2}$

= 1.03 x 2.14

= 2.20

$$C_{water} = \frac{15.1}{2.20 \times 1.75 \times 2.13}$$

= 1.84 µg/L

1.4.4 Example 4

Fathead minnow (*Pimephales promelas*) in a river that consume approximately ⅔ copepods and ⅓ aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods and aquatic insects (TTF^{TL2}) Copepods = 1.41 Average of all aquatic insects = 2.14 $TTF^{TL2} = \sum_{i=1}^{n}(TTF_i \times w_i)$ $= (1.41 \times ⅔) + (2.14 \times ⅓)$ $= 1.65$	1.65
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$

= 1.57 × 1.65

= 2.59

$$C_{water} = \frac{15.1}{2.59 \times 0.85 \times 1.40}$$

= 4.90 µg/L

1.5.5 Example 5

Flathead chub (*Platygobio gracilis*) in a river with a diet of approximately 80% aquatic insects and 20% algae:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of flathead chub: Lowest matching taxon is the family Cyprinidae. Therefore, the TTF value of Cyprinidae is used (TTF^{TL3})	1.20
Trophic transfer factor for insects (TTF^{TL2}) Average of all aquatic insects = 2.14	2.14
Egg-ovary to whole-body conversion factor for flathead chub (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	1.95
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times w_2]$$

Where:
w_1 = Proportion of fathead chub diet from insects; and
w_2 = Proportion of fathead chub diet from algae

$$TTF^{comb} = [1.20 \times 2.14 \times 0.8] + [1.20 \times 0.2]$$
= 2.29

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{2.29 \times 0.85 \times 1.95}$$

= 3.98 µg/L

1.5.6 Example 6

Largemouth bass (*Micropterus salmoides*) in a large river that consume mostly Western mosquitofish (*Gambusia affinis*) that consume approximately ¾ insects and ¼ crustaceans:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of largemouth bass (TTF^{TL4})	1.39
Trophic transfer factor of Western mosquitofish (TTF^{TL3})	1.21
Trophic transfer factor for insects and crustaceans (TTF^{TL2}) Median all Insects – 2.14 Median all Crustaceans – 1.41 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i^{TL2} w_i\right)$ = (2.14 x 0.75) + (1.41 x 0.25) = 1.96	1.96
Egg-ovary to whole-body conversion factor for largemouth bass (species-specific value not available, so median CF for genus Micropterus is used) (CF)	1.42
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$
$= 1.39 \times 1.21 \times 1.96$
$= 3.30$

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{3.30 \times 0.85 \times 1.42}$$

= 3.79 µg/L

2.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING BIOACCUMULATION FACTORS (BAF)

2.1 Summary of the BAF Approach

A bioaccumulation factor (BAF) is the ratio (in milligrams/kilogram per milligrams/liter, or liters per kilogram) of the concentration of a chemical in the tissue of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). BAFs are used to relate chemical concentrations in aquatic organisms to concentrations in the ambient media of aquatic ecosystems where both the organism and its food are exposed and the ratio does not change substantially over time. The BAF is expressed mathematically as:

$$BAF = \frac{C_{tissue}}{C_{water}} \qquad \textit{(Equation K-8)}$$

Where:

BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)
C_{tissue}	=	concentration of chemical in fish tissue (mg/kg)
C_{water}	=	ambient concentration of chemical in water (mg/L)

The site-specific BAF can then be applied to the tissue criterion to solve for a target site-specific water column criterion (C_{target}):

$$C_{target} \times \frac{C_{egg-ovary\ criterion}}{BAF} \qquad \textit{(Equation K-9)}$$

Where:

C_{target}	=	site-specific water criterion concentration (mg/L)
$C_{egg\text{-}ovary\ criterion}$	=	national egg-ovary tissue criterion (15.1 mg Se/kg dw)
BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)

To translate a fish tissue criterion to a water concentration value, a site-specific, field-measured BAF for the waterbody could be developed, and then a water concentration criterion could be calculated using Equation K-9. Detailed information about how to derive a site-specific, field-measured BAF is provided in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume 3: Development of Site-specific Bioaccumulation*

Factors (U.S. EPA 2009). Although this guidance was developed for deriving human health criteria, the methodological approach is also applicable to the derivation of aquatic life criteria. The following example illustrates the calculation of a site specific water column criterion using the BAF approach.

2.1.1 Example: Derivation of a site specific water column criterion for a waterbody impacted by selenium

Available data for a hypothetical site indicate that the average egg/ovary tissue concentration of selenium for the bluegill (*Lepomis macrochirus*) is 22 mg/kg (dw). This concentration exceeds the USEPA proposed egg/ovary criterion of 15.1 mg/kg (dw). The ambient selenium water column concentration at that hypothetical site is 4.0 µg/L. The following calculation shows how to derive a target water column that would achieve a site-specific criterion using the bioaccumulation factor (BAF) approach.

Site specific selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.1
Ambient selenium water column concentration (µg/L)	4.0
Target water column concentration (µg/L)	X

Set up proportional equation to solve for allowable water column concentration:

$$\frac{Site\ specific\ egg/ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Site\ specific\ water\ concentration\ (\frac{\mu g\ Se}{L})} = \frac{Criterion\ egg\ ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Solve for the target water concentration that will achieve a site-specific criterion:

$$\frac{22.0\ (\frac{mg\ Se}{kg\ dw})}{4.0\ (\frac{\mu g\ Se}{L})} = \frac{15.1\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Target water concentration = 2.75 µg/L.

2.2 Managing Uncertainty using the BAF Approach

Uncertainty can be introduced when using the BAF approach to derive a water concentration value from a fish tissue criterion concentration. Inaccurate water concentration values can result when BAFs are derived from water and fish tissue concentration measurements that are obtained from sources that do not closely represent site characteristics, or from field data collected from large-scale sites that encompass multiple water bodies or ecosystems. Most of this uncertainty results from differences in the

bioavailability of selenium between the study sites where measurements are made to derive the BAF, and the site(s) to which the BAF is used to derive needed water concentration values.

Because of uncertainties associated with the BAF approach, EPA does not recommend developing BAFs from data extrapolated from different sites or across large spatial scales. EPA's Framework for Metals Risk Assessment (U.S. EPA 2007) outlines key principles about metals and describes how they should be considered in conducting human health and ecological risk assessments due the the effects of water chemistry on bioavilability of such chemicals. The current science does not support the use of a single, generic threshold BAF value as an indicator of metal bioaccumulation. The use of BAFs are appropriate only for site-specific applications where sufficient measurements have been taken from the site of interest and there is little or no extrapolation of BAF values across differing exposure conditions and species.

The preferred approach for using a BAF to implement the selenium fish tissue criterion is to calculate a site-specific, field-measured BAF from data gathered at the site of interest, and to apply that BAF to that site. A site-specific, field-measured BAF is a direct measure of bioaccumulation in an aquatic system because the data are collected from the aquatic ecosystem itself and thus reflects real-world exposure through all relevant exposure routes. A site-specific, field-measured BAF also reflects biotic and abiotic factors that influence the bioavailability, biomagnification, metabolism, and biogeochemical cycling of selenium that might affect bioaccumulation in the aquatic organism or its food web. Appropriately developed site-specific, field-measured BAFs are appropriate for all bioaccumulative chemicals, regardless of the extent of chemical metabolism in biota from a site (U.S. EPA 2000).

Although a site-specific, field-measured BAF is a direct measure of bioaccumulation, its predictive power depends on a number of important factors being properly addressed in the design of the field sampling effort. For example, sampling in areas with relatively long water residence times should be a priority because selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as wetlands, oxbows, and estuaries) and with fine particulate sediments high in organic carbon. In addition, migratory species should generally not be used because their exposure to selenium could reflect selenium concentrations in areas other than where the fish were caught. Fish may also need to be sampled and BAF values recalculated if selenium levels significantly change over time because BAFs are known to be affected by the ambient concentration of the metals in the aquatic environment (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). States and tribes should refer to *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume* (U.S. EPA 2009) for guidance on appropriate methods for developing a site-specific, field-derive BAF.

The advantage of using the BAF approach is its relative simplicity, especially when the data necessary to derive the BAF is already available. Furthermore, the BAF approach is completely empirical and does not require any specific knowledge about the physical, chemical, or biological characteristics of the waterbody. The relationship between the concentration of selenium in fish tissue and water is directly determined by direct measurements in these media. This may be advantageous when there are uncertainties with how to collect a particulate sample that is representative of the base of the food web, or dilution concerns (e.g., sandy streams with little surface area for algae sampling and high potential for sand contamination of a benthic sediment sample).

Limitations of the BAF approach should be considered before deciding if this method is appropriate for translating the selenium FCV to a water concentration value. One disadvantage of the BAF approach is the considerable effort and resources necessary to collect sufficient data to establish the relationship between tissue and water concentrations. Resource use increases as the spatial scale and complexity of the aquatic system increases. Furthermore, the BAF approach does not allow extrapolation across species, space, and large time scales because the site-specific factors that might influence bioaccumulation are integrated within the tissue concentration measurements and thus cannot be individually adjusted to extrapolate to other conditions. Thus, site-specific, field-measured BAFs only provide an accounting of the uptake and accumulation of selenium for an organism at a specific site and point in time. This is more important in lotic habitats, since the kinetics of selenium bioaccumulation may be very different at a site upstream or downstream from the site of interest.

As noted previously, NPDES permitting regulations at 40 CFR § 122.45(c) require WQBELs for metals be expressed as total recoverable metal unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Guidance for converting expression of metals in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996). Whether or not a water concentration value derived from a site-specific, field-derived BAF requires conversion from dissolved to total recoverable selenium depends on how the BAF is developed. Generally, conversion would not be necessary if the BAF is derived from water concentration values that measure total selenium; however, conversion would be necessary if the BAF was derived from water concentration values that measured dissolved selenium. Table K-4 compares some of the principle characteristics of the mechanistic bioaccumulation modeling approach or the BAF approach for translating the selenium FCV to a water concentration.

3.0 COMPARISON OF MECHANISTIC BIOACCUMULATION MODELING AND BAF APPROACHES

Data from Saiki et al. (1993) are used here to illustrate an example comparison of the two translation approaches, the mechanistic bioaccumulation modeling approach and the bioaccumulation factor (BAF) model approach. Definitive selenium measurements for all ecosystem compartments (e.g., water, algae, etc.) are available for two species, bluegill and largemouth bass, at four sites. Food web pathways were calculated using results of gut content analysis. Although Saiki et al. (1993) satisfies the minimum requirements for a site specific translation, it represents a sparse dataset, with only two measurements per ecosystem compartment, one for the spring and fall of 1987, respectively. For purposes of this exercise, samples from the same site collected at different time periods will be treated as replicate data; however, EPA recommends using larger sample sizes collected during the same time period when calculating a site specific criterion.

Selenium data used to calculate site specific water criteria are included in Table K-4. Median concentrations and coefficients of variation for each ecosystem compartment at each site are included in Table K-5. Because at most only two concentrations were available for each ecosystem, site median are equal to site averages. Site specific translations for both approaches will be calculated using median selenium concentrations.

Table K-4. Selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw.

Site	Date	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	Fall 1987	3	7.40	22	4.6	8.9	5.2	2.4	6.4	6.8
Mud Slough at Gun Club Road	Spring 1987	9	1.60	7.9	3.3	7.2	4.4	5.4	5	6.9
Salt Slough at the San Luis National Wildlife Refuge	Fall 1987	3	0.38	8.9	3.4	5.4	3.1	4.5	4.5	4.7
Salt Slough at the San Luis National Wildlife Refuge	Spring 1987	13	2.40	7.9	3.7	6.9	3.2	4.4	4.3	4
San Joaquin R. above Hills Ferry Road	Fall 1987	3	1.20	6.6	3.8	6	1.7	2.6	3.3	2.2
San Joaquin R. above Hills Ferry Road	Spring 1987	11	1.30	3.4	2.8	4.1	1.9	4.3	2.7	2.4
San Joaquin R. at Durham Ferry State Recreation Area	Fall 1987	1	0.39	1.2	1.5	1.5	0.77	1.6	2	1.8
San Joaquin R. at Durham Ferry State Recreation Area	Spring 1987		0.50	1.3	1.1	1.6	1.3	1.8	1.9	1.7

Table K-5. Median selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
For purposes of this exercise, spring and fall samples measured at the same site are treated as replicates. Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw. Coefficients of determination included in parentheses.

Site	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	6.0 (0.71)	4.50 (0.91)	14.95 (0.67)	3.95 (0.23)	8.05 (0.15)	4.80 (0.12)	3.90 (0.54)	5.70 (0.17)	6.85 (0.01)
Salt Slough at the San Luis National Wildlife Refuge	8.0 (0.88)	1.39 (1.03)	8.40 (0.08)	3.55 (0.06)	6.15 (0.17)	3.15 (0.02)	4.45 (0.02)	4.40 (0.03)	4.35 (0.11)
San Joaquin R. above Hills Ferry Road	7.0 (0.81)	1.25 (0.06)	5.00 (0.45)	3.30 (0.21)	5.05 (0.27)	1.80 (0.08)	3.45 (0.35)	3.00 (0.14)	2.30 (0.06)
San Joaquin R. at Durham Ferry State Recreation Area	1.0 (na)	0.45 (0.17)	1.25 (0.06)	1.30 (0.22)	1.55 (0.05)	1.04 (0.36)	1.70 (0.08)	1.95 (0.04)	1.75 (0.04)

3.1 Translation using the BAF Approach

Site specific BAFs were calculated for bluegill and largemouth bass at each of the four sites (Table K-6). A site-specific water criterion was calculated for each species at the four sites using equation K-8, which is equivalent to the BAF example shown in the previous section. The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$BAF = \frac{C_{tissue}}{C_{water}} = \frac{4.4\ \mu g/g}{8\ \mu g/L} = 0.55\ L/g$$

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{BAF} = \frac{8.5\ mg/kg}{0.55\ L/g} = 15.5\ \mu g/L$$

The whole body tissue criterion of 8.5 mg/kg is used here because whole body fish tissue selenium measurements were made. If site specific egg ovary fish tissue had been measured, then the egg ovary tissue criterion of 15.1 mg/kg would have been used.

Table K-6. Site and species specific translated water concentrations using the BAF translation approach.

		Bluegill:			**Largemouth Bass:**		
Site	**Water (µg/L)**	**WB Se (mg/kg)**	**BAF (L/g)**	**Water SSC[a] (µg/L)**	**WB Se (mg/kg)**	**BAF (L/g)**	**Water SSC[a] (µg/L)**
Mud Slough at Gun Club Road	6.0	5.70	0.95	8.95	6.85	1.14	7.45
Salt Slough at the San Luis National Wildlife Refuge	8.0	4.40	0.55	15.45	4.35	0.54	15.63
San Joaquin R. above Hills Ferry Road	7.0	3.00	0.43	19.83	2.30	0.33	25.87
San Joaquin R. at Durham Ferry State Recreation Area	1.0	1.95	1.95	4.36	1.75	1.75	4.86

a – Site specific criterion based on BAF for respective species.

At each site, the lowest translated water criterion for all species is used as the site specific criterion. At site "Mud Slough at Gun Club Road," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the BAF approach range from 4.4 to 19.8 µg/L Table K-6).

3.2 Translation using the Mechanistic Bioaccumulation Modeling Approach

The first step in the bioaccumulation modeling approach is the calculation of site specific enrichment factors (*EF*s). Because both algae and detritus selenium concentrations were available, the first step was the calculation of separate *EF*s for algae and detritus at each site, following the procedures described in section 1.2.4.1. Algal and detrital *EF*s, respectively, were calculated using the median of all Se concentrations in algae (or detritus) at a site by the median of all Se concentrations in water at the same site. After calculating separate algal and detrital *EF*s, the final *EF* at each site was calculated as the geometric mean of the algal and detrital *EF* at a given site. Algal, detrital, and site *EF*s are shown in Table K-7.

Table K-7. Se concentrations in water, algae, detritus, and site specific *EF*s.

Site	Water (µg/L)	Algae (mg/kg)	Detritus (mg/kg)	EF (L/g)
Mud Slough at Gun Club Road	6.0	4.50	14.95	1.37
Salt Slough at the San Luis National Wildlife Refuge	8.0	1.39	8.40	0.43
San Joaquin R. above Hills Ferry Road	7.0	1.25	5.00	0.36
San Joaquin R. at Durham Ferry State Recreation Area	1.0	0.45	1.25	0.75

As an example, the EF calculation for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$EF_{algae} = \frac{C_{algae}}{water}\ ;\ EF_{detritus} = \frac{C_{detritus}}{water}$$

$$EF_{site} = \sqrt{\left(EF_{algae} \times EF_{detritus}\right)}$$

$$EF_{algae} = \frac{1.39\ mg/kg}{8.0\ \mu g/L};\ EF_{detritus} = \frac{8.4\ mg/kg}{8.0\ \mu g/L}$$

$$EF_{site} = \sqrt{(0.17 \times 1.05)}$$

$$EF_{site} = 0.43\ L/g$$

The second step in the bioaccumulation modeling approach is the calculation of site specific composite trophic transfer factors ($TTF^{composite}$). Based on gut content analysis, bluegill diets consisted of

47% amphipods, 23% chironomids, and 30% zooplankton, while largemouth bass diets consisted of 73% bluegill and 27% crayfish.

The composite TTF for bluegill was calculated using the following equation:

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times TTF^{TL2} \times w_2] + [TTF^{TL3} \times TTF^{TL2} \times w_3]$$

Where:

W_1 = proportion of diet from amphipods,

W_2 = proportion of diet from chironomids, and

W_3 = proportion of diet from zooplankton.

For each of the 3 species in the bluegill diet, site specific TTF^{TL3} and TTF^{TL2} were calculated separately. Using median concentrations from Table K-5, $TTF^{composite}$ were calculated for each of the sites and are included in Table K-8.

Table K-8. Trophic transfer factors (TTFs) for bluegill and bluegill prey.

	TL2 TTFs:			**TL3 TTFs:**			**$TTF^{composite}$:**
Site	**Amphipod**	**Chironomid**	**Zooplankton**	**BG-Amph**	**BG-Chiro**	**BG-Zoo**	**Bluegill**
Mud Slough at Gun Club Road	0.41	0.83	0.40	1.44	0.71	1.46	0.59
Salt Slough at the San Luis National Wildlife Refuge	0.73	1.26	0.91	1.24	0.72	0.99	0.90
San Joaquin R. above Hills Ferry Road	1.06	1.62	1.10	0.91	0.59	0.87	0.96
San Joaquin R. at Durham Ferry State Recreation Area	1.53	1.83	2.01	1.50	1.26	1.15	2.30

As an example, the bluegill $TTF^{composite}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$TTF^{composite} = [1.24 \times 0.73 \times 0.47] + [0.72 \times 1.26 \times 0.23] + [0.99 \times 0.91 \times 0.30]$$

$$TTF^{composite} = 0.90$$

The composite TTF for largemouth bass was calculated using the following equation:

$$TTF^{composite} = [TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times TTF^{TL2} \times w_2]$$

Where:

W_1 = proportion of diet from bluegill, and

W_2 = proportion of diet from crayfish

For the proportion of the largemouth bass diet consisting of bluegill, TTF^{TL3} x TTF^{TL2} was equal to the $TTF^{composite}$ for bluegill. As was the case for bluegill, site specific TTFs were calculated for each species, and are included in Table K-9.

Table K-9. Trophic transfer factors (TTFs) for largemouth bass and largemouth bass prey.

	Crayfish dietary fraction:		Bluegill dietary fraction:		$TTF^{composite}$:
Site	**Crayfish**	**LMB-Cray**	**Bluegill[a]**	**LMB-BG**	**LMB**
Mud Slough at Gun Club Road	0.49	1.43	0.59	0.70	0.49
Salt Slough at the San Luis National Wildlife Refuge	0.64	1.38	0.90	0.89	0.82
San Joaquin R. above Hills Ferry Road	0.58	1.28	0.96	0.74	0.71
San Joaquin R. at Durham Ferry State Recreation Area	1.22	1.69	2.30	2.06	4.03

a – $TTF^{composite}$ for bluegill.

As an example, the largemouth bass $TTF^{combined}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$TTF^{composite} = \left[TTF^{TL4} \times TTF_{bluegill}^{composite} \times w_1\right] + [TTF^{TL3} \times TTF^{TL2} \times w_2]$$

$$TTF^{composite} = [0.89 \times 0.90 \times 0.73] + [1.38 \times 0.64 \times 0.27]$$

$$TTF^{composite} = 0.82$$

After calculating site and species specific EF and $TTF^{combined}$, site specific water criterion concentrations were calculated using a modified version of equation K-1, shown below.

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{EF\ x\ TTF^{composite}}$$

The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$C_{water\ criterion} = \frac{8.5\ mg/kg}{0.43\ L/g\ x\ 0.90} = 22.1\ \mu g/L$$

Because the selenium in fish tissue at these sites were measured as whole body concentrations, the whole body criterion of 8.5 µg/L is used, and an egg-ovary to whole body conversion factor is not required. As with the BAF approach, the lowest translated water criterion for all species is used as the site specific criterion. At site "San Joaquin R. at Durham Ferry State Recreation Area," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the mechanistic bioaccumulation modeling approach are more variable than concentrations calculated using the BAF approach, and range from 2.8 to 33.3 µg/L Table K-10). At all sites using both methods, the translated site specific water concentration criteria were higher than the measured water concentrations.

Table K-10. Site and species specific translated water concentrations using the mechanistic bioaccumulation modeling approach.

		Bluegill:			**Largemouth Bass:**		
Site	**EF (L/g)**	**WB Se (mg/kg)**	**TTF**	**Water SSC (µg/L)**	**WB Se (mg/kg)**	**TTF**	**Water SSC (µg/L)**
Mud Slough at Gun Club Road	1.37	5.70	0.59	10.61	6.85	0.49	12.65
Salt Slough at the San Luis National Wildlife Refuge	0.43	4.40	0.90	22.14	4.35	0.82	24.18
San Joaquin R. above Hills Ferry Road	0.36	3.00	0.96	24.79	2.30	0.71	33.31
San Joaquin R. at Durham Ferry State Recreation Area	0.75	1.95	2.30	4.95	1.75	4.03	2.83

3.3 Summary Comparison of the Mechanistic Bioaccumulation and BAF Approaches

A comparison of the mechanistic bioaccumulation and BAF approaches is included in Table K-11.

Table K-11. Comparison of mechanistic bioaccumulation and BAF approaches.

Mechanistic bioaccumulation modeling	Bioaccumulation Factor (BAF)
Knowledge of the aquatic system needed	No information on aquatic system needed
Choice of input parameters at discretion of state or tribe	No input parameters to choose
Species-specific	Species-specific
Can be applied at different sites if site *EF* can be estimated.	Site-specific
Fish tissue sampling not required for translation	Fish tissue and water sampling required

APPENDIX L: ANALYTICAL METHODS FOR MEASURING SELENIUM

The Clean Water Act (CWA) establishes an EPA approval process for certain analytical methods used in the National Pollutant Discharge Elimination System (NPDES) program and for section 401 certifications. EPA has several approved methods for measuring selenium in water under 40 CFR § 136. EPA generally requires the use of EPA-approved methods for the NPDES program and for CWA section 401 certifications issued by states and tribes (40 CFR § 136.1). However, since there are no EPA approved methods for the analysis of selenium in fish tissue, states and tribes may use analytical methods not approved by EPA to evaluate the attainment of water quality standards or to develop or implement Total Maximum Daily Loads provided that these methods are scientifically sound (40 CFR 122.21(g)(7)).

Implementation of a water quality standard for selenium may require the ability to detect and measure the concentration of selenium in effluent, ambient water, tissue, and other media that is below the detection limit or limit of quantitation that some analytical methods can provide. States and tribes should choose an analytical method that is sufficiently sensitive to implement its water quality standard for selenium. Below are descriptions of some of the methods available for measuring selenium concentrations with sufficient sensitivity to implement EPA's recommended selenium criterion. Complete descriptions of analytical methods appropriate for analyzing selenium in different media can be found in the National Environmental Methods Index at http://www.nemi.gov.

1.0 General Considerations when Measuring Concentrations of Selenium

The oxidation states of selenium dissolved in surface water are usually selenate (+6), selenite (+4), and organo-selenium (-2). The presence of selenium in different oxidation states complicates some analytical methods (Presser and Ohlendorf 1987). EPA recommends using standard reference samples to check for the percentage recovery of each species of selenium (selenate, selenite and organo-selenium) during initial testing of selenium methodologies for quality control and assurance.

If water samples are not filtered, particulate species such as elemental selenium and particulate organo-selenium will also be measured. In addition, federal regulations at 40 CFR §122.45(c) generally requires considering total recoverable metals when establishing effluent limits and reporting requirements.

2.0 Analytical Methods Recommended for Measuring Selenium in Water

EPA has several approved analytical methods under 40 CFR § 136 specifically for measuring total selenium in water. These regulations state that measurements for NPDES permit applications and permittee reporting should be made using analytical methods approved by EPA. Because EPA has

approved methods for analyzing selenium in water, these methods must be used for NPDES permits (40 CFR § 122.21(g)(7), 122.41(j), 136.1, 136.3, and 136.6).

A complete list of EPA-approved analytical methods for selenium can be found at: http://www.epa.gov/waterscience/methods/method/. Three EPA-approved methods that may be sufficiently sensitive[4] for the purposes of implementing a selenium water quality criterion are listed below (Table L-1).

Table L-1. Suggested EPA-Approved Methods for Selenium in Water

Method	Technique	Method detection limit
American Public Health Standard Method 3114 B (2009) or 3114 C (2009)	Hydride generation atomic absorption spectrometry (HG-AAS)	2 µg/L
EPA Method 200.8, Rev 5.4 (1998)	Inductively coupled plasma mass spectrometry (ICP-MS)	7.9 µg/L
EPA Method 200.9, Rev.2.2 (1994)	Stabilized temperature graphite furnace atomic absorption (STGF-AA)	0.6 µg/L

2.1 American Public Health Standard Method 3114 B

For measuring selenium in water, American Public Health Standard Method 3114 B uses the HG-AAS technique. Method 3114 B has a method detection limit (MDL) of 2 µg/L. Samples for dissolved analytes should be filtered on-site through 0.45-micron capsule filters certified free of trace-element contamination or other appropriate filtering equipment (Wilde et al. 1999). Dissolved samples should be preserved with high purity hydrochloric acid or nitric acid to a pH less than 2.

For measuring total selenium, samples should not be filtered. In addition, all selenium in the sample should be in the form of selenite (+4). Thus, the following pre-treatment steps to convert all selenium in the sample to selenite are critical when using the HG-AAS method:

1. Boiling with persulfate to oxidize and digest organic material.
2. Boiling with hydrochloric acid to reduce selenate species to selenite.
3. Reduction by sodium borohydride to hydrogen selenide in the quartz tube of the AAS.

[4]For more information on choosing a sufficiently sensitive method, see the memorandum *Analytical Methods for Mercury in National Pollutant Discharge Elimination System (NPDES) Permits* from James A. Hanlon, Director of the Office of Wastewater Management, dated August 23, 2007, available at http://www.epa.gov/npdes/pubs/mercurymemo_analyticalmethods.pdf.

Optimal conversion conditions are essential for accurate results because too mild a reduction could lead to incomplete reduction of selenate and too rigorous a reduction could lead to plating out of elemental selenium (Cutter 1987, 1983; Presser and Barnes 1984, 1985).

Method 3114 B has the advantage that it is a fully validated method, is commonly used by many laboratories, is relatively inexpensive, is less susceptible to background interference (Cutter 1987, 1983; Presser and Barnes 1984, 1985), and has sufficient sensitivity to accurately measure what can be expected in many lotic aquatic systems. However, this method may not be sufficiently sensitive for some lentic aquatic systems where relatively lower selenium concentrations may need to be measured. If no selenium is detected in a lentic system using this method, EPA recommends using a more sensitive analytical method.

2.2 EPA Method 200.8

EPA method 200.8 has a MDL of 7.9 µg/L using the ICP-MS analytical technique. This method has the advantage that no pre-treatment steps are necessary. However, this method may not be sufficiently sensitive in many applications of the selenium criterion (Lamothe et al. 1999). If no selenium is detected using this method, EPA recommends monitoring with a more sensitive method.

2.3 EPA Method 200.9

Method 200.9 has a MDL of 0.6 µg/L using the STGF-AA analytical technique. This method has the advantage that it can detect selenium at very low concentrations. However, graphite furnace techniques require careful matrix matching.

Of these three EPA approved methods, Method 3114B using the HG-AAS technique is the most cost-effective, with sufficient sensitivity and relatively low risk of interference in most cases. EPA Method 200.8 may be used where appropriate, such as when selenium concentrations in effluent are known to be higher than 7.9 µg/L. EPA Method 200.9 may be used if a very low MDL is needed. Some additional methods not approved by EPA that states and tribes might consider are:

- Collision/Reaction Cell Inductively Coupled Plasma Mass Spectroscopy (cICP-MS) (Garbarino et al. 2005) - A relatively new technique that is a sensitive and selective detector for metal analysis. However, isobaric interference can cause problems for quantitative determination as well as identification based on the analyte pattern. Collision cells, reaction cells or other interfaces reducing sample matrix effects that might otherwise interfere in the mass selective determinative step are allowed in CWA analyses provided the method performance specifications relevant to ICP-MS measurements are met

- Fluorometric Analysis - a wet chemistry technique using diaminonapthalene. This method also achieves acceptable precision and accuracy on standard reference samples (Olson 1969; Olson et al. 1975; American Public Health Association Standard Method 3500, on-line version).

Methods for measuring different species of selenium dissolved in water are also available. These methods determine the species of dissolved selenium present in a sample through differential digestion and hydride generation atomic adsorption spectrophotometry (Cutter 1978, 1983; Presser and Barnes, 1984; 1985; May et al. 2007). Selenite can be measured in samples with no pre-treatment. Selenate plus selenite can be measured in samples subjected to boiling with hydrochloric acid. Subtraction of the measured selenite fraction from the measured combined fraction would yield a measure of the selenate fraction. If a sample is analyzed to measure total dissolved selenium as described above, then measurements of the combined fraction can be subtracted to yield measurements of the dissolved organo-selenium fraction.

3.0 ANALYTICAL METHODS AVAILABLE FOR MEASURING SELENIUM IN FISH TISSUE

EPA does not have approved methods under 40 CFR § 136 for measuring selenium in fish tissue. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in fish tissue if the samples are made soluble. Tissue samples are homogenized and digested prior to analysis using strong acid or dry-ashing digestion as described below. A review of sample digestion techniques has been published (Ihnat 1992). Standard reference materials, analytical duplicates, and matrix spike samples are recommended to determine the applicability of a selected digestion procedure.

3.1 Strong acid digestion

Solid samples can be subjected to strong acid digestion to break down mineral and organic matrices. Samples are typically dried and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Note that some strong acid digestion methods may not be suitable for fish tissue. Strong acid digestion methods are categorized by the type of material or amount of organic material present (e.g., solid waste; biological tissue, plants, soil, sediment, rock, coal) and degrees of tissue solubilization needed (extraction, leachate, or complete destruction). Methods differ in acid mixture and degree and type of heating (EPA Method 3050B, Revision 2, 1996; EPA Method 200.2,

Revision 2.8, 1994; Briggs and Crock, 1986; Taggart, 2002, chapters I, J, and K). High boiling acids (perchloric and sulfuric) may lead to a loss of selenium if solutions are heated to dryness.

3.2 Dry-ashing digestion

Dry-ashing digestion is applicable to biological samples (Brumbaugh and Walther, 1989; May et al., 2007). Biological samples are normally lyophilized (freeze-dried) and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Dried solid samples are:

1. Boiled in nitric acid for solubilization and oxidation
2. Ashed at 500º C with magnesium nitrate to complete oxidation and decompose remaining organic material
3. Heated with hydrochloric acid to dissolve the ash and reduce selenium to the selenite (+4) state required for detection by HG-AAS.

3.3 Analytical methods available for measuring selenium in particulate material

There are no 40 CFR § 136 methods for analyzing selenium in particulate material. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in particulate material after the sample has been separated from the water and pre-treated using the same methods used for fish tissue. In order to obtain a particulate material sample, a water column sample should be filtered to separate the particulate material and bed sediment. Various techniques for collection of suspended particulate material using filtration are available from the EPA (e.g. Method 1669) and the U.S. Geological Survey (Moulton et al. 2002; USGS, Britton and Greeson 1987). These techniques include:

- EPA Method 1669 (1996) includes filtration through a 0.45 µm capsule filter at the field site.
- USGS protocols for collection of phytoplankton and seston in rivers and streams as part of their National Water Quality Assessment Program for watershed and habitat assessment (http://water.usgs.gov/nawqa/protocols.html).
- Textbooks such as *Limnological Analyses* address sampling of lakes using traditional techniques including phytoplankton nets. (Wetzel and Likens 1991).
- Sampling of suspended material from estuaries where particulates are a substantial part of the ecosystem is described in Doblin et al. (2005) as part of their work on the San Francisco Bay-Delta Estuary.
- Separating suspended sediment using high-speed centrifugation and decantation when the concentration of particulate material is relatively low (Horowitz et al. 1989).

APPENDIX M: ABBREVIATIONS

REFERENCE AND SITE ABBREVIATIONS

Reference	Site		Species	
Bi:	22	Miller's Lake, Wellington CO	FM	Fathead minnow
Birkner 1978	27	Sweitzer Lake, Delta CO	FM	Fathead minnow
	23	Twin Buttes Reservoir, Laramie WY	FM	Fathead minnow
	20	East Allen Reservoir, Medicine Bow WY	ID	Iowa darter
	7	Galett Lake, Laramie WY	ID	Iowa darter
	22	Miller's Lake, Wellington CO	ID	Iowa darter
	23	Twin Buttes Reservoir, Laramie WY	ID	Iowa darter
	30	Larimer Highway 9 Pond, Fort Collins CO	NPK	Northern plains killfish
	3	Meeboer Lake, Laramie WY	NPK	Northern plains killfish
	27	Sweitzer Lake, Delta CO	NPK	Northern plains killfish
	23	Twin Buttes Reservoir, Laramie WY	NPK	Northern plains killfish
Bu91:	4	Uncompahgre River at Colona	BhS	Bluehead sucker
Butler et al. 1991	4	Uncompahgre River at Colona	BnT	Brown trout
	4	Uncompahgre River at Colona	FS	Flannelmouth sucker
	4	Uncompahgre River at Colona	MS	Mottled sculpin
	4	Uncompahgre River at Colona	RT	Rainbow trout
	4	Uncompahgre River at Colona	WS	White sucker
Bu93:	SP2	Spring Creek at La Boca	BhS	Bluehead sucker
Butler et al. 1993	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BT	Brown trout
	SP2	Spring Creek at La Boca	BT	Brown trout
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BB	Black bullhead
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	ChC	Channel catfish
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	CC	Common carp

Reference	Site		Species	
	SP2	Spring Creek at La Boca	FM	Fathead minnow
	SP2	Spring Creek at La Boca	SD	Speckled dace
	SP2	Spring Creek at La Boca	WS	White sucker
Bu95:	ME2	McElmo Cr., downstream from Alkali Canyon	BhS	Bluehead sucker
Butler et al. 1995	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BhS	Bluehead sucker
	NW	Navajo Wash near Towaoc	BhS	Bluehead sucker
	SJ1	San Juan R. at Four Corners	BhS	Bluehead sucker
	SJ3	San Juan R. at Mexican Hat Utah	BhS	Bluehead sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BB	Black bullhead
	SJ1	San Juan R. at Four Corners	ChC	Channel catfish
	SJ3	San Juan R. at Mexican Hat Utah	ChC	Channel catfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	CC	Common carp
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	CC	Common carp
	SJ1	San Juan R. at Four Corners	CC	Common carp
	SJ3	San Juan R. at Mexican Hat Utah	CC	Common carp
	HD2	Hartman Draw near mouth, at Cortez	FM	Fathead minnow
	ME1	McElmo Cr. at Hwy. 160, near Cortez	FM	Fathead minnow
	ME2	McElmo Cr., downstream from Alkali Canyon	FM	Fathead minnow
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FM	Fathead minnow
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FM	Fathead minnow
	WC	Woods Canyon near Yellow Jacket	FM	Fathead minnow
	SJ1	San Juan R. at Four Corners	FS	Flannelmouth sucker
	HD2	Hartman Draw near mouth, at Cortez	FS	Flannelmouth sucker
	ME2	McElmo Cr., downstream from Alkali Canyon	FS	Flannelmouth sucker
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	SJ3	San Juan R. at Mexican Hat Utah	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	GnS	Green sunfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	RSh	Red shiner
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	RSh	Red shiner

Reference	Site		Species	
	SJ1	San Juan R. at Four Corners	RSh	Red shiner
	ME1	McElmo Cr. at Hwy. 160, near Cortez	SD	Speckled dace
	ME2	McElmo Cr., downstream from Alkali Canyon	SD	Speckled dace
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	SD	Speckled dace
	NW	Navajo Wash near Towaoc	SD	Speckled dace
	SJ1	San Juan R. at Four Corners	SD	Speckled dace
	HD2	Hartman Draw near mouth, at Cortez	Su	Sucker
Bu97:	MUD2	Mud Cr. at Hwy. 32, near Cortez	BhS	Bluehead sucker
Butler et al. 1997	MNP2	Large pond south of G Road, southern Mancos Valley	FM	Fathead minnow
	MUD2	Mud Cr. at Hwy. 32, near Cortez	FM	Fathead minnow
	WCP	Pond on Woods Canyon at 15 Road	FM	Fathead minnow
	CH1	Cahone Canyon at Hwy. 666	GnS	Green sunfish
	MUD2	Mud Cr. at Hwy. 32, near Cortez	GnS	Green sunfish
	MNP3	Pond downstream from site MNP2, southern Mancos Valley	SB	Smallmouth bass
Ca:	DC	Deerlick Creek	RT	Rainbow trout
Casey **2005**	LC	Luscar Creek	RT	Rainbow trout
Fo:	CC-1A	Crow Creek – 1A	BnT	Brown trout
Formation 2012	CC-3A	Crow Creek – 3A	BnT	Brown trout
	CC-150	Crow Creek – 150	BnT	Brown trout
	CC-350	Crow Creek – 350	BnT	Brown trout
	CC-75	Crow Creek – 75	BnT	Brown trout
	DC	Deer Creek	BnT	Brown trout
	HS	Hoopes Spring	BnT	Brown trout
	HS-3	Hoopes Spring – 3	BnT	Brown trout
	LSV-2C	Sage Creek – 2C	BnT	Brown trout
	LSV-4	Sage Creek – 4	BnT	Brown trout
	SFTC	South Fork Tincup Creek	BnT	Brown trout

Reference	Site		Species	
	CC-1A	Crow Creek – 1A	Sc	Sculpin
	CC-3A	Crow Creek – 3A	Sc	Sculpin
	CC-150	Crow Creek – 150	Sc	Sculpin
	CC-350	Crow Creek – 350	Sc	Sculpin
	CC-75	Crow Creek – 75	Sc	Sculpin
	DC	Deer Creek	Sc	Sculpin
	HS	Hoopes Spring	Sc	Sculpin
	HS-3	Hoopes Spring – 3	Sc	Sculpin
	LSV-2C	Sage Creek – 2C	Sc	Sculpin
	LSV-4	Sage Creek – 4	Sc	Sculpin
	SFTC	South Fork Tincup Creek	Sc	Sculpin
Gr:	17	Arapahoe Wetlands Pond	FM	Fathead minnow
Grasso et al. 1995	17	Arapahoe Wetlands Pond	WS	White sucker
HB:	LEMC	Lower East Mill Creek	CT	Cutthroat trout
Hamilton and				
Buhl 2004				
Le:	BA	Badin Lake	BB	Black bullhead
Lemly 1985	BE	Belews Lake	BB	Black bullhead
	HR	High Rock Lake	BB	Black bullhead
	BA	Badin Lake	CC	Common carp
	BE	Belews Lake	CC	Common carp
	HR	High Rock Lake	CC	Common carp
	BA	Badin Lake	FM	Fathead minnow
	BE	Belews Lake	FM	Fathead minnow
	HR	High Rock Lake	FM	Fathead minnow
	BA	Badin Lake	GnS	Green sunfish
	BE	Belews Lake	GnS	Green sunfish
	HR	High Rock Lake	GnS	Green sunfish

Reference	Site		Species	
	BA	Badin Lake	WM	Western mosquitofish
	BE	Belews Lake	WM	Western mosquitofish
	HR	High Rock Lake	WM	Western mosquitofish
	BA	Badin Lake	RSh	Red shiner
	BE	Belews Lake	RSh	Red shiner
	HR	High Rock Lake	RSh	Red shiner
Sa87:	KP11	Kesterson Pond 11	WM	Western mosquitofish
Saiki and	KP2	Kesterson Pond 2	WM	Western mosquitofish
Lowe 1987	KP8	Kesterson Pond 8	WM	Western mosquitofish
	SLD	San Luis Drain	WM	Western mosquitofish
	VP26	Volta Pond 26	WM	Western mosquitofish
	VW	Volta Wasteway	WM	Western mosquitofish
Sa93:	GT4	Salt Slough at San Luis Wildlife Refuge	Bg	Bluegill
Saiki et al. 1993	GT5	Mud Slough at San Luis Wildlife Refuge	Bg	Bluegill
	SJR2	San Joaquin R. above Hills Ferry Rd.	Bg	Bluegill
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	Bg	Bluegill
	GT4	Salt Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	GT5	Mud Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	SJR2	San Joaquin R. above Hills Ferry Rd.	LMB	Largemouth bass
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	LMB	Largemouth bass
	GT4	Salt Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	GT5	Mud Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	SJR2	San Joaquin R. above Hills Ferry Rd.	WM	Western mosquitofish
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	WM	Western mosquitofish
St:	M4720	Marsh 4720	BB	Black bullhead
Stephens et al. 1988	M4720	Marsh 4720	CC	Common carp

APPENDIX N: COMPARISON OF APPROACHES FOR CALCULATING SELENIUM TISSUE CONVERSION FACTORS

1.0 COMPARISON OF THE MEDIAN RATIO AND REGRESSION APPROACHES

Regression analysis and the application of median ratios are two approaches that can be used to quantify the relationship between two variables, such as the concentration of selenium within two tissue types. When concentrations in the two tissues are plotted, each point represents the ratio of one tissue type to another. A regression analysis calculates the line that best fits those tissue concentrations, which is characterized by both a slope and a y-intercept. In contrast, the median ratio is a single value representing the 50^{th} centile of all ratios. Conversion factors (CFs) are presently calculated as the median ratio of two tissue types. The use of median ratios grew out of the goal of patterning the translation procedure after the Luoma and Presser selenium bioaccumulation model, where field derived factors representing the transfer of selenium from one ecosystem compartment to another were represented as single values calculated using constrained (y-intercept passes through the origin) regression. Median ratios were implemented to produce a single value that was operationally similar to a constrained regression slope, but that was free from the issues associated with constrained regression, particularly cases where the y-intercept was notably different from zero, which would result in slopes that were highly divergent from slopes derived using conventional regression. Both median ratios and conventional regression (with or without log transformation) are far superior to constrained (no y-intercept) regression. The following discussion will compare median ratios and conventional linear regression.

A median is a measure of central tendency that is free from all parametric assumptions associated with linear regression. As the 50^{th} centile of all y/x ratios, it is independent of the effects of outliers or the overall distribution of ratios. As implemented in the criterion document, median ratios were assumed to be representative of the linear relationship between the concentration in tissue y to the concentration in tissue x. This assumption was tested with a pre-screening procedure using conventional linear regression. If the regression model had a positive slope and was statistically significant at $P<0.05$, then the relationship was assumed to be positive and linear, and a median ratio was used as representative of that linear relationship.

A log-log regression includes both a slope and a y-intercept. Because they apply to log space, these parameters mean something different than similar parameters in arithmetic space. Linear relationships in log space translate back to power functions in arithmetic space. That is, the log space straight line function:

$$\log(EO) = m \cdot \log(WB) + b \qquad (1)$$

translates to:

$$EO = a \cdot WB^{m} \qquad (2)$$

where the coefficient $a=10^{b}$. The log-log plot intercept b represents the value of log EO when WB=1 mg/kg (that is when log(WB)=0).

The key point when comparing log-log regression to the median ratio approach is that when log-log slope m=1, then Equation 2 reduces to a simple direct proportion EO = a·WB in arithmetic space**.** Figure N-1 illustrates the behavior of CF (that is, the ratio EO/WB), depending on whether the log-log slope m of the plot of log(EO) versus log(WB) has a value 1, >1, or <1.

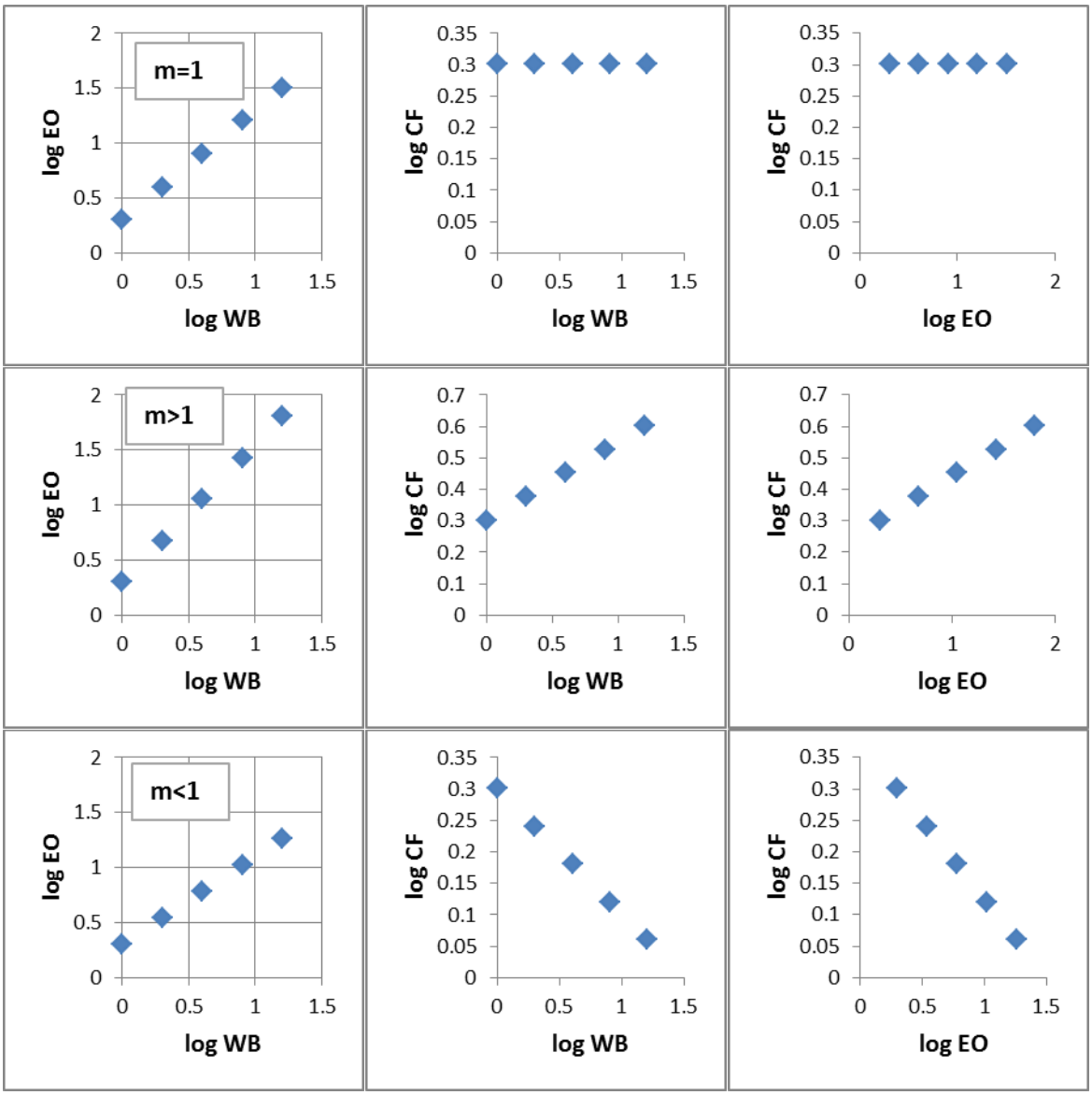


Figure N-1. Idealized illustration (sans scatter) of the effect of log-log slope, m, on whether CF is stable, increases, or decreases with concentration (whether measured as WB in column 2 or EO in column 3).

Top row: m=1. Second row: m=1.25. Bottom row: m=0.8. In all cases, CF=EO/WB, but the three rows were not designed to yield the same median CF. Were the five idealized data points replaced by a large number of well-behaved real-world data points, the straight lines would tend to be replaced by oval clouds of points having the illustrated slopes.

When the log-log slope m≈1, CF does not change with concentration. In that case, CF is the simple proportionality constant as assumed in all previous versions of the criterion document. When m is noticeably different from 1, CF changes with concentration, and we would solve for its value at the EO criterion concentration. If the EO criterion concentration is not near the median EO value in the graphed data, then the regression-calculated CF value may differ somewhat from the median CF.

While both a median ratio and a linear regression account for all of the plotted values within a particular relationship, the regression model is derived from the specific locations of every data point, whereas the median is derived independent of the specific distribution of the data points. In this way, a regression contains more information about the entire data distribution, and as such, is more affected by deviations from linearity. This second point can be an advantage or a disadvantage, depending on the data distribution. For some CF relationships in the database, there is evidence of slight non-proportionality, in which the y/x ratios at higher concentrations are different than the y/x ratios at lower concentrations. In these instances, a log transformation of the tissue concentrations will serve to better linearize the overall relationship, so that the resulting regression model will better capture the y/x relationship across the full concentration range. The median ratio of these models will be the same regardless of whether or not the data are transformed. However, because the use of median ratios is based on the assumption of proportionality, CFs calculated using regression of log transformed values will provide slightly more accurate representations of the relationship across the full concentration range than a median ratio, for those datasets that show some evidence of non-proportionality. An exception would be a case where the midpoint of the data distribution, where the median ratio is more likely to be located, is similar to the criterion concentration. In these instances the median ratio would be expected to be similar the regression based CF regardless of slope. Finally, for those datasets that do not show this effect, selection of either the median ratio or the regression based CF approach are both equally valid approaches.

Another source of uncertainty can occur for species with a CF derived from a narrow concentration range that does not encompass the criterion concentration. In these instances, the slope of the regression model may not be representative of the slope had there been concentrations bracketing the criterion. Similarly, the median of the concentration ratios within this small range may not be representative of the median ratio if there had been concentrations bracketing the criterion. However, it may be preferable, or “safer”, to use a non-parametric median rather than the result of an extrapolated regression equation, particularly when the regression is based on few data points (no matter how good r^2 is).

To conclude, CFs calculated from median ratios have the advantages of simplicity, being easier to explain and implement, and they are “safer” in the sense that they are not affected by outliers or the distribution of variance across the data range. CFs calculated from log regression include more

information about the entire data distribution, but can be sensitive to outliers. CFs calculated from the two approaches can diverge in cases where the data range does not encompass the criterion concentration, particularly in cases those where the log transformed slope is also much greater or less than one. Overall, the median ratio and log regression approaches generate similar CFs for this dataset, and have little effect on the translated water criterion elements.

In general, as indicated by the idealized data in Exhibit A, the median and the TLS regression-predicted CF will be similar under either of the following conditions: (a) log-log EO/WB slope near 1.0, or (b) criterion near the middle of the observed data range of tissue concentrations for the species. They are likely to differ from each other when both of the following conditions simultaneously occur: (c) log-log slope distant from 1.0, and (d) criterion distant from the center of the data range.

2.0 COMPARISON OF THE ORDINARY LEAST SQUARES AND TOTAL LEAST SQUARES REGRESSION APPROACHES

The calculation of conversion factors using linear regression following log transformation addresses the issues associated with non-proportional relationships between Se in different tissues, and is the approach recommended by several public commenters. Conventional ordinary least squares (OLS) regression results can vary depending on which tissue type is assigned to the x and y axis, respectively. This is because OLS regression assumes that the variable on the y axis is dependent on the variable on the x axis, and the resulting regression is the line that minimizes the sum of the squared distances between observed y-values and predicted y-values. OLS regression assumes that the values on the x-axis have no uncertainty. For datasets such as the paired tissue concentrations used to calculate CF, there is no dependency between the selenium concentrations in one tissue type to another tissue type, and concentrations in both tissue types are equally uncertain. Because of this, we could assign either tissue type to either axis, and the resulting CF would be slightly different. By convention, we assign egg-ovary to the y-axis when comparing it to whole-body or muscle, and we assign muscle to the y-axis when comparing it to whole-body, because these are the ratios used in the translation equations. While CFs using median ratios are not affected by axis assignment, CFs using OLS are, for reasons described above.

An alternative regression approach that corrects this issue is total least squares (TLS) regression. TLS regression is preferable to OLS regression in cases where there is error associated with each of the variables, and there is no dependency of one variable on the other. With TLS, the regression is the line that minimizes the sum of the squared distances between observed predicted x- and y-values, and produced the same result regardless of which variable is assigned to which axis. Curves drawn by eye tend to mimic TLS, not OLS. Without thinking about it, the person drawing the line naturally attempts to

minimize both vertical and horizontal errors. However, a significant disadvantage of TLS regression is that Excel has no built-in function to perform it, and many readers will be unfamiliar with it.

Table N-1 shows the effect of the different calculation procedures (median ratio, log OLS regression - xyOLS, log OLS regression with reversed axes - yxOLS, and log TLS regression) on all directly measured CFs. Median ratio CFs tend to diverge from regression based CFs for datasets where log-log slopes are markedly different than 1 and the criterion is not near the center of the observed concentrations. CFs calculated from TLS regression nearly always fall between CFs calculated from OLS regression with and without the axes reversed, and are not affected by axis order.

	Directly calculated conversion factors for each tissue ratio, by method											
	EO/WB				M/WB				EO/M			
Species	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS
bluegill	2.13	1.90	2.04	1.98	1.32	1.36	1.37	1.36	1.38	1.11	1.24	1.18
bluehead sucker	1.82	1.41	1.50	1.45	1.23	1.70	1.67	1.59	1.48	0.82	0.91	0.85
brook trout									1.09	0.96		0.99
brown trout	1.45	1.53	1.77	1.74								
common carp	1.92	1.62	1.63	1.62	1.61	1.36	1.41	1.45	1.14	1.06	1.18	1.14
creek chub	1.99	2.05	2.01	2.03								
cutthroat trout	1.96	1.37	1.67	1.48					1.81	1.97	1.83	1.89
desert pupfish	1.20	1.14	1.14	1.14								
dolly varden									1.26	1.64	1.52	1.59
fathead minnow	1.40	1.71	1.56	1.64								
flannelmouth sucker	1.41	1.14	1.84	1.49	1.46	1.94	1.89	1.85	1.08	0.51	1.06	0.69
green sunfish	1.45	1.35	1.45	1.40	1.23	1.28	1.32	1.24	1.21	1.08	1.17	1.12
mountain whitefish									5.80	10.47	4.98	7.35
northern pike									1.88	1.65	1.78	1.70
rainbow trout									1.92	1.82	1.88	1.82
razorback sucker									2.31	1.93	1.89	1.90
roundtail chub	2.07	2.22	2.26	2.24	1.05	1.08	1.10	1.05	2.04	1.99	2.10	2.06
smallmouth bass	1.42	1.31	1.68	1.52	1.23	1.88	1.97	1.68	1.19	0.67	0.88	0.72
white sturgeon									1.33	0.97	1.07	1.01
white sucker	1.38	1.02	1.25	1.12	1.34	1.43	1.54	1.45	1.00	0.59	0.84	0.67

Table N-1. Comparison of all directly calculated conversion factors by method.
Methods include median ratio (Ratio), log ordinary least squares (xyOLS), log ordinary least squares with axes reversed (yxOLS), and log total least squares (logTLS). Regression based CFs were calculated at the egg ovary criterion concentration of 15.1 mg/kg. Muscle to whole body (M/WB) CFs were calculated at the muscle concentration at the egg-ovary criterion.

The following examples illustrate the differences between OLS and TLS regressions, and the effect of axis assignment on CF.

2.1 Example 1 – Flannelmouth Sucker (Egg-ovary/Whole-body)

CF by approach: (1.41 –median ratio, 1.13 – log OLS, 1.86 – log OLS with reversed axes, 1.48 – log TLS)

Model comparison 1a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log (Egg-ovary) = (0.7966 x (log Whole-body)) + 0.2857

log (Whole-Body) = (log (16 mg/kg) – 0.2857)/0.7966 = 1.153

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.13

TLS: log (Egg-ovary) = (0.9877 x (log Whole-body)) + 0.1843

log (Whole-Body) = (log (16 mg/kg) – 0.1843)/0.9877 = 1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

In Figure N-2, the fitted regression lines do not appear particularly divergent; however, these points cover a relatively narrow (and low), concentration range. At the criterion concentration (log E/O = 1.204), the predictions lines are more divergent, resulting in the differences between the CFs. Also, note that the TLS slope is close to 1. The resulting TLS-derived CF is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in a CF for the OLS model that is notably different than the median ratio CF (1.13 vs 1.41).

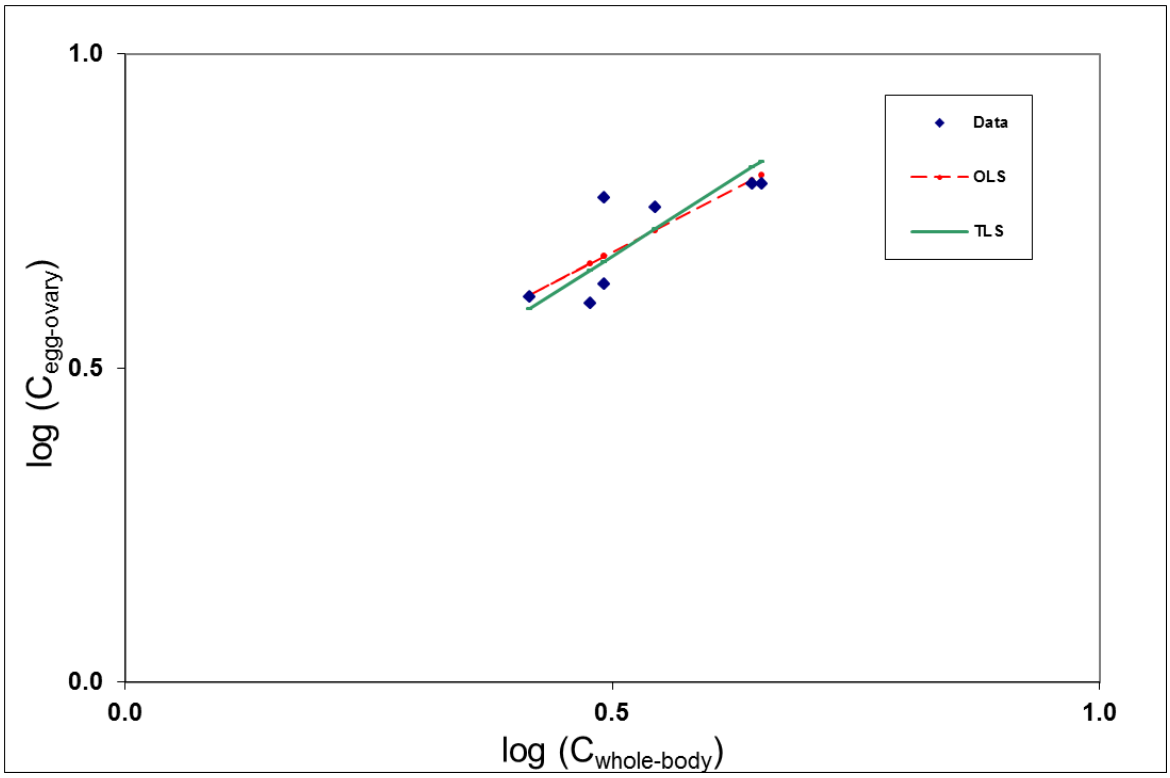


Figure N-2. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 1b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.8126 x (log Egg-ovary)) - 0.0450 = 0.9335

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.86

TLS: log(Whole-body) = (1.012 x (log Egg-ovary)) - 0.1866 =1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

At first glance, Figure N-3 appears very similar to Figure N-2. However, note that the axes are reversed, and because we are now solving for y (whole body concentration at egg-ovary criterion concentration), the shallower slope of the reverse OLS figure results a whole body concentration at the egg-ovary criterion lower than in the upper figures, which in turn results in a larger CF. Also, note that the TLS model is a mirror image of the model in Figure N-3, and as such has the same calculated CF. As above, the TLS slope is close to 1, with a TLS-derived CF that is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in an OLS-derived CF that is notably different than the median ratio CF (1.86 vs 1.41).

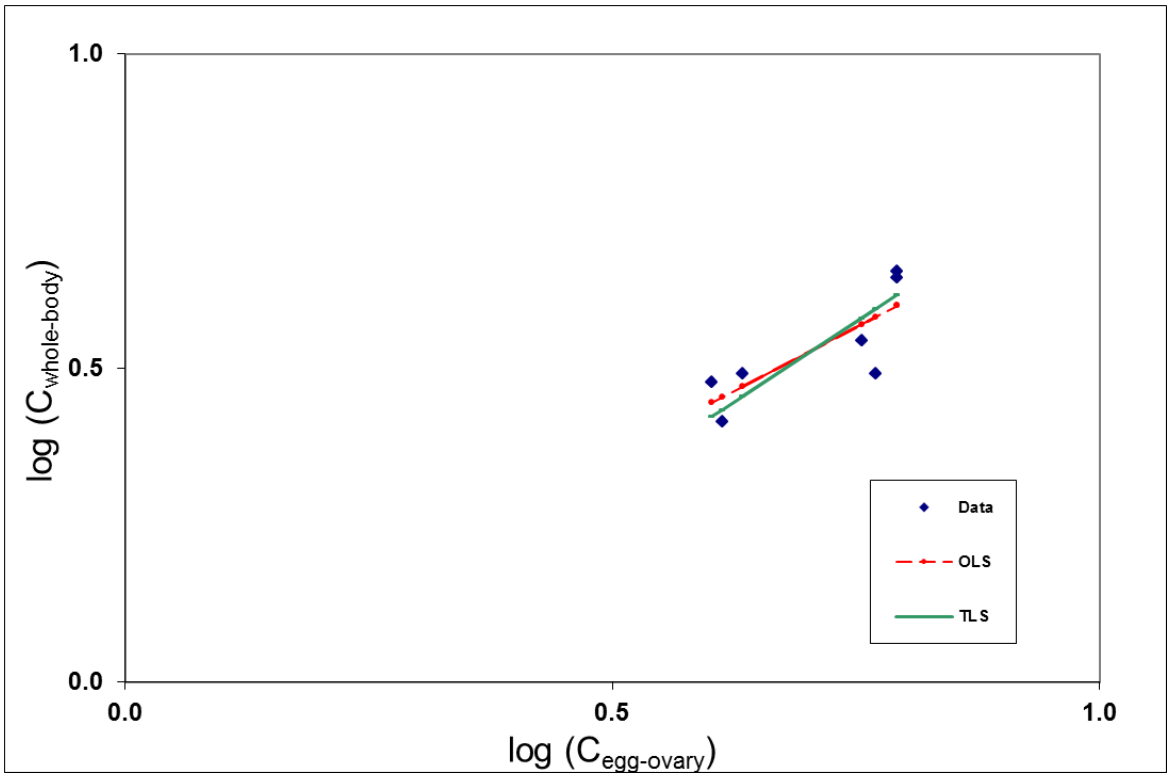


Figure N-3. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

2.2 Example 2 – Bluegill (Egg-ovary/Whole-body)

CF by approach: (2.13 –median ratio, 1.90 – log OLS, 2.07 – log OLS with reversed axes, 2.01 – log TLS)

Model comparison 2a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log(Egg-ovary) = (1.061 x (log Whole-body)) + 0.2227

log (Whole-Body) = (log (16 mg/kg) – 0.2227)/1.061 = 0.9250

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.90

TLS: log(Egg-ovary) = (1.240 x (log Whole-body)) + 0.0.0861

log (Whole-Body) = (log (16 mg/kg) – 0.0861)/1.240 = 0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly larger calculated CF (Figure N-4). Even though the slopes are larger than 1, the data range encompasses the criterion concentration, which is close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

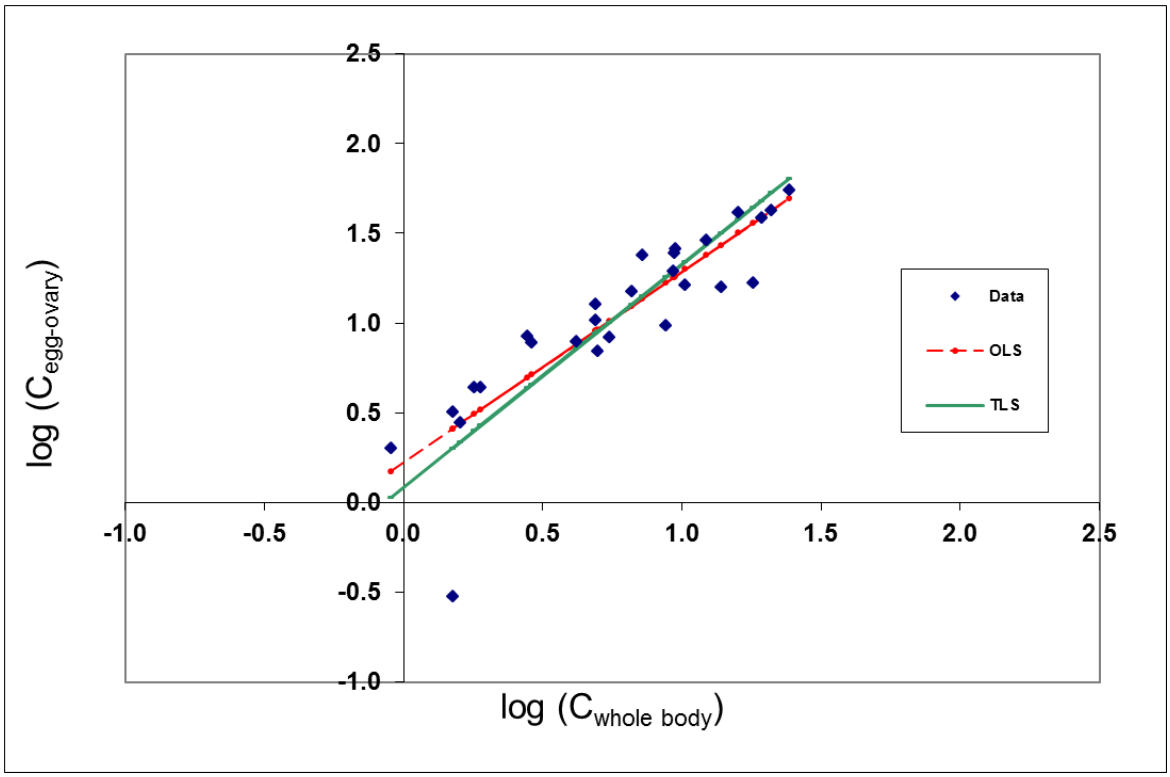


Figure N-4. OLS vs. TLS regression model fits for bluegill. Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 2b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.7269 x (log Egg-ovary)) + 0.0129 = 0.8883

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.07

TLS: log(Whole-body) = (0.8066 x (log Egg-ovary)) - 0.0695 =0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly smaller calculated CF (Figure N-5). Even though the slopes are less than 1, the data range encompasses the criterion concentration, which is also close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

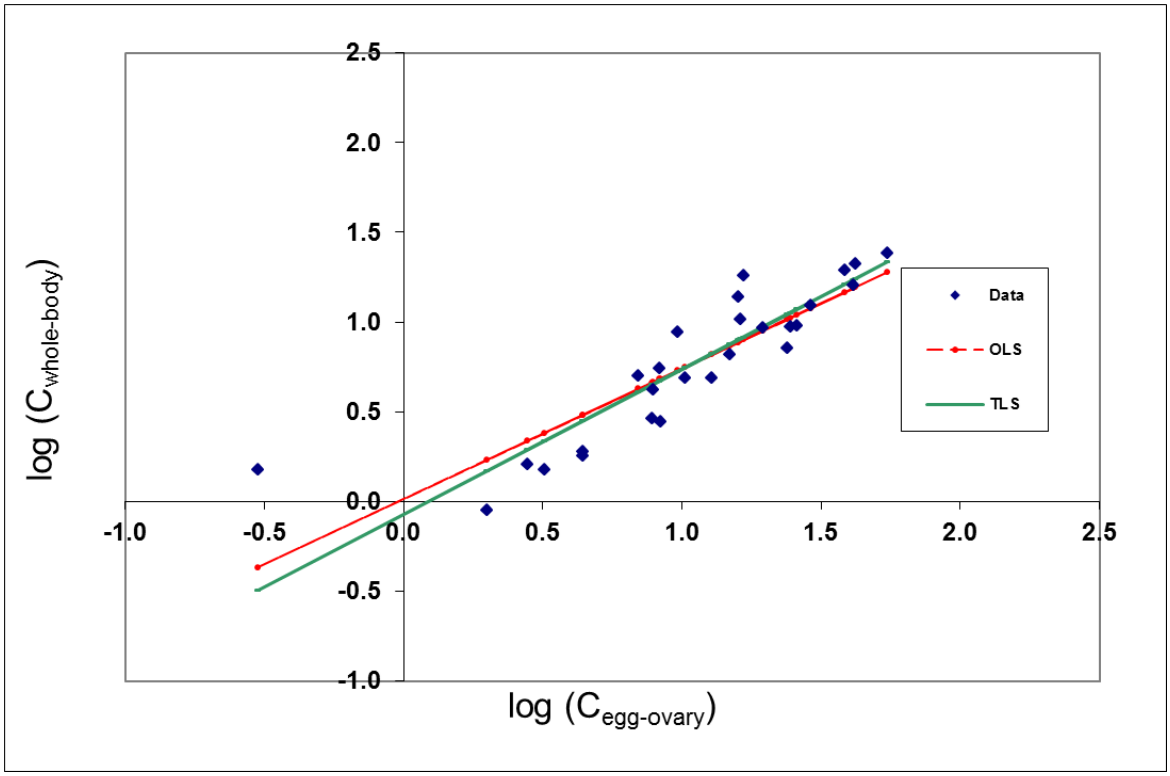


Figure N-5. OLS vs. TLS regression model fits for bluegill.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

The effect of the CFs calculated by the different approaches has a minor effect on the final translated water criterion elements. Compared to the median ratio method, translated water criterion element concentrations are slightly higher using CFs calculated from the log OLS regression methods, CFs calculated from the reverse axis log OLS are slightly lower. Lentic translated water criterion element

concentrations are the same using CFs from median ratios and log TLS regression methods, while lotic concentrations calculated from log TLS CFs are slightly lower compared to those calculated using median ratio CFs (**Table N-2**).

Table N-2. Translated water concentration criterion element criterion concentrations by CF calculation method.

Method	Lentic (µg/L)	Lotic (µg/L)
Median Ratio	1.5	3.1
log OLS regression	1.6	3.3
inverse log OLS regression	1.4	2.9
log TLS regression	1.5	2.9

3.0 COMPARISON OF MEDIAN- AND REGRESSION-BASED CONVERSION FACTORS TO CALCULATE CHRONIC VALUES FOR MUSCLE AND WHOLE BODY TISSUES

Besides being used in the translation of the egg-ovary (EO) tissue criterion to water, conversion factors (CF) were also used to convert egg-ovary (EO) chronic values (CV) to muscle or whole body tissue concentrations. These conversions were done when the data from a reproductive toxicity study did not have muscle or whole body selenium concentrations or if the latter tissue data was not usable to determine a chronic value. Directly calculated CVs using either muscle or whole body selenium measurements from a study was preferred over converted CVs in the determination of the final chronic value (= criterion).

Table N-3 provides a comparison of median-based and regression-based CFs when they are used to convert an EO selenium concentration to muscle or whole body. Regression-based CFs used total least squares (TLS) regression for the reasons stated above. The table lists each taxon in the reproductive toxicity data set and presents CVs that are either directly calculated or converted from the EO CV using either the median or TLS CF. Generally, the median-based and TLS-based CFs were similar for both tissue types and this similarity resulted in similar criteria (bottom row). The muscle criterion for the data set that contained directly calculated CVs and converted CVs was similar whether median or TLS CFs were used, 11.3 and 10.6, respectively. The whole body criterion was also similar using these two approaches, 8.5 and 9.6, respectively. The median-based CFs were selected based on reasons stated in the previous section.

Table N-3. Comparison of muscle and whole body chronic values when calculated directly and converted from egg-ovary concentrations using median- and TLS regression-based conversion factors.

Taxon	EO CV	Muscle chronic values (CV) and conversion factors (CF)						Whole body chronic values (CV) and conversion factors (CF)					
		Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV	Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV
Salvelinus	56.22	44.48	35.36	1.26	44.48	1.59	35.36	34.92	24.34	1.61	34.92	2.31	24.34
Esox	34	21.70[d]	21.70[d]	1.88	18.13	1.70	20.00	14.23	13.77	2.39	14.23	2.47	13.77
Cyprinodon	27	28.72	34.18	0.94	28.72	0.79	34.18	22.50	23.68	1.20	22.50	1.14	23.68
O. mykiss	24.5	12.79	13.46	1.92	12.79	1.82	13.46	10.04	9.28	2.44	10.04	2.64	9.28
O. clarkii, Rudolph	24.7	16.6[d]	16.6[d]	1.81	13.65	1.89	13.07	12.60	16.69	1.96	12.60	1.48	16.69
O. clarkii, Nautilus	27.7	15.30	14.66	1.81	15.30	1.89	14.66	14.13	18.72	1.96	14.13	1.48	18.72
Oncorhynchus	25.31	14.28	14.49	NA	13.59	NA	13.65	11.58	12.81	NA	11.58	NA	12.81
Micropterus	26.3	22.16	36.53	1.19	22.16	0.72	36.53	18.52	17.30	1.42	18.52	1.52	17.30
L. macrochirus, Coyle	26.3	19.13	22.29	1.38	NA	1.18	NA	8.6[d]	8.6[d]	NA	NA	NA	NA
L. macrochirus, Doroshov	22.6	15.7[d]	15.7[d]	NA	NA	NA	NA	10.61	11.41	2.13	NA	1.98	11.41
L. macrochirus, Hermanutz	14.7	13.4[d]	13.4[d]	NA	NA	NA	NA	10.6[d]	10.6[d]	NA	NA	NA	NA
Lepomis	20.60	15.91	16.74	1.38	14.98	1.18	17.45	9.890	10.13	2.13	9.656	1.98	10.40
Salmo	21	18.50	17.50	1.14	18.50	1.20	17.50	13.2[d]	13.2[d]	1.45	14.48	1.74	12.07
Acipenser	15.6	11.9[d]	11.9[d]	1.33	11.73	1.01	15.45	9.209	10.68	1.69	9.209	1.46	10.68
Criterion	**15.10**	**11.34**	**11.57**	NA	**10.99**	NA	**13.35**	**8.538**	**9.567**	NA	**8.189**	NA	**9.879**

[d] directly calculated from muscle or whole body selenium concentrations

Appendix H

Final
Written Findings for the Site-Specific Water Column Selenium Standard for Lake Koocanusa, MT

June 2022

Prepared by:
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1.0 INTRODUCTION

On December 11, 2020, the Board of Environmental Review (board), voted to adopt the Department of Environmental Quality's (department) proposed selenium water quality standard for Lake Koocanusa into state law and determined the proposed standard was no more stringent than federal guidelines. Therefore, there was no requirement for the completion of written findings as described in MCA 75-5-203. The multi-part standard includes fish tissue and water column components with the following numeric values: 15.1 mg/kg dry weight (dw) egg/ovary, 11.3 mg/kg dw muscle, 8.5 mg/kg dw whole body, and 0.8 µg/L total dissolved selenium in Lake Koocanusa and 3.1 µg/L in the Kootenai River mainstem. See ARM 17.30.632. The frequency and duration of the fish tissue standards are instantaneous measurements, not to be exceeded. The water column standard is computed as a 30-day average and shall not be exceeded more than once in three years, on average.

The standards were adopted into state law on December 25, 2020, and codified in ARM 17.30.632. The water quality standards were approved by the U.S. Environmental Protection Agency (EPA) on February 25, 2021. Petitions were filed to the board by Teck Coal Limited (Teck) on June 30, 2021, and by Lincoln County on October 14, 2021, with both petitions calling for a review of the stringency determination for the site-specific water column standard for Lake Koocanusa in ARM 17.30.632(7)(a). On February 25, 2022, the board reversed its previous stringency determination and determined that the site-specific water column standard for Lake Koocanusa is more stringent than comparable federal guidelines. The department will, therefore, comply with MCA 75-5-203 by making the written findings set forth in 75-5-203(2), MCA. Presented in this document are the written findings supporting ARM 17.30.632(7)(a) required under MCA 75-5-203(2) and MCA 75-5-203(3).

2.0 WRITTEN FINDINGS

The purpose of this document is to complete the written findings described in MCA 75-5-203 for the site-specific water column selenium standard for Lake Koocanusa. The document has been structured to address each statutory requirement individually. MCA 75-5-203 states that the written findings must be based on the record. Citations to the board's rulemaking record on the matter are included with the prefix "RR" followed by the page number, and the full record can be found on the DEQ Standards website at https://deq.mt.gov/files/Water/WQPB/Standards/pdf/BER_Record.pdf

<u>75-5-203(2)(a) The proposed state standard or requirement protects public health or the environment of the state</u>

Selenium Toxicity

The selenium water quality standard for Lake Koocanusa is necessary to protect aquatic life from the toxic effects of selenium (**RR_000001**). The water quality standard is a multi-part standard comprised of egg/ovary, muscle or whole body, and water column components (**RR_000074**). The multi-part water quality standard accounts for diet as the primary pathway of selenium exposure (**EPA, 2016; RR_000317**). EPA (2016) states that traditional methods for predicting toxicity on the basis of exposure to water column concentrations do not work for selenium because the behavior and toxicity of selenium in aquatic systems is highly dependent on site-specific factors (**RR_000310**). Therefore, the protection of the egg/ovary

selenium concentration levels are the foundational basis of the federal EPA guideline (EPA, 2016). From the egg/ovary tissue the other fish tissue (muscle and whole body) standards and water column standard were derived (EPA, 2016). The most influential step in selenium bioaccumulation in the food web is the uptake of dissolved selenium into particulate selenium at the base of the food web and this can be characterized as the Kd. This is operationally defined as the concentration of selenium in particulate material divided by the concentration in the water column (**Presser and Luoma, 2010; RR_001111**). As selenium is further transferred through the food chain, a variety of toxic effects can manifest with the most well-documented being reproductive teratogenesis and larval mortality of egg- laying vertebrates (**EPA, 2016; RR_000328**). While selenium is an essential nutrient, a narrow margin exists between the amount of selenium required by an organism and the amount that can cause toxicity with egg-laying vertebrates being more sensitive to selenium than mammals (**EPA, 2016; RR_000327-RR_00329)**. For Lake Koocanusa, larval survival of egg-laying vertebrates, specifically fish, were determined to be the most sensitive endpoint (**RR_000093**). Presser and Luoma (2010) describe some of the effects in fish being larva and fry survival and growth and deformity (**RR_002945**). EPA (2016) outlines a variety of lethal and sublethal deformities that can occur in developing fish exposed to selenium where those fish with deformities can die shortly after hatching or their overall fitness is reduced to a level whereby their rate of survival is less than normal young fish. EPA (2016) further states that the percentage of deformed adults observed during surveys will likely underestimate the underlying percentage of deformed young (**RR_000329**). Biologists with Fish Wildlife and Parks (FWP) have corroborated this for the Lake Koocanusa system, stating that selenium affects fish at the reproductive level which can be challenging to detect beyond sampling selenium concentrations in egg/ovary tissues and that as a result of the sampling methods used in routine FWP fish surveys adult fish are preferentially captured and they would not expect to see deformities in surviving adult fish in their sampling (**RR_002146**). At the center of many technical discussions in the process of standards setting for Lake Koocanusa was the topic of a lag time before effects are fully realized in fish tissues (**RR_004233**). EPA (2016) notes that in a system with new inputs, concentrations in fish tissue can take years to be detected at stable concentrations in a lentic system (**RR_000417**).

Selenium Inputs and Scientific Approach

Jenni et al. (2017) reports annual selenium loads entering Lake Koocanusa have increased from 2,600 kilograms (kg) in 1992 to over 13,000 kg in 2012, representing more than a fivefold increase over 20 years (**RR_002844**). Presser and Naftz (2020) show a continuing increase in selenium concentrations for decades recorded at Highway 93 on the Elk River, British Columbia, a tributary to Lake Koocanusa. The Canadian data presented in Presser and Naftz (2020) shows concentrations below 1 μg/L in 1985 with a steady increasing trend to over 8 μg/L by 2020 (**RR_001146**). Furthermore, Presser and Naftz (2020) show cross sectional areas of Lake Koocanusa with selenium concentrations greater than1 μg/L increasing from 2016-2019 (**RR_001170-001171**). The Lake Koocanusa Monitoring and Research Group and Selenium Technical Subcommittee that guided the department's work, was established, in part based on the increasing levels of selenium entering Lake Koocanusa.

In 2013 the British Columbia Minister of Environment issued a Ministerial Order (**RR_003994-RR_004002**) under the Environmental Management Act to remediate water quality effects of past mining activities and to guide environmental management of future mining activities in the Elk Valley, including the Canadian portion of Lake Koocanusa (**RR_000080**). The Order led to the Elk Valley Area Based Management Plan (ABMP) and established a Technical Advisory Committee (TAC) with agencies from Canada and the United States participating (**RR_000087**). That work taking place in Canada, eventually led to the formation of the bi-national Lake Koocanusa Monitoring and Research Working Group that

brought together Montana and British Columbia regulatory agencies and stakeholders. Jenni et al. (2017) describes the establishment of the Lake Koocanusa Monitoring and Research Working Group in 2015 by Montana DEQ and British Columbia Ministry of the Environment and Climate Change Strategy (BC-ENV) to address current and future water quality concerns in the Lake Koocanusa watershed and to work towards joint solutions for managing potential selenium contamination including the development of a site-specific selenium criteria for the protection of aquatic life and wildlife (**RR_002844**). The department co-led the Lake Koocanusa Monitoring and Research Working Group engaging a diverse group of stakeholders, first nation governments, federal agencies, and industry. A Selenium Technical Subcommittee was established comprising leading experts in the field of selenium toxicology from both the United States and Canada (**RR_005231**). Members of the Selenium Technical Subcommittee were identified and invited to participate. This made up a team of bi-national selenium experts, most with careers of over 20 years of expertise in selenium toxicity – collectively over 200 years of direct work with selenium. Member affiliations of the Selenium Technical Subcommittee include: state and provincial co-chairs, tribal and First Nations, federal agencies (EPA, U.S. Fish and Wildlife Service, U.S. Geological Survey), academia (University of Saskatchewan) and an Industry Consultant. The Selenium Technical Subcommittee undertook the following steps to guide the ultimate standards decision: identifying objectives, peer-reviewed conceptual model framework, supplemental targeted data collection by U.S. entities, BC-ENV, and Teck, a peer-reviewed Lake Koocanusa ecosystem model report and supplemental peer-reviewed data and model results, and recommendations on the standard based on the model results. The department worked over many years soliciting feedback from stakeholders and the collective knowledge of the selenium experts throughout the development of a site-specific selenium standard for Lake Koocanusa. (**RR_002109**).

The department followed federal guidance for developing a site-specific water column standard for Lake Koocanusa. The process followed is defined in EPA (2016) Appendix K: Translation of a Selenium Fish Tissue Criterion Element to a Site-Specific Water Column Value (**RR_001035-001078**). The water column component is translated from the fish tissue component utilizing the mechanistic Presser and Luoma (2010) bioaccumulation modeling approach and tailoring it to the Lake Koocanusa ecosystem (**RR_002514**). The department worked with the U.S. Geological Survey (USGS) to complete the modeling effort **(RR_002514; RR_000119-RR_000127)**. The outcome of that work was the peer-reviewed Presser and Naftz (2020) report which utilized the Presser and Luoma (2010) ecosystem model and calibrated it to the Lake Koocanusa ecosystem (**RR_002971-003017**). The final interpretative report was subject to the USGS rigorous scientific protocols for peer-review (**RR_002134**). The department worked with this scientifically peer-reviewed and published model, the associated peer-reviewed report and dataset, and peer-reviewed interactive spreadsheets including different modeling scenarios for the derivation of a site-specific water column standard for Lake Koocanusa (**RR_001175; RR_004062**).

The Presser and Naftz (2020) peer-reviewed report, dataset, and interactive spreadsheets with various modeling scenarios all provided the rationale and food web modeling structure for the quantitative derivation of a site-specific guideline for Lake Koocanusa (**RR_004062**). Model predictions from the peer-reviewed work for a range of protective dissolved selenium concentrations were specific to the EPA national guideline of 8.5 mg/kg whole body criterion element. Modeling assumptions used for modeled scenarios were guided by the goals defined by the Selenium Technical Subcommittee and are summarized here as:

- Consideration of ecologically significant species and those important to stakeholders,
- Protection of ecosystems during maximum dietary exposure (i.e., feeding within a benthic food web),

- Protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding in an ecosystem that functions as a lentic reservoir, and
- Long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all Se loading profiles, and all water years (**Presser and Naftz 2020; RR_002980**

Derivation of Site-Specific Water Column Standard

The collective scientific expertise of the Selenium Technical Subcommittee guided the development of Lake Koocanusa's site-specific water column Se standard, from data collection to the final recommendations for ecosystem-scale modeling factors (**RR_000124**). Based on the Presser and Naftz (2020) modeling work, the recommendations by the Selenium Technical Subcommittee, Lake Koocanusa Working Group, and the professional judgement of DEQ staff, the final model selected by the department utilizes the EPA recommended whole body guideline of 8.5 mg/kg, the trophic fish model with a 100% aquatic insect diet, 60% bioavailability, and the Kd selected at the 75th percentile which results in a protective water column value of 0.8 µg/L (**RR_000127**). This model was one of the options put forward in the peer-reviewed interactive spreadsheets (Jenni and Schmidt, 2020) that accompanied the Presser and Naftz (2020) report. Specifically, this correlates with the "W6 TFM L3" model listed in Jenni and Schmidt (2020). This model was selected by the department to protect all fish including those piscivores (bull trout and burbot) that may be consuming prey fish with a 100% aquatic insect diet (**RR_004060; RR_004065**). For modeling, Presser and Naftz (2020) characterized the fish species in the reservoir with a 100% insectivore diet as rainbow trout, westslope cutthroat trout, redside shiner, and longnose sucker (**RR_001166**). The adopted 8.5 mg/kg dw fish tissue standard in ARM 17.30.632 is the same value that was used in modeling that led to the site-specific water column standard (**RR_002532**). The department then reviewed the full range of model results provided in the interactive and peer-reviewed spreadsheet and made a risk decision to select at the 75th percentile of the Kd distribution resulting in a protective dissolved selenium concentration of 0.8 µg/L (**RR_004065**). This level of protection meets the protection goals defined for Lake Koocanusa (**RR_002354**). The standards are consistent with the best available science for selenium toxicity and will protect the selenium-sensitive aquatic life in this watershed (**RR_000069**).

With water column concentrations currently near 1 µg/L, data available at the time of rulemaking showed there have been 9 individual fish found with concentrations greater than 15.1 mg/kg dw spanning three species. Moreover, the downstream Kootenai River in Idaho has been listed impaired due to selenium found at high levels in fish tissue. Water quality standards are set to protect the beneficial use (to prevent impacts) and to protect downstream uses (**RR_001538**).[1]

<u>75-5-203(2)(b) The state standard or requirement to be imposed can mitigate harm to the public health or environment and is achievable under current technology</u>

The water column selenium standard for Lake Koocanusa of 0.8 µg/L can mitigate harm to the environment and is achievable under current technology.

[1] Following rulemaking, the 2020 fish tissue data was made publicly available by Fish Wildlife and Parks and posted to the Lake Koocanusa Monitoring and Research Working Group wiki website. Fish egg/ovary data through 2020 show 17 individual fish spanning four species at levels at or above 15.1 mg/kg dw. EPA has indicated that levels at or above 15.1 mg/kg dw in egg/ovary tissue can have toxicological effects on the fish at the reproductive level.

As explained above, the water column selenium standard for Lake Koocanusa is necessary and can mitigate harm to aquatic life in Lake Koocanusa. The site-specific water column standard for Lake Koocanusa is achievable under current technology. EPA (2016) states that two main anthropogenic activities are known to cause increased mobilization of selenium into the aquatic environment. Those two activities include mining of metals, minerals and use of fossils fuels and irrigation of selenium rich-soils (**RR_000320**). The department found no public or private entities discharging to Lake Koocanusa with Montana Pollutant Discharge Elimination System (MPDES) permit effluent limits for selenium. There are no permitted sources of selenium in the Kootenai Watershed (**RR_001213**). This watershed does not have the same selenium rich geologic strata that are found in areas such as the Elk Valley, BC (**RR_001213; RR_002400; RR_002519**). Even if selenium was found in the watershed, this standard is site-specific and only applicable to permits discharging directly to Lake Koocanusa (**RR_002415**). There are no pending discharge permit applications nor has the department heard of any future permits for Lake Koocanusa. FWP's Murray Springs Fish Hatchery (MTG130001) is the only currently permitted facility discharging directly into Lake Koocanusa and selenium is not a pollutant of concern and that permit contains no effluent limits for selenium. There are no other general or individual permits authorizing discharges to or around Lake Koocanusa. The only hard rock permit near Lake Koocanusa is the McGilvary Rock quarry (OP#00167) which is inactive.

Current treatment technologies for activities around Lake Koocanusa include best management practices (BMPs) such as: measures that prevent storm water from coming into contact with pollutants; measures that minimize impervious surface area and retain runoff where it can be treated through infiltration; and measures that provide riparian buffers and reduce erosion to protect surface water from direct site runoff that may contain pollutants. Additionally, mines and industrial sites must document potential pollutants in a storm water pollution prevention plan (SWPPP) and provide adequate control measures to avoid impact to water quality. Existing and future land disturbing and industrial operations are already subject to storm water permitting requirements and BMPs to avoid impacts to surface water and no owner/operator/permittee should incur substantially different or increased treatment costs as a direct result of the site-specific water column standard for Lake Koocanusa. There is no evidence to suggest this site-specific standard will result in increased treatment costs for owners and operators of activities or facilities that discharge to surface water (**RR_002497**).

This standard is achievable under current technology. There are no current or planned point source dischargers to Lake Koocanusa with selenium as a pollutant of concern. Based on evidence in the record there is no significant geological source of selenium in Montana contributing to selenium concentrations in Lake Koocanusa and the two main anthropogenic activities that cause selenium mobilization to the aquatic environment (mining and irrigation of selenium-rich soil) do not occur on or around Lake Koocanusa. However, if there were a need for treatment in the future, this would be achieved through one of the current technologies listed by EPA which include reverse osmosis, iron reduction/precipitation, active biological treatment, aerobic wetlands, and/or biochemical reactors or anaerobic wetlands. It is more likely that any contribution of selenium related to or arising from land disturbing activities would be addressed through BMPs required under applicable General Permits such as the General Permit for stormwater discharges associated with construction activity (MTR100000), the Multi-Sector General Permit for storm water discharges associated with Industrial Activity (MTR000000), or the General Permit for Sand and Gravel Operations (MTG490000).

75-5-203(3) The written finding must reference pertinent, ascertainable, and peer-reviewed scientific studies contained in the record that forms the basis for the department's conclusion.

The department referenced the following pertinent, ascertainable, and peer-reviewed scientific studies contained in the record. Full citations are listed in the reference section:

- EPA (2016)
- Jenni et al. (2017)
- Presser and Luoma (2010)
- Presser and Naftz (2020) and accompanying peer-reviewed database and interactive spreadsheets by Jenni and Schmidt (2020)

EPA (2016) and Presser and Luoma (2010) address selenium toxicology. Jenni et al. (2017), Presser and Luoma (2010), Presser and Naftz (2020) address model development. Jenni et. al. (2017), Presser and Naftz (2020) and accompanying peer-reviewed database and interactive spreadsheets address model development specifically to the Lake Koocanusa ecosystem.

75-5-203(3) The written finding must also include information from the hearing record regarding the costs to the regulated community that are directly attributable to the proposed state standard or requirement.

The department has reviewed permits and activities on and around Lake Koocanusa and determined that this standard is achievable for Montana point source dischargers as there are no point source dischargers with selenium as a pollutant of concern. There is no cost to the regulated community directly attributed to the Lake Koocanusa selenium standard. The regulated community is within Montana because this is a site specific water column standard for Lake Koocanusa, Montana that only applies within Montana's borders. The only potentially affected dischargers are those discharging to Lake Koocanusa, Montana.

No Montana permittee will incur additional costs to treat wastewater for selenium as a direct result of the adoption of ARM 17.30.632(7)(a). Larger land development activities, such as surface mining and construction are already subject to general discharge permit requirements including implementation and maintenance of best management practices (BMPs). The department foresees no additional or different treatment requirements associated with these land disturbing activities directly attributable to the adoption of ARM 17.30.632(7)(a). Available treatment technology and economic cost of treatment can be further considered in future use attainability determinations and in variance development (**RR_002964**).

3.0 PUBLIC INVOLVEMENT

As part of the original rulemaking, MAPA provides for public review and comment of the proposed rule. That public comment period was held from October 9 to November 23 in 2020 with a public hearing held on November 5, 2020. During that time, commenters had an opportunity to provide comment on the rulemaking package, including the Board of Environmental Review's stringency determination. A subset of commenters did comment on that, and the board provided a response during the response to comment.

Public comment on the department's draft written stringency findings will be received between April 4 and May 4, 2022. A public hearing will be held on April 26, 2022. Public notice will be published in the local newspapers in Libby, Troy, and Eureka to promote local awareness and participation in the public comment period.

4.0 CONCLUSION

The rulemaking record provides sufficient evidence to support the 0.8 μg/L site-specific water column standard in ARM 17.30.632(7)(a) as necessary to protect the environment. The site-specific water column standard for Lake Koocanusa will not have a substantial economic impact on the regulated community. There will be no additional costs to the regulated community directly attributable to ARM 17.30.632(7)(a) and the standard is achievable under current technology. These findings will be made a part of the rulemaking record. These findings will be complete upon DEQ's response to substantive comments on ARM 17.30.632(7)(a) received from the public during the public comment period.

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Appendix I

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Article

Growth of Coal Mining Operations in the Elk River Valley (Canada) Linked to Increasing Solute Transport of Se, NO_3^-, and SO_4^{2-} into the Transboundary Koocanusa Reservoir (USA–Canada)

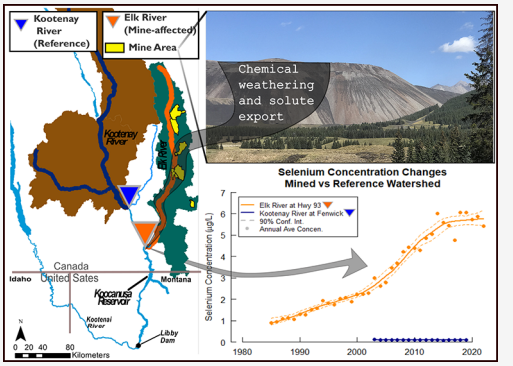
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ABSTRACT: Koocanusa Reservoir (KOC) is a waterbody that spans the United States (U.S.) and Canadian border. Increasing concentrations of total selenium (Se), nitrate + nitrite (NO_3^-, nitrite is insignificant or not present), and sulfate (SO_4^{2-}) in KOC and downstream in the Kootenai River (Kootenay River in Canada) are tied to expanding coal mining operations in the Elk River Watershed, Canada. Using a paired watershed approach, trends in flow-normalized concentrations and loads were evaluated for Se, NO_3^-, and SO_4^{2-} for the two largest tributaries, the Kootenay and Elk Rivers, Canada. Increases in concentration (SO_4^{2-} 120%, Se 581%, NO_3^- 784%) and load (SO_4^{2-} 129%, Se 443%, NO_3^- 697%) in the Elk River (1979–2022 for NO_3^-, 1984–2022 for Se and SO_4^{2-}) are among the largest documented increases in the primary literature, while only a small magnitude increase in SO_4^{2-} (7.7% concentration) and decreases in Se (−10%) and NO_3^- (−8.5%) were observed in the Kootenay River. Between 2009 and 2019, the Elk River contributed, on average, 29% of the combined flow, 95% of the Se, 76% of the NO_3^-, and 38% of the SO_4^{2-} entering the reservoir from these two major tributaries. The largest increase in solute concentrations occurred during baseflows, indicating a change in solute transport and delivery dynamics in the Elk River Watershed, which may be attributable to altered landscapes from coal mining operations including altered groundwater flow paths and increased chemical weathering in waste rock dumps. More recently there is evidence of surface water treatment operations providing some reduction in concentrations during low flow times of year; however, these appear to have a limited effect on annual loads entering KOC. These findings imply that current mine water treatment, which is focused on surface waters, may not sufficiently reduce the influence of mine-waste-derived solutes in the Elk River to allow constituent concentrations in KOC to meet U.S. water-quality standards.



KEYWORDS: *trends, load, water quality, WRTDS, selenium*

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1. INTRODUCTION

Worldwide, more than 260 river basins are divided and shared by multiple nations. Managing and preserving transboundary watersheds, their water resources, and cultural heritage is exceptionally difficult because political borders rarely coincide with watershed boundaries, and governments may have conflicting regulatory approaches.[1−3] Without cooperative resource management between governments, transboundary waterways are uniquely vulnerable to the influences of human land use on water quality and ecosystem integrity. One example is large scale mining and the alteration of land surfaces and aquifers due to placement of waste rock, which are known to profoundly influence groundwater and surface water quality.[4−6] Mining supports regional and national economies but has been shown to alter water, solute, and sediment dynamics and harm aquatic ecosystems.[7] Understanding environmental and water-quality impacts from mines located near borders provides information that may be used to protect, restore, and manage natural resources in the complex regulatory setting of transboundary watersheds.[8]

Koocanusa Reservoir (KOC, also called "Lake Koocanusa") is a transboundary reservoir that is split between northwestern Montana (MT), United States (U.S.), and southeastern British Columbia (B.C.), Canada (CA). The reservoir was impounded in 1972 by the Libby Dam, near Libby, MT (Figure 1). KOC encompasses the headwaters of the Kootenai (Kootenay in CA) River Basin. Including KOC, the Kootenai River crosses the U.S./CA border twice and drains into the Columbia River just north of where the river crosses the international border a third time (Figure 1). The Kootenai and Columbia Rivers have significant cultural importance—the watershed itself is the basis for the Ktunaxa Creation Story.[9] Ecologically and

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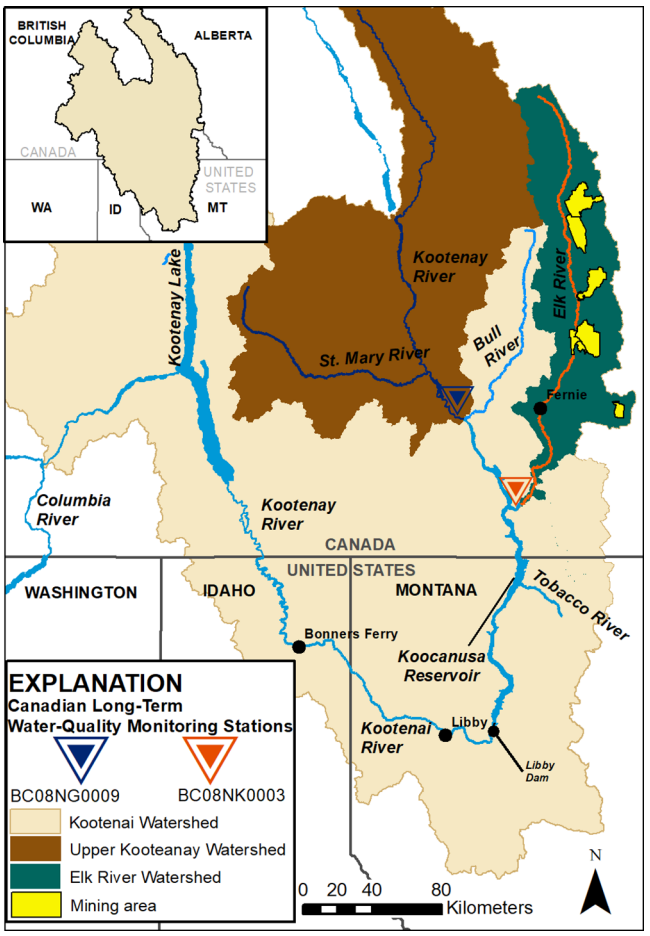


Figure 1. Study area map showing the Kootenai Basin (and partial Columbia River Basin) including the Elk River and Kootenay River Watersheds and other major tributaries; the Bull River, and the Tobacco River. Mine areas in the Elk River Watershed are shown in yellow, sampling locations are shown as triangles.[11,12]

culturally important fish resources in the Kootenai Watershed (U.S.) include the federally endangered Kootenai River White Sturgeon (*Acipenser transmontanus*), the threatened Bull Trout (*Salvelinus confluentus*), and two species of concern, Burbot (*Lota lota*) and Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*).[10] The headwater drainages for KOC are present on both sides of the border. However, the three largest tributaries are in B.C., and the second largest is the Elk River, which drains a watershed that contains several open-pit, coal mining operations (Figure 1).[11,12]

Coal mines have operated in the Elk River Watershed since 1897 and are known sources of contaminants to the transboundary waters in the Kootenai River Basin (Figure 2).[5,13−15] Current coal mines in the Elk River Watershed (Elk River Mines, ERM) are classified in Canada as open-pit mines, but they are analogous to mountain top removal coal mines in the U.S.[7] where mining operations create abundant volumes of waste rock that are deposited in valley areas more than 100-m thick.[4,13]

Open-pit coal mining in the Elk River Watershed results in the removal of coal and waste rock and subsequent waste rock dump generation (valley fill)—these mining operations alter the slopes, physical landscape, hydrologic, hydrogeologic, and geochemical functions of the mountain headwater systems where the mines are present (Figure 2).[5,14] As a result, these

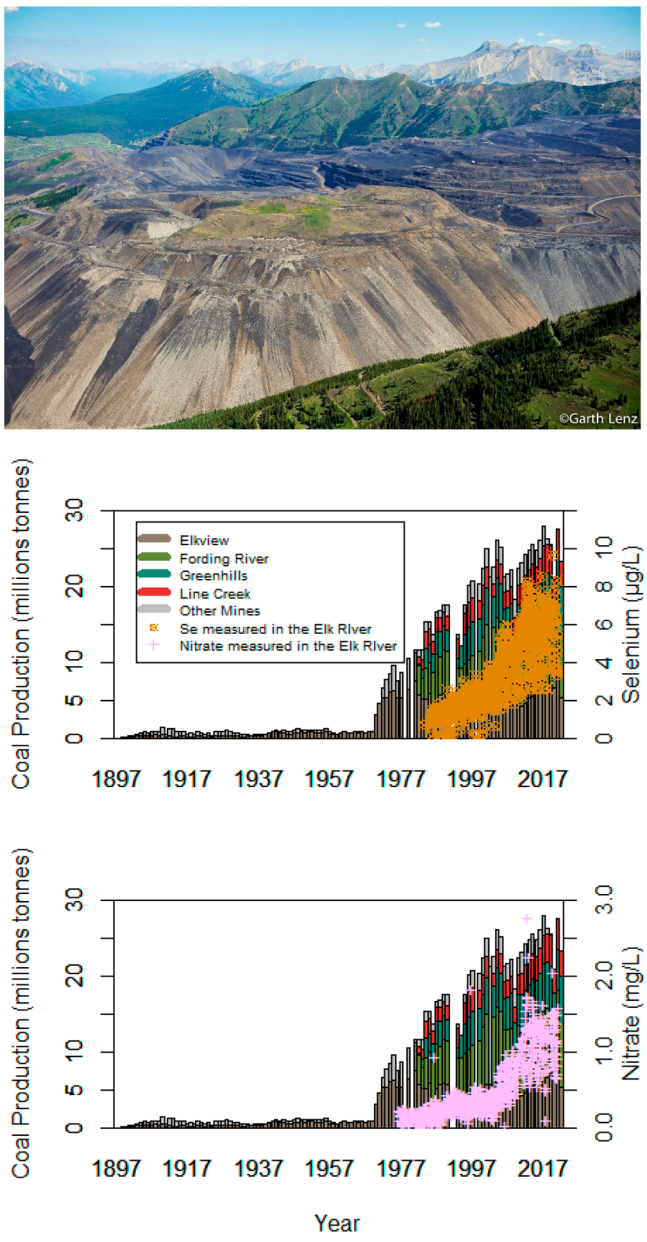


Figure 2. Coal production in the Elk River Watershed. Aerial image of one area of mine operations within the watershed. Copyrighted, used with permission via licensing agreement with Garth Lenz. Plots illustrate yearly coal production by mine, represented by different colors,[16] and measured concentrations of total Se and NO_3^- in the Elk River and Highway 93.[22]

mountain headwaters have less relief and more low gradient surfaces, which alters infiltration and runoff processes.[5,14] Geochemical processes are also altered when solid bedrock is removed and replaced with porous crushed waste rock. Waste rock dumps change the porosity and transmissivity of the system and increase the surface area and exposure of waste rock to air[16] and water, enabling more rapid chemical weathering.[4,5,17] Changing mining practices, including the development of new technologies, treatments, and best managements also further complicate the landscape and runoff and infiltration mechanisms, including lag times that affect solute movement through waste rock.[18−21]

Past literature has correlated Se and NO_3^- concentrations in the Elk River with the volume of waste rock produced in the

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ERMs (Figure 2); however, the mechanisms driving the changing concentrations (e.g., the increase in concentrations around 2005) are not well understood.[5,13] Likewise, Se, NO_3^-, and SO_4^{2-} concentrations measured in the Elk River where it enters KOC have been increasing since concentrations were first measured in 1984, and concentrations are also increasing in KOC (station no. 12300110) (Figure 3).[22−24] Water-quality sampling on the Elk River was initiated roughly 80 years after coal mining began, but it is likely that mining affected water quality before monitoring started.[15]

Coal-associated waste rock contains sulfide minerals (pyrite, most commonly) and organosulfide compounds which are associated with Se and other trace elements.[25−27] Se is of particular importance because its crustal concentrations can be enriched up to 82 times in coal-bearing geologic formations.[27] Chemical weathering through the oxidation of sulfide minerals releases SO_4^{2-} and associated trace elements to both surface water and groundwater.[28] Increasing NO_3^- concentrations in the Elk River have been tied to blasting practices from coal mining operations,[13] and excess ammonium may originate directly from coal.[29] Release of these mine-waste-derived solutes has negative implications for beneficial uses, including the potential to harm ecosystems in downstream water bodies.

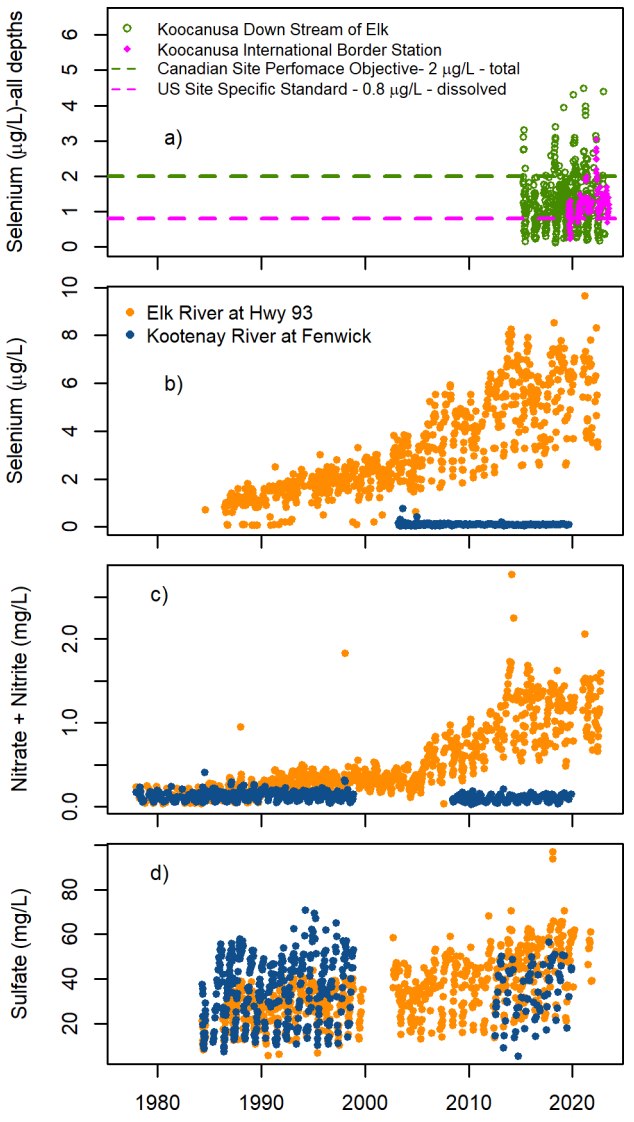


Figure 3. Solute concentration measurements in Koocanusa Reservoir, the Elk River at Highway 93 and the Kooteanay River at Fenwick.[22] (a) Koocanusa Reservoir total Se concentrations measurements at a Canadian compliance point (Lake Koocanusa South of Elk River, green)[46] and dissolved Se on the United States side of the international boarder (U.S. Geological Survey Site ID 12300110, magenta)[24] with their respective regulatory criteria.[45,46] Concentration measurements of total Se (b), NO_3^- (c), and SO_4^{2-} (d) at the Elk River at Highway 93 (orange) and the Kootenay River at Fenwick (blue).[22]

KOC is oligotrophic due to low background phosphorus concentrations and limited sediment delivery to the downstream section of the reservoir because of the Libby Dam. Increasing NO_3^- loads from the Elk River coupled with limited phosphorus has the potential to alter food webs through an imbalance of nutrients.[30−33] Food web effects from the nutrient imbalance have known ecological consequences downstream of the reservoir in the Kootenai River in MT and Idaho (ID), where phosphorus is now being added to improve food webs for fisheries.[30,34]

Se is an essential trace element for life, but excess Se can be toxic to vertebrates and invertebrates.[35] In egg-laying organisms, Se substitutes for sulfur in proteins causing teratogenesis (deformities) in early life stages, among other effects.[31,36−39] Thus, elevated Se entering from the Elk River poses a risk to organisms and the ecological function of KOC and the entire Kootenai Watershed.

In 2012, KOC and the Kootenai River were listed as impaired for Se on Montana's Clean Water Act section 303(d) list.[40] Idaho's listing for Se occurred in 2020 with a second section of the Kootenai listed in 2022.[41,42] From 2015 to 2020, the State of MT and province of B.C. worked to develop site-specific Se standards to protect water resources in KOC. The water column Se criterion of 0.8 ug/L (dissolved Se) was set for the U.S. portion of KOC (along with fish tissue criteria) (Figure 3) and 3.1 μg/L (dissolved Se) for the Kootenai River in MT and ID.[43−45] However, as of September 20, 2023, B.C. has yet to officially establish a site-specific water-quality guideline for the CA portion of the reservoir. B.C. is the primary regulatory entity, and they have specified water-quality performance objectives (i.e., CA regulatory criteria for water column concentrations) at order stations (i.e., CA regulatory compliance points) associated with mining permits, which are site-specific objectives that differ from CA water-quality guidelines.[46] The water-quality performance objectives for Se, at the order station within the CA portion of KOC, match the provincial recommended guideline value of 2 μg/L (total) (Figure 3a).[47] Water-quality criteria for Se are now being regularly exceeded on both sides of the border. At the time of publication, water Se concentrations collected at the international boundary (USGS Site ID 12300110) have not been less than the U.S. site-specific regulatory standard since July, 2020 (Figure 3a).[24]

Here, we aimed to gain a better understanding of how the history of ERM operations has influenced the quality of waters flowing into the U.S. via a retrospective analysis of Canadian water-quality and discharge records. Our analysis included three constituents (Se, NO_3^-, and SO_4^{2-}) in the two largest tributaries of KOC (The Kootenay River and Elk River), near their entries to the reservoir. For all three constituents in each river, we built empirical models of concentration, using weighted regression as a function of time trend, discharge, and season (WRTDS).[48] Based on these models, we estimated

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daily and annual concentrations and loads and evaluated trends in flow-normalized concentrations and loads. Through a paired watershed study with three objectives, our goal was to address the following research question: How does the export of potential contaminants such as Se, NO_3^-, and SO_4^{2-} reflect the history of coal mining land use and waste rock management in watersheds? Our first objective was to model and estimate the masses of Se, NO_3^-, and SO_4^{2-} that enter KOC annually from the two major tributaries and assess how the annual delivery of solutes has changed through time via flow-normalized trends in concentration and load. Our second objective was to determine the relative contributions of Se, NO_3^-, and SO_4^{2-} over time, from the two tributaries into KOC. Lastly, we looked for evidence of changing solute dynamics in the two tributaries in relation to the coal mining operational history, including the recent implementation of water treatment at the ERM.

While public concern and scientific study related to mining contaminants in the Elk River have been previously documented in government reports, Canadian and U.S. national news sources, and primary literature,[5,14,27,49−52] this study provides the first comprehensive and comparative analysis in the primary literature of historical water-quality effects to U.S. waters from solutes derived from coal mine operations in the Elk River Valley. Our efforts demonstrate how flexible analysis techniques (WRTDS) can be used to improve our understanding of changing solute dynamics and the fate and transport of mine-waste-derived solutes (Se, NO_3^-, and SO_4^{-2}).

2. METHODS

2.1. Site Information. KOC is 145 km long and is bisected by the international border in southeastern B.C., CA, and northwestern MT, U.S. (Figure 1).[53] There are four gauged tributaries that contribute water to KOC; the three largest are Canadian tributaries, the Kootenay, Elk, and Bull Rivers—which together supply approximately 87% of the inflow to the reservoir, with roughly 50% coming from the Kootenay River and 25% from the Elk River (Figure 1).[23,54,55]

Large growth of mining operations in the Elk River Watershed occurred in the 1970s, with the transition from underground mining to large scale surface mining with valley-fill waste rock dumps (Figure 2).[16,56] In 2020, ERM was responsible for producing 80% of Canada's annual steelmaking coal exports and 43% of B.C.'s mining revenues. In 2021, ERM produced 24.6 million tons of coal, with the majority exported to the Asia Pacific region.[57,58] In 2022, the ERM encompassed over 145 km^2 (3%) of the Elk River Watershed (SI Figure 1 and SI Table 1a,b). At present (2023), there are also three additional proposed mines and one proposed mine expansion.[56] As of 2020 the ERM had generated over 6.74 billion bank (*in situ*) cubic meters (B.B.C.M) of waste rock,[29] a 43% increase from the 4.7 B.B.C.M of waste rock that was present in 2010.[59] Current permitted mine operations allow 11.03 B.B.C.M of waste rock, so volumes could nearly double from their present sizes under existing mine permits (SI Table 2).[29] The areal extent of waste rock dumps is specified by permitting,[5,60] so future waste rock generation from existing operations is likely to alter the geometry of the dump areas by increasing their depth.

In 2013, based on rising concentrations of five constituents (Se, NO_3^-, SO_4^{2-}, cadmium and calcite) in the Elk River, B.C. began requiring ERM to address mining contamination.[61] Remedial measures implemented by ERM included piloting of water treatment technologies to remove Se and NO_3^- beginning in late 2014, leading to 4 operating treatment facilities by the end of 2022 (SI Table 4).[29]

2.2. Data Compilation. Water-quality and discharge data were obtained from the online B.C. Water Tool (downloaded on 7/7/2022)[55] and the Environment and Climate Change Canada (ECCC) data portal (downloaded on 11/12/2022).[62] Water-quality data for the Kootenay River were obtained from the Kootenay River near the Fenwick site (B.C.08NG0009), which has a drainage area of 11,754 km^2. Discharge and water-quality sampling locations were not coincident, so discharge data from the Kootenay River at Fort Steele (station ID 08NG065)[55] were adjusted based on the drainage area ratio between the discharge monitoring and the water-quality sampling locations (SI Figure 2).[22] The Elk River is 220 km long and has a drainage area of 4,450 km^2 to the water-quality monitoring site located at Highway 93 Near Elko (B.C.08NK0003).[23] Because the discharge and water sampling locations were not coincident, the Maintenance of Variance Extension, Type 2 (MOVE.2)[63] for record extension was used to extend the daily discharge record at the Elk River at Phillips Bridge site (08NK005),[55] which is the closest gauge, but discharge measurements were discontinued after 1996. Record extension for Phillips Bridge was based on the discharge relationship with the Elk River at the Fernie site (08NK002) (correlation coefficient 0.9805) (SI Figure 2).[63,64] A drainage area ratio correction was then applied to adjust for the differences in contributing area between the Phillips Bridge discharge records and the Highway 93 water-quality monitoring location.[22] Additional information is in the SI Methods, including additional site information and geology. Concentration and discharge input files, in addition to the models, associated metadata, and additional details are available in ref 22.

Both the Elk and Kootenay Rivers have snowmelt-dominated flow regimes, generally characterized by peak discharges in late May through early June, transitioning to baseflow recession in the late summer and early fall, and finally steady low discharge dominated by baseflow contributions throughout the winter months (SI Figure 3).[5] Water-quality samples at each location were generally collected two times each month over the period of record for each solute and generally spanned the range of flow conditions (SI Figure 4). Additional information regarding data quality screening, and corrections, are available in ref 22.

2.3. Analysis Methods. Weighted Regression on Time, Discharge and Season (WRTDS) was implemented using R Studio (version 1.4.1106), and the EGRET (version 3.0.7), and EGRETci (Version 2.0.4) packages.[48,65,66] Six individual models were generated, with one model for each constituent of interest at each site. Additional details, including the governing equation are included in the Supporting Information (SI Analysis Methods).

2.3.1. Evaluation of Flow Stationarity. To determine the appropriate implementations of WRTDS (stationary flow-normalization or generalized flow-normalization), eight metrics of variation in annual stream discharge (SI Figures 5 and 6) were examined for monotonic trends at each river location.[67] The statistics were evaluated using the nonparametric Mann-Kendall trend test with Theil−Sen slope (SI Figures 5 and 6). In addition, Quantile-Kendall plots were examined for changes in discharge quantiles for the periods of record with discrete

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water-quality data (Elk 1979−2021; Kootenay 1979−2019) (SI Figure 7).[68] These statistical tests suggested that trends in discharge were small and not statistically significant; therefore, we implemented WRTDS using stationary flow-normalization.

2.3.2. Flow-Normalized Trends. Trends in the flow-normalized concentration and load were examined visually and quantitatively for each model using the WRTDS framework. WRTDS was developed to evaluate the combined effects of discharge, season, and interannual trends in water quality. It does this by creating a statistical model of concentrations for each day in the record as a function of discharge, trends in time (expressed in decimal years), and season (SI Analysis Methods; Kalman). The model results were integrated over the frequency distribution of the discharge to compute flow-normalized estimates. These estimates are designed to allow a comparison of year-to-year variations in concentration and load that are independent of the effects of variations in discharge. Removing this source of variation facilitates the identification and estimation of long-term, nonmonotonic trends.[48,69]

The EGRETci package was used to estimate the uncertainty associated with these trends through block bootstrapping.[70] This method constructs confidence intervals around a trend by randomly subsampling the data set and recreating the WRTDS model over many iterations to capture variability in the trend estimate. Uncertainty was described using likelihood terminology following Hirsch, Archfield and De Cicco,[70] where a trend likelihood is described based on the percentage of increasing or decreasing trends from hundreds of bootstrapped iterations, with higher likelihoods corresponding to higher percentages.[70]

Trend estimates for load are generally presented in the Results and Discussion section as yield (i.e., area normalized load). Loads are presented as yields to make results more directly comparable because the Kootenay Watershed has more than twice the contributing area of the Elk Watershed (Figure 1).

Additionally, we chose to explore general effects that the recent implementation of ERM water treatment (post 2015) may have had on the contaminant loading trends at the Elk River at the Highway 93 sampling location (80−120 km downstream of mining operations). Daily mass removal data from ERM water treatment for Se and NO_3^- through September 2022 are available in ref 22. We carried out a mass balance calculation for Se using the combined daily Se mass removals from all treatment locations. The calculations make two simplifying assumptions. The first is that the Se removed by the treatment plants would have otherwise been transported conservatively (i.e., in its entirety) 80−120 km downstream to the Highway 93 sampling location. The second assumption is that the load reduction at the monitoring location on any given day is equal to the running mean of Se removal for the prior 30 days. This averaging was selected to account for different treatment locations upstream and to account for surface water advection and dispersion as well as exchange of the solute between the surface water and groundwater systems. This second assumption results in a smoothing of the overall treatment effect assessment but does not have a significant effect on the concentration reduction estimates at longer time scales (e.g., seasonal to annual). The smoothed data were used to construct a concentration surface using the same indexing (for time and logQ) as the original Se concentrations computed from the monitoring data within the Elk Se WRTDS model.[22]

2.3.3. Load Estimates. The EGRET package[65] was used to provide daily estimates of concentration and load, which were then summarized into a time series of annual mean concentration and annual load using a state space modeling approach with the WRTDS_Kalman method.[71,72] The WRTDS_Kalman method uses the WRTDS model and the measured sample values to compute optimal estimates for each day in a manner that accounts for serial correlation in the model residuals. It is a method that has been shown to generate some of the most accurate estimates of load based on comparisons with measurements.[71,72] Comparisons between observed and modeled daily loads and WRTDS and WRTDS_Kalman annual estimates are in SI Figures 8 and 9.

2.3.4. Concentration−Discharge Relationships. Concentration−discharge relationships (C−Q) were plotted and assessed for variation with respect to seasons and over time, providing a summary of solute dynamics and insights into changing watershed flushing and dilution processes. WRTDS captures changes in C−Q relationships over time and generates a modeled 3D surface that shows expected concentrations across a range of possible discharge values for each day in the period of record (SI Figure 10). Information contained within this 3D surface allows for inferences about the effects of changing hydrologic conditions, evidenced through changing behavior in the C−Q relationship over time.[48,73,74]

Two different visualizations were explored. The first set of visualizations are like traditional C−Q plots, but on an arithmetic scale, and relationships at three different days over time are shown on the same graph. Multiple iterations of these graphs were examined looking at historical changes during high and low discharge times of year. The second set of plots looked at changes in concentration over time for each constituent at specific discharges based off a flow duration curve generated for 60 days surrounding a specific date (high, 95th quantile; intermediate, 50th quantile; and low discharges, 5th quantile), during peak discharge (June 13) and low discharge (January 1) times of the year. These two graphical tools provide a visual assessment of patterns that can illustrate potential changes in solute delivery dynamics over time because patterns in the relationship of C−Q provide perspective on the mechanisms related to the mixture of precipitation water and stored water in streamflow generation.

There are three common patterns that are frequently described for C−Q relationships: dilution, mobilization, and chemostasis. Often these patterns vary between individual solutes and watersheds, so they are useful for characterizing the average behavior of a watershed.[75−80] As such, a change in C−Q behavior over time may indicate hydrologic shifts, such as changes in land use that affect basin characteristics, changes or transformations in constituents and sources, or altered flow paths (i.e., solute delivery dynamics).[76,81,82]

3. RESULTS AND DISCUSSION

3.1. Concentration. Within the two watersheds, both land use and bedrock geologies are generally different, suggesting the possibility that the background concentrations of solutes might vary (SI Methods, Geology). Background Se concentrations have the potential to be higher in the Elk River than the Kootenay River, based on bedrock geologic sources independent of mining.[83] The mean Se concentration on the Kootenay River (2003−2019) is near 0.1 μg/L and is likely to have decreased 10% over this time (Table 1). The annual

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Table 1. Trends in Loads and Concentrations (Flow-Normalized) For Select Time Periods for the Elk River at Highway 93 Bridge and the Kootenay River at Fenwick[22,a]

	Elk River at Highway 93							
	Constituent	First year	Last year	Percent change over period: Entire period	Start to 2002	2002 to end	first year mean	Last year mean
Se	Annual load (t/yr)	1985	2022	+443%	+146%	+121%	1.97	10.72
	Concentration (μg/l)			+551%	+178%	+134%	0.89	5.77
NO_3^-	Annual load (t/yr)	1979	2022	+697%	+155%	+213%	279	2226
	Concentration (mg/l)			+784%	+165%	+234%	0.13	1.18
SO_4^{2-}	Annual load (t/yr)	1985	2022	+129%	+57%	+46%	35872	82165
	Concentration (mg/l)			+120%	+51%	+45%	22.40	49.30
	Kootenay River at Fenwick							
	Constituent	First year	Last year	Percent change over period: Entire period	Start to 1998	1998 to end	first year mean	Last year mean
Se	Annual load (t/yr)	2003	2019	-15%	NA	NA	0.50	0.43
	Concentration (μg/l)			-10%	NA	NA	0.10	0.09
NO_3^-	Annual load (t/yr)	1979	2019	-4.60%	-2.50%	-2%	607.79	580.55
	Concentration (mg/l)			-8.50%	+2.1%	-11%	0.12	0.11
SO_4^{2-}	Annual load (t/yr)	1985	2019	+19%	+18%	+0.58%	113974	135136
	Concentration (mg/l)			+7.7%	+13%	-5%	35.50	38.20

Likelihood category name	Likelihood results from bootstrap confidence interval analysis
Highly likely downward trend	>95% likelihood trend is downward
Likely downward trend	70% to 95% likelihood trend is downward
Highly uncertain	30% to 70% likelihood trend is downward
Likely upward trend	70% to 90% likelihood trend is upward
Highly likely upward trend	>95% likelihood trend is upward

[a]Uncertainty is described in terms of the likelihood from bootstrapped replicates.

mean concentration for Se on the Elk River at Highway 93 in 2022 was 5.77 μg/L, but in 1985 it was 0.89 μg/L (Table 1).[22] The 1985 value is 295% greater than concentrations on the Elk River upstream of the mines and in the neighboring Flathead River watershed, based on values that were measured by Hauer and Sexton[15] (0.3 μg/L). There is limited literature surrounding background concentrations in the Elk River, but values upstream of the mines and the concentration increases early in the record (SI Table 3) suggest that concentrations of mine-related contaminants may have been increasing since before concentrations were initially measured.

Like Se, NO_3^- and SO_4^{2-} concentrations in the Elk River have followed a trajectory where average, maximum, and minimum concentrations are all increasing, in addition to increases in amplitude in annual patterns among high and low concentrations (Figure 3b,c,d). NO_3^- concentrations have remained stable or decreased marginally in the Kootenay River (Figure 3b,c,d).[84] NO_3^- and SO_4^{2-} concentrations entering KOC do not exceed current CA regulatory guidance for water quality.[85,86] However, NO_3^- concentrations in the Elk River have increased 784% between 1979 and 2022, more than any other solute (Table 1). NO_3^- concentrations in the Elk River were like those in the Kootenay River when concentrations were first measured in the 1980s, but recently measured high values each year are nearing half the 3 mg/L CA guidance level (Table 1).[85]

Since the year 2000, measured Se concentrations in the Elk River have constantly exceeded recommended ambient water-quality guidelines to protect aquatic life set by both the Canadian Council for Ministers of the Environment (1 μg/L) and the British Columbia Ministry of Environment and Climate Change Strategy (alert concentration: 1 μg/L; guideline: 2 μg/L).[47,87] In KOC, dissolved Se concentrations have exceeded U.S. regulatory criteria since July, 2020 (Figure 3a).[45] In contrast, Se concentrations in the Kootenay remain 1−2 orders of magnitude lower and stable (Figure 3b).

The Elk River location at Highway 93 is not a CA regulatory compliance point; therefore, concentrations here are being compared to the CA recommended federal and provincial Se guidelines (total).[47,87] In 2021, the average annual measured Se concentration in the Elk River where it enters KOC was 5.87 μg/L, the maximum was 9.65 μg/L, and the minimum was 3.51 μg/L. We used the WRTDS model output to calculate the expected number of days that modeled concentrations exceeded the guideline criteria (SI Methods, Exceedance Probability). For the federal guideline of 1 μg/L, exceedances increased from 70 days a year in 1984, to 252 in 1990, to 350 in 2000, and by 2006 exceedances were greater than the federal/provinical alert guideline for more than 360 days per year, for each year until the present. Similarly, for the 2 μg/L provincial guideline, the expected number of exceedances per year rose from 7 days in 1984, to 31 days in 1990, to 217 days in 2000, and by 2010 it is greater than the CA recommended guidance concentration for more than 360 days per year for every year since 2010.

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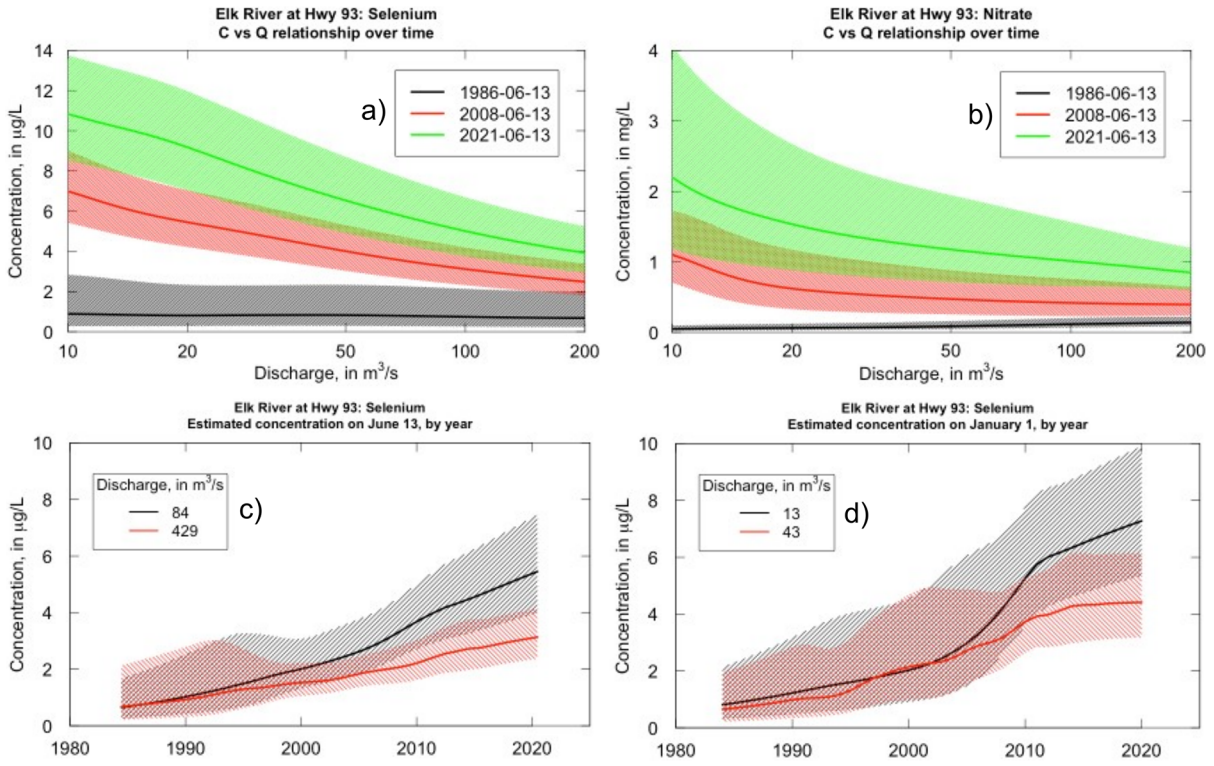


Figure 4. Concentration vs discharge relationships for total Se (a) and NO_3^- (b) in the Elk River at Highway 93.[22,65] Colored lines (a,b) show modeled C−Q relationships for three different dates, illustrating changes in the solute behavior over time. Shading (a, b) shows the 90% prediction interval for each date. (c,d) illustrates changes in concentration over time at two different discharges and two times of the year. (c) illustrates patterns at the high discharge time of year (June 13) and (d) shows a low discharge time of year (Jan 1). Lines (c, d) represent the 5th (black) and 95th (red) percentiles for flow duration curves for 60 days around each date (30 days before and after). Shading (c, d) shows the 90% prediction interval for each flow. Additional solutes and Kootenay River data are shown in SI Figures 11 and 12.

3.2. Loads. Loads discussed in this section are based on estimates optimized for accuracy (WTRDS_Kalman) and not flow-normalized.[71,72] Within the Elk and the Kootenay Watersheds, snowmelt runoff is the time of year when most of the discharge moves through each system and with it most of the mass of solutes is delivered to KOC over the water year. The mean annual discharge for the Kootenay River is roughly twice that of the Elk River (SI Figure 3 and SI Table 3), and generally, the proportion of water from the two tributaries discharged into KOC did not change over the last 40 years (SI Table 3). On average, the Elk River contributed 29% of the combined discharges, but in the past decade contributed 95.3% of the Se, 76% of the NO_3^- and 38% of the SO_4^{2-} to KOC from the two tributaries combined (SI Table 3). Annual variations in loads for both rivers were driven by year-to-year variations in discharge, and within the Elk River variation in loads were also coupled with the large increase in solute concentrations from mining operations.

The masses of SO_4^{2-} delivered to KOC from these rivers were the most similar relative to the other solutes (SI Table 3). Mining in the Elk River Watershed has added additional mass to background weathering and other SO_4^{2-} sources, which was evident by the increase in annual load estimates over time, from 25% of the combined load in the late 1980s to 38% in the 2010s (SI Table 3).

NO_3^- load increased more than the other two constituents within the Elk River over its respective period of record. This increase is likely driven by ammonium nitrate used in mine blasting[13] and to some extent geogenic ammonium ions in the coal-bearing strata.[29] In 1979, when NO_3^- was first measured, roughly 30% of the NO_3^- entering KOC was coming from the Elk River, a proportion like the discharge volume (concentrations were similar in both rivers), but by 2014, it had increased up to 83% of the combined NO_3^- contributed to KOC (SI Table 3).

Annual loads of Se have grown by over 1 order of magnitude over the past 35 years (SI Table 3). When water-quality monitoring was initiated in the Elk River in 1984, estimated loads were already 3−4 times greater than loads entering the KOC from the Kootenay River (SI Table 3).

The mass of NO_3^- and Se being delivered to KOC is now dominated by contributions from the Elk River, despite its much lower discharge volume (SI Table 3). Discharge in the vicinity of waste rock dumps in the Elk River Watershed has been shown to be attenuating, with decreasing peak and stormflows and increasing baseflows.[58] Thus, the timing of solute mass delivery may shift toward a greater proportion during baseflow periods, due to increased transient storage and solute sources from growing volumes of waste rock.[4] Likewise, several studies have shown that transport is the limitation for solute delivery out of waste rock within the Elk River Watershed.[5,13,14] This limitation is present despite increased infiltration capacities that can be three times greater than the infiltration capacity of the natural catchment.[60] This suggests that if more flow through waste rock occurred, more solute mass would be mobilized—an important consideration given changing precipitation patterns and form (rain vs snow) as a result of climate change.

A portion of the most contaminated surface waters are now being treated, and the volume of water to be treated is planned

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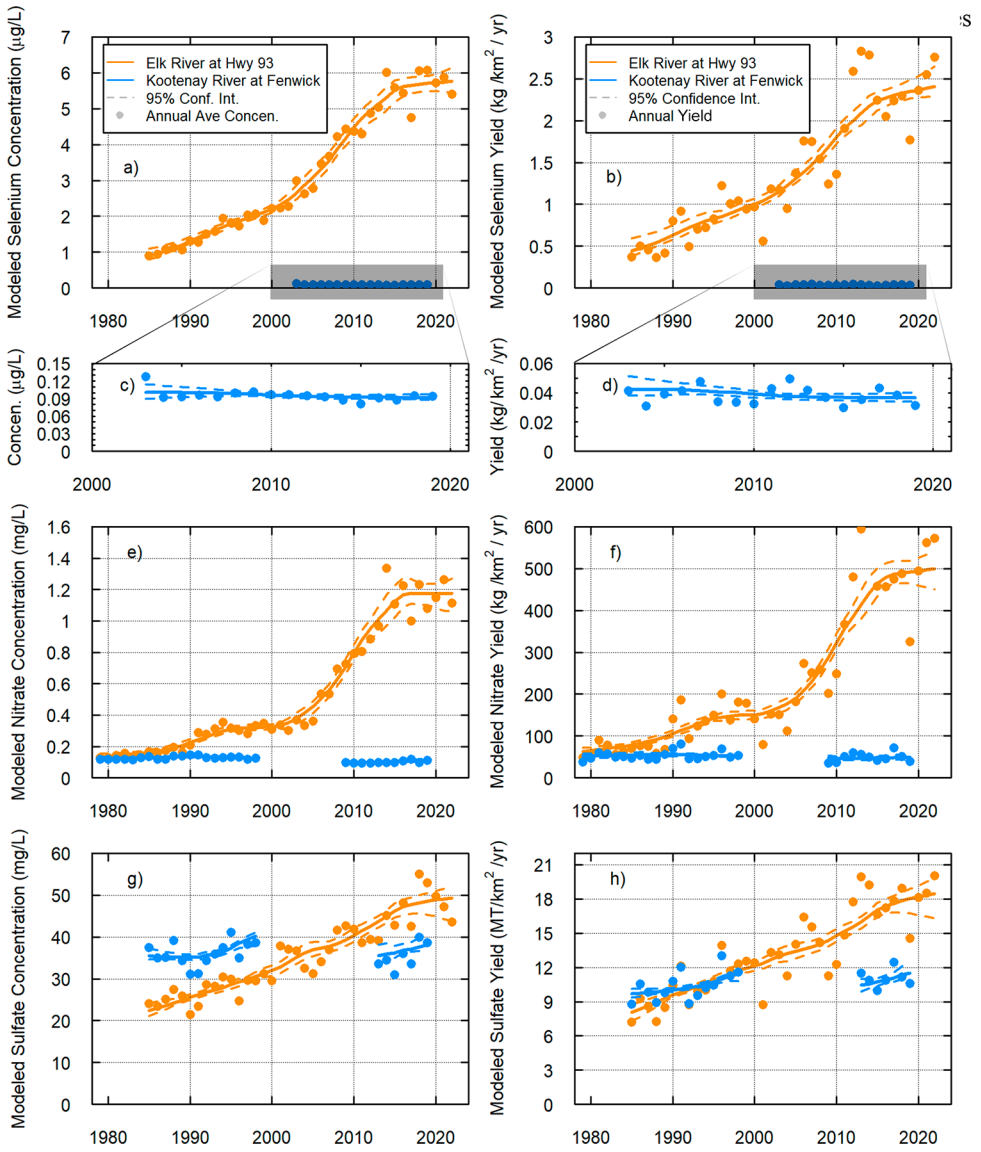


Figure 5. Flow-normalized trends for the Elk River (orange) and the Kootenay River (blue).[22,48,65] Concentration trends for each constituent are on the left (a, c, e, g), and trends in yield (i.e., area normalized load) are on the right (b, d, f, g).[22] Each row is a constituent: (a, b) total Se, (c, d) total Se for the Kootenay River only, (e, f) NO_3^-, and (g, h) SO_4^{2-}. Solid dots are mean annual concentration or annual yield. Dashed lines are 90% confidence intervals.[65]

to increase through 2027.[88] In 2015[14] and 2018,[5] Wellen, Shatilla and Carey found that mining practices, including surface reclamation, the shape of waste rock piles, and the age of waste rock dumps, also have the potential to reduce Se delivery to water bodies, suggesting operational practices, which change in time, are likely to influence loads (increase or decrease). Water treatments may reduce loads to KOC going forward, but the magnitude and timing of that reduction are not well understood. Likewise, there has been little published study of surface water−groundwater interaction in mine-affected areas and areas downstream. A recent update to the Regional Water Quality Model (RWQM) added a surface water−groundwater partitioning component, suggesting solute movement in groundwater is an important mechanism that needs to be understood in the context of downstream water quality.[29,89,90] Loads from both shallow and deep groundwater are a source of uncertainty in this system, and very little is known about the effects on deep groundwater from waste rock dumps on top of bedrock limestone with high karst potential.[89,91] Presently only surface water is treated, and current plans are to treat only surface waters into the future.[29] With limited knowledge surrounding surface water−groundwater interaction and potential groundwater contamination, it is unclear if treatment of surface water alone will sufficiently reduce the mass of solutes moving downgradient in the watershed and into KOC to meet U.S. water-quality regulations (Figure 3a).

3.3. C−Q Relationships. For each of the three solutes in the Kootenay River, the C−Q relationships for individual solutes were consistent over time, suggesting consistency in the solute delivery mechanisms for the watershed (SI Figure 11a−c). Se and SO_4^{2-} both showed a dilution signal. Se shows a slight decrease across all concentrations from 2003 to 2019, while SO_4^{2-} shows an increase across all discharges between 1986 and 2019, but more so at lower discharges (SI Figure 11a,c). NO_3^- exhibited a mobilization pattern, suggesting that

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the mechanisms behind the Kootenay's delivery of NO_3^- to KOC is driven by runoff solute delivery mechanisms, coupled with an overall decrease across all concentrations between 1986 and 2019 (SI Figure 11b).

In contrast to the Kootenay River, the Elk River C−Q relationships for Se and NO_3^- changed with time (Figure 4), while SO_4^{2-} maintained a consistent dilution signal, but the magnitude of concentrations increased across all discharges (SI Figure 11f). In 1986 Se and NO_3^- in the Elk River exhibited C−Q relationships that were indicative of mobilization or chemostasis solute delivery mechanisms (Figure 4). However, by 2008 and into 2021, the solute delivery for both Se and NO_3^- changed to dilution (Figure 4a,b). Like SO_4^{2-}, as time progressed both Se and NO_3^- exhibited an overall increase in concentrations across all discharges, with the largest magnitude of increase occurring at the lowest flows.

In addition to more traditional perspectives on C−Q relationships, we used information within the WRTDS models to evaluate how concentrations have changed at different discharges (i.e., seasonal high, average, or low discharge) during different seasons (Figure 4c,d and SI Figure 12).[65] In the Elk River for all three constituents, we observed that solute concentrations at lower discharges increased faster than those at higher discharges (SI Figure 12). This was the case during both high discharge (June 13) and low discharge (January 1) times of the year (Figure 4c,d). However, the magnitude of concentration increase was greatest during the lowest discharges, at the baseflow time of the year (Figure 4c,d). Patterns shown in Figure 4 display a notable change in relative rates of increase (slope) that occurred in the mid-2000s, where the concentrations began to increase more rapidly across the different discharges—this is particularly evident in Se during the low flow time of year (Figure 4d). In contrast, patterns across discharges in the Kootenay River for all three solutes during high and low discharge periods are relatively flat over time (not shown). The shift in Se and NO_3^- C−Q signals (Figure 4a,b), as well as the largest concentration increase during low flows (Figure 4), suggests that processes controlling solute delivery to surface water have changed over the last 40 years in the Elk River, with a notable increase in the lowest flows relative to higher flows taking place in the early to mid-2000s (Figure 4d).

Concentrations of solutes at baseflow conditions are generally a good indicator of the chemistry of shallow groundwater; existing reports suggest that most streams in the Elk Valley gain shallow groundwater.[58] Therefore, shifts in C−Q behavior (increased concentrations during baseflow) may reflect increased solute loadings to streams from shallow groundwater. However, increasing concentrations at low discharges are also consistent with observations made by Nippgen et al.,[4] who suggest waste rock dumps may be altering the hydrologic storage and release of solutes within Appalachian coal mine affected watersheds, causing increased solute mobilization during the baseflow period via surface water drainage from dumps. The switch in Se and NO_3^- C−Q behavior to dilution and the largest increase in concentrations at the lowest baseflows in Figures 3 and 4 suggest that the ERM may now behave like a point source, with a near continuous release of higher concentration solutes that are being diluted by water from unmined portions of the Elk River Watershed. The changing solute behavior in the Elk River could be the result of contaminated groundwater downgradient of waste rock dumps and or increases in surface water sources (concentration and/or discharge) emerging from waste rock dumps—suggesting that additional investigation to understand the mechanisms driving the change in solute delivery in the Elk River Watershed may support understanding the source(s). In contrast, solute dynamics in the Kootenay River have remained relatively consistent over time.

3.4. Trends. *3.4.1. Differing Trends from the Paired Watersheds.* Trends in load are presented in this section as yields, to facilitate direct comparisons between the two watersheds, which are different in size. In the Kootenay River there are gaps in the SO_4^{2-} and NO_3^- record and a shorter Se record due to discrepancies in concentration measurements after methods changes; thus, the time periods for comparison vary slightly (Figure 5). However, the flow-normalized trends (concentration and yield) for these constituents in the Kootenay River either decreased (Se and NO_3^-) or increased marginally (SO_4^{2-}) (Figure 5 and Table 1).[22]

The flow-normalized trends of Se, NO_3^-, and SO_4^{2-} for both concentrations and yields in the Elk River document significant water-quality changes (Figure 5 and Table 1); Se concentrations in the mine-affected tributaries to the Elk River are among the largest in published literature, and the increases in Se and NO_3^- in the Elk River at Highway 93 are the largest percent increases documented in the primary literature that are known to the authors.[35,92] Flow-normalized concentrations of Se, NO_3^-, and SO_4^{2-} in the Elk River increased over their respective periods of record by 551% (Se), 784% (NO_3^-), and 120% (SO_4^{2-}). Trends in yield mirrored those in concentration but were slightly smaller in magnitude for Se (443%) and NO_3^- (697%) and slightly larger for SO_4^{2-} (129%) (Table 1). This occurred while marginal decreasing trends in Se and NO_3^- and a slight increase in SO_4^{2-} were observed in the Kootenay River.

Modeled annual average NO_3^- concentrations in the Kootenay River and Elk River were nearly the same when monitoring began in 1984 (Figure 5c). Since 1984, concentrations of NO_3^- have been declining in the Kootenay. There were likely significant declines after the closure of a fertilizer manufacturing plant (ammonia phosphate) on the Saint Mary River in 1987 (a major tributary to the Kootenay, Figure 1).[93] Conversely, NO_3^- concentrations have been on an upward trajectory in the Elk River from mining operations.

SO_4^{2-} concentrations in the Elk River in 1984 were lower than concentrations in the Kootenay River; concentrations were comparable between the two rivers in the mid 2000s but have been higher in the Elk River ever since (Figure 5e). Generally, SO_4^{2-} and Se weather from the same parent material; however, the difference in concentration trends over time (Figure 5a, g) suggests that different geochemical or biogeochemical mechanisms affect these solutes in different ways within the Elk River Watershed.

In the Kootenay River, percent change for SO_4^{2-} and Se yields is greater than the respective changes in concentrations. However, NO_3^- is the opposite, again suggesting that mechanisms delivering NO_3^- are different from the other two solutes (Table 1 and Figure 5), which aligns with the mobilization signal that was observed in the C−Q relationship for NO_3^- on the Kootenay River (SI Figure 11). Overall concentration and yields entering KOC from the Kootenay River have been consistent historically, especially in comparison to the large magnitude increase in all three solutes from the Elk River.

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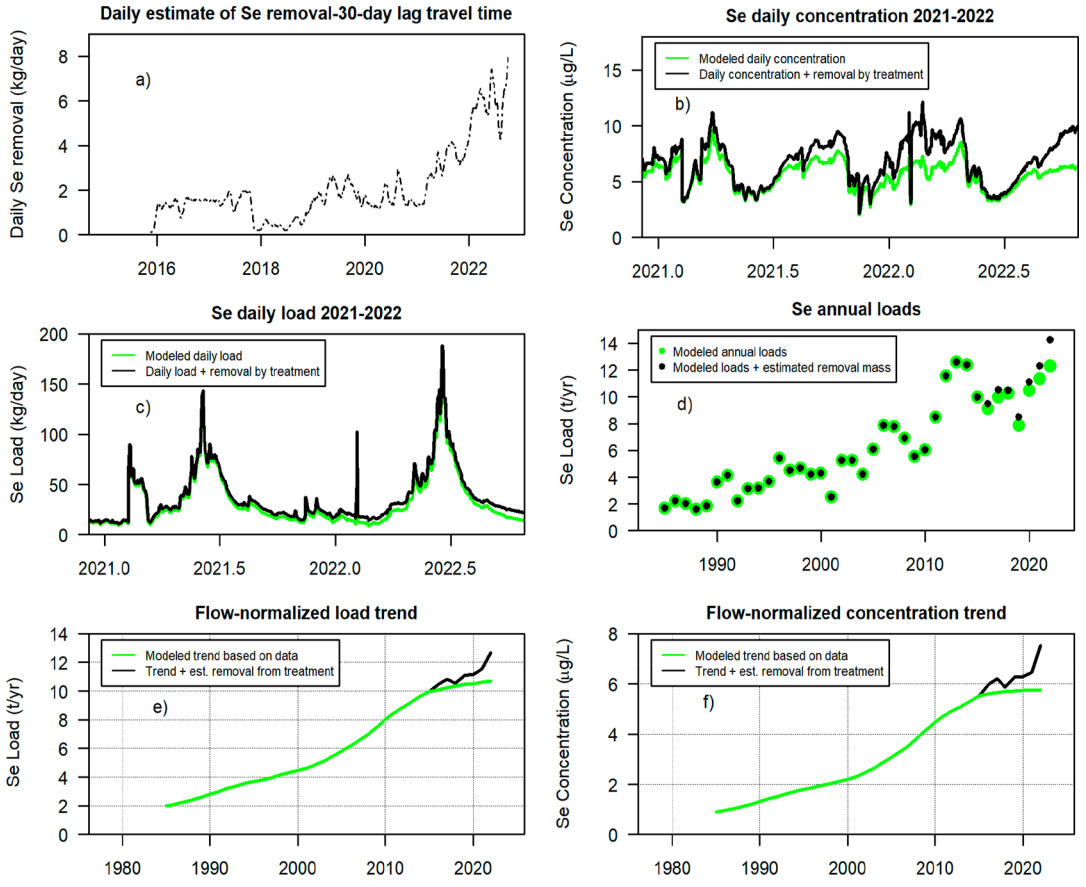


Figure 6. Modeled influence of Se mass removal from three mine water treatment facilities in the Elk River Watershed.[22] Estimated Se removal by water treatment in the Elk River at Highway 93, using a 30 day lagged running mean (a). Estimated daily concentration (b) and load (c) of Se in the Elk River at Highway 93 (only 2021 and 2022 are shown for temporal resolution). The green line (b,c,e,f) is an estimate based on the model optimized for accuracy (WRTDS_Kalman).[71,72] The black line (b,c,e,f) is based on this same model with the addition of the estimated amount of Se that was removed by treatment. Green dots (d) are the estimated annual load of Se for the Elk River WRTDS_Kalman model. Black dots (d) are the annual loads if there was no treatment. The flow-normalized trends for load (e) and concentration (f) in black, are an estimate of the trends in the absence of treatment, compared to what was observed (modeled) in green.

For NO_3^- and Se in the Elk River, the increase in concentration has been larger in percentage terms than the corresponding increase in yield, but this pattern was the opposite for SO_4^{2-} (Table 1 and Figure 5a–d). This is driven by the larger relative increases in concentration during baseflow, but due to low discharge volumes during those times, trends in load have not increased at the same rate. The relative magnitude of the increase in SO_4^{2-} (concentration and yield) was less than the other two constituents, and the trend was more consistent in terms of the magnitude of the slope through time (Figure 5e, f). The difference in the SO_4^{2-} trends in the Elk River (compared to NO_3^- and Se) may have been driven by chemical weathering of other geologic sources of sulfate, delivering higher baseline concentrations (SI Methods, Geology).

3.4.2. Mining Operations Drive Trends in the Elk River. Three distinct time periods of increasing trends in the NO_3^- and Se concentrations were observed in the Elk River (Figure 5a–d). The first period was from the start of the record through the early 2000s and was characterized by a moderate (relative) slope. The second period showed an increase in the slope around 2002, suggesting the concentrations and corresponding solute delivery were increasing at a faster rate. The third period coincided with a taper in the slope around 2015. The 2015 inflection is noteworthy for NO_3^- and Se as the first reduction in the slope of the trend that has been underway for 3 to 4 decades. What caused the taper in both the concentration and yield trends around 2015 is uncertain, but we pose three hypotheses that could explain changes in hydrologic processes that could cause a slowing in the rate of increase late in the record:

(1) The RWQM[58] incorporates lag times to account for the time it takes for weathering or release of solutes after waste rock deposition to begin appearing in surface waters. Those times in the RWQM average to 7 years across all watersheds. Similar work in the primary literature has also shown that 8 year lag times were necessary to model solute delivery within the West Line Creek mine site. Past work has tied concentration with waste rock volume and areal extent.[5,13,14] Other work has also shown the possibility of some depletion to occur in this time scale for NO_3^-; however, it is unlikely that depletion of Se is occurring on the same time scale. The similarity in trends between Se and NO_3^- suggests that the pattern in trends is not driven by depletion alone.[27,94,95] Thus, we hypothesize that the lag times coupled with decreases in waste rock production from economic conditions[16] could explain the inflection in trend near 2015, as they began to present themselves roughly 7 years after the economic recession of 2008 when there was a decrease in coal production (Figure 2). This hypothesis could be tested by

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evaluating annual waste rock production over time; however, those data are not publicly available since 2010.[96]

(2) Alternatively, other mine engineering processes could have affected the trend. A transition to deeper waste rock dumps with fixed aerial extent could increase flow path lengths and limit or delay solute transport temporarily.[5,13,25] Past work has shown that solute loss in waste rock dumps is driven by vertical percolation and not lateral inputs of water.[5] Similarly, Villeneuve et al.[60] describe three stages in the evolution of the dump footprint for the West Line Creek operation, which is part of the Line Creek Mine in the ERM: first a linear increase in dump area from 1981 to 1998, followed by decreasing rates of areal expansion between 1998 and 2003, and a relatively constant dump area from 2003 to 2014, suggesting an increase in thickness (longer vertical flow paths) during this time.[5] Here we hypothesize that the inflection point could occur because of the lagged effect of these longer flow paths through waste rock, resulting in longer time periods before solutes will enter downstream surface waters. However, longer flow paths are also associated with more oxidation of sulfide minerals and may cause a subsequent increase in concentrations and loads later in time.[5,13,25]

(3) The third hypothesis is that the reduction in the trend slope is a result of water treatment. ERM have invested in active and passive water treatment technologies to mitigate NO_3^- and Se concentrations into the Elk River Watershed; pilot efforts began as early as 2014, but the first treatment system was not fully operational until 2018 (SI Table 4).[29] To explore the effect that the treatment may have had on the contaminant loading at the Elk River at the Highway 93 Bridge sampling location (80−120 km downstream of mining operations), we carried out a mass balance calculation (described here in terms of Se) using daily mass removals from ERM water treatment facilities between October 2015 and September 2022.[22]

Figure 6a shows the sum of the 30 day running mean of the daily Se removal from all treatment locations. Figure 6b shows modeled daily concentrations at Elk River and the estimated concentrations that could have occurred if there were no treatment removals upstream, assuming our assumptions are reasonable. There were notable reductions in simulated concentration during times of low discharge (late summer through early spring). But, at times of high discharge (June), the decreases due to treatment were limited. Figure 6d shows a load perspective; the simulated effect of the treatment plants approaches a 40% reduction for portions of the months of lowest discharge in 2022 but is limited in the months of the highest discharge. For high discharge months, the amounts removed are minor (less than 5%) compared to the total amount of Se being transported by the Elk River (SI Figure 13a). Additional perspective on the effect of treatment can be seen in SI Figure 13b,c (like the patterns in Figure 4); concentrations are generally higher in the months of lower discharges. Concentration increases between 2006 and 2015 and 2016−2022 were substantial for high and low discharge segments of the year. The effect of the treatment was greater for the months of low discharge than it was for the months of high discharge.

In general, with our assumptions in mind, our analysis suggests that water treatment has been successful in reducing concentrations during the months of lower discharge (when concentrations tend to be highest). But, for the high discharge months, the effect of treatment has been modest. This translates directly to treatment effects on loads, suggesting that treatment will have less effect on annual loads and more on some seasonal and annual average concentrations. Accurate understanding of treatment effects on concentrations downstream may be supported by a more complete understanding of the hydrologic and hydrogeologic system between the ERM and the outlet to KOC than is currently available. How mass removals by treatment actually affected concentrations and loads at the Highway 93 site (without our two simplifying assumptions) is not yet clear.

Incorporating masses removed from treatment back into the WRTDS model of Se in the Elk River provides support for a combination of hypotheses. The plateau in the Se trends is still present even with mass removals incorporated (i.e., without treatment), and we see an increase in the delivery rate after 2021 (Figure 6e,f). This suggests that the reduction in rate around 2016 may not only be the result of treatment but may be more likely attributed to hypothesis 1, showing a possible lagged rebound in production postrecession, or hypothesis 2, showing how dump construction practices that are focused on increasing height of dumps have resulted in longer flow paths and further lagged times for solutes to enter downstream surface waters. The absence of the rise in solute concentration and load around 2021 (Figures 5a,b and 6e,f) suggests that in recent years concentrations could have resumed a more rapid rate of increase without treatment, which aligns with increases in solutes from longer flow paths in hypothesis 2. The modeled trends (Figure 5a−d) and trends incorporating removal (Figure 6e,f) suggest that the overall patterns in trends may be more likely attributed to hypotheses 2 and 3, with the plateau driven by operational practices that increased flow path lengths and coincident timing that would have resulted in another rapid increase in the trend around 2021 if treatment had not been present at its current capacity. Fundamentally the combination of changes in waste rock production, waste rock management, and water treatment are likely to drive the magnitude of downstream solute delivery.

4. IMPLICATIONS AND FUTURE OUTLOOK

Through a retrospective analysis of water-quality and discharge data, this study indicates that increasing mine waste derived solutes have been exported from ERM operations to the transboundary KOC, while solute delivery from the Kootenay River has remained largely unchanged. The large differences in load contributions from these two watersheds point to the potential further increase in solutes into the Elk River with coal mine expansions or new coal mines and for solutes to continue to be released after the end of mining.[20,95] The physiographic setting of the ERM and their operational practices have resulted in tons of Se and thousands of tons of other mine wastes (NO_3^-, SO_4^{2-}) entering U.S. waters (SI Table 3) that are now resulting in exceedances of U.S. and CA water-quality regulations in KOC (Figure 3a).[22,24,45,46,97]

Within the mining affected watershed of the Elk River, beginning in the early 1980s, there have been large, documented increases in both load and concentration for Se and NO_3^-.[5,14,22,23] There have been large relative increases in the concentration of SO_4^{2-} as well. In the case of Se, the Elk River is now delivering on average 95% of the combined annual Se mass to KOC. Large increases and recent plateaus in the concentration and load trends suggest that changes may be driven by waste rock production, waste rock dump geometry (vertical vs lateral expansion), and mine surface water

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treatment in the last 1−2 years; these processes seemingly dictate the magnitude of downstream delivery of solutes.

The changes in the C−Q relationships and increasing baseflow concentrations in the Elk River indicate increased chemical weathering of solutes due to increased waste rock volume and year-round mobilization of solutes into surface water. Solutes may be transported to the Elk River via waste rock dump discharge to surface water and/or through groundwater discharge to surface water. Changes in the solute delivery dynamics in the Elk River suggest that the hydrologic processes responsible for delivering solutes have changed over the past four decades. In the 1980s C−Q relationships for Se and NO_3^- showed patterns indicative of surface runoff mechanisms exhibited through mobilization and chemostatic behavior but are now dominated by dilution. This is further evidenced by the largest increase in concentrations of all three solutes in the Elk River during the lowest discharges during the late fall and winter baseflow months. However, a better understanding of surface water−groundwater interaction may support observations in the study by filling a knowledge gap as the increase in solute concentrations at low discharges could be partially attributed to a contaminated groundwater source.

Past studies have shown that lower topographic relief, and higher infiltration rates, coupled with increased transmissivity of material from waste rock dumps (i.e., valley fill) can alter the flow paths and residence time of water within mined watersheds.[4] What is surprising is that we can observe this through changing solute dynamics 80−120 km downriver from the ERM, at the outflow of the larger Elk River Watershed, indicating the profundity of the change that large scale mining is having on solute transport.

The spring freshet is still the period when the largest mass of solutes is delivered to KOC. However, the baseflow is the time when the highest concentration waters flow into the reservoir. Based on the patterns we have observed in the changes in solute dynamics, increasing transient storage and solute generation from the growing volume of waste rock has raised baseflow solute concentrations more than those occurring at other times in the year, which may increase further with additional mining. High base-flow concentrations are likely to persist unless water treatment can increase and outpace growing solute delivery from additional mining and address all major sources. Our analyses suggest that current ERM water treatment has the potential to decrease concentrations in the Elk River during low discharge portions of the year; however, treatment will have much less effect on the annual loads being delivered to KOC—meaning how solute concentrations are distributed in time and space within the reservoir may have significant implications for downstream ecosystems, fisheries, and water-quality targets. This is an important finding that could assist in the management of beneficial uses in KOC because the mass of contaminants being delivered to KOC post ERM water treatment may not have the same proportional reduction that annual average concentrations may have. Likewise, there is limited primary literature on the effects on the aquatic ecosystem in KOC thus far. But this work is a first step in quantifying masses of solutes entering the reservoir over time, which provides context for ecological studies going forward.

Mining operations have changed the solute delivery dynamics within the Elk River Watershed between 1984 and 2022, while the Kootenay River has remained largely unchanged. With the introduction of water treatment and planned treatment expansions, understanding how treatment further changes the hydrologic and geochemical systems in conjunction with planned and proposed mining operations may support management decision making. There are still many areas that could benefit from further research, including: surface water−groundwater interaction in the Elk Valley and its mine-affected tributaries, an understanding of the magnitude and extent of groundwater contamination, the long-range transport potential of Se, and a better understanding of how treatment will affect downstream concentrations and loads. In addition, an assessment of the Se and NO_3^- mass balance in KOC is needed. Including developing an understanding of spatial and temporal distribution of contaminants within KOC, both current and historic, which may improve understanding of the downstream effects on solute concentrations and loads from mining operations and associated water treatment—an important consideration given the potential for effects to aquatic ecosystems and fisheries. Given current and planned mine operations and water treatment, it is unclear whether current and planned surface water treatment will be sufficient to meet downstream water-quality regulations. This transboundary system presents a unique challenge for managers and decision makers on both sides of the border and has implications for water quality throughout the Kootenai Basin and the broader Columbia River system.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c05090.

Method details, additional site information, WRTDS model governing equation, and additional tables and figures (PDF)

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■ ABBREVIATIONS

KOC	Koocanusa Reservoir
ERM	Elk River Mines
RWQM	Teck Resources Regional Water Quality model
WRTDS	weighted regression on time discharge and season
MT	Montana
ID	Idaho
USGS	United States Geologic Survey
B.C.	British Columbia
CA	Canada
U.S.	United States
ECCC	Environment and Climate Change Canada

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Supporting Information for:

Growth of coal mining operations in the Elk River Valley (B.C. Canada) linked to increasing solute transport of Se, NO_3^-, and SO_4^{2-} into the transboundary Koocanusa Reservoir (USA-Canada)

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Methods

SI Geospatial Analysis for Mine Area

Total area of disturbance (waste rock deposits) was estimated using remote sensing and geospatial analysis techniques (SI Figure 1). Specifically, Sentinel 2 data [1] for August, 2022 were mosaicked and composited for the Elk River Watershed using Google Earth Engine. [2] A function to mask clouds from the image using bands 10 and 11 was applied and pixel values for all images included in the composite were reduced to the median value for each band. Random forest classification was then done on the composite image. [3] The classification model was trained with ten trees using the training classes and associated sample sizes shown in table SI 1a. Fifty percent of the training data were held out for validation for each tree. Total mean error from validation on holdout sites was 12.7%. The classified image was then imported to ArcPro[4] for postprocessing. The image was converted to a multipart polygon using the conversion tools. Next, the Region Group tool was used to eliminate polygons less than 5,000 square meters, as these small polygons likely represented misclassified pixels rather than actual landcover features. Additionally, because the objective of this exercise was to extract the perimeter of the waste rock area, small polygons located within the waste rock polygons associated with a class other than waste rock were eliminated on the assumption that while the surface reflectance in the imagery might indicate the presence of another landcover (e.g., water or vegetation) it is likely still underlain by waste rock. The final areas of waste rock are shown in table SI 1b.

Additional site information

The Kootenay River near Fenwick Station (BC08NG0009) is 13 km downstream of the Fort Steele discharge station (08NG065) and downstream of the confluence with the Saint Mary's River, but upstream of the confluence with the Bull River (Figure 1). [5] Sample collection at this

site was discontinued in September 2019. The mean daily discharge for the Kootenay at Fort Steele (WSC site 08NG065) between 1963 and 2022 was 148 m^3sec^{-1}. [5] Because the discharge and water sampling locations are not concurrent, flow was corrected by the difference in contributing area between the two sites, meaning flow at the Fort Steele location was increased by 2.82% to estimate flows at the Fenwick location. Adjusted flows for the Fenwick site are available in Lange and Storb [6]

There is a historical Water Survey of Canada (WSC) site the Elk River at Phillips Bridge (08NK005), 6.7 km upstream of the Highway 93 sampling location[5]. Flow at Phillips Bridge was measured from 1924-1996. The closest flow measurement location that is currently in operation is the WSC Elk River at Fernie site (08NK002). Thus, Move2 streamflow record extension techniques [7, 8] were implemented in R[55] using the smwrStats package[9] to extend the daily flow record at Phillips Bridge, based on the flow relationship between the two sites post 1970 (Correlation coefficient 0.9805). Once the record extension was complete, an area correction factor was applied to account for the difference in contributing area between the Phillips Bridge and the Highway 93 sampling location, flows from Phillips Bridge were increased by 0.49%. Adjusted flows for the Highway 93 site are available in Lange and Storb [6]

Geology

The Elk and Kootenay Watersheds are both contained within the Canadian Cordillera and bisected by faults that run north to south. In both watersheds faults have generated the valley bottoms where their respective rivers are present, however the bedrock geologies are different. [10] The Upper Kootenay Watershed is generally comprised of carbonate and silicate geology, the eastern side is primarily limestones and dolomites, and the western side is dominated by carbonate

and siliciclastic formations. The Kootenay River overlays quaternary alluvium, and the riverbed mirrors the Rocky Mountain Trench fault [10]. The Elk River Watershed generally runs north to south, with the Elk River overlaying a quaternary alluvial aquifer and mirrors the Bourgeau thrust fault from its northern extent to the town of Fernie. The Elk Valley contains two of the three, structurally separate, major coalfields in British Columbia (B.C.) [11]. Mining in the Elk River Watershed is focused on bituminous coal from the Mist Mountain Formation which is part of the Jurassic-Cretaceous Kootenay Group (deposited 150-130 M years ago; 450-550 m thick) and is underlain by limestone bedrock, which has high karst potential in some areas [12, 13].

Coal has been mined in the Elk River Watershed since 1897, with large-scale mining in the valley beginning in the 1970's with the transition to open pit drill and blast methods [14]. The Kootenay River Watershed is also home to mining operations, including a smelter and several gypsum and silica mines. Several metal explorations are also occurring [10] and the Sullivan Metal Mine (operated by Teck Resources for Pb, Zn and silver (Ag) production) near the town of Kimberly operated for almost a century, but closed in 2001. [15]

Analysis methods

WRTDS Model Governing Equation

The Weighted Regression on Time Discharge and Seasons (WRTDS) model is based on using statistical smoothing by partitioning the variation present in constituent concentration values into three components and an error term. The four components are related to season within the year, the watershed hydrologic condition or discharge component, long-term trend, and the random unexplained portion of the variation [16]. The basic form of the underlying WRTDS model is below, where c is concentration; Q is discharge; t is time in years; and ε is unexplained variation:

$$\ln(c) = \beta 0 + \beta 1\, t + \beta 2 \ln(Q) + \beta 3 \sin(2\pi t) + \beta 4 \cos(2\pi t) + \varepsilon \quad (1)$$

The equation is a weighted regression and is fit in the form of a weighted Tobit model (i.e., survival regression). The model accommodates the incorporation of non-detect data because each concentration value can be expressed as a single number for a data point with a detection or as an interval between 0 and the reporting limit for non-detect values [17, 18].

Likelihoods were determined from 250 bootstrap intervals assuming stationary flow normalization.

Kalman

Performance of WRTDS_Kalman[19] depends on the AR1 coefficient (ρ), and that relationship varies with constituents and sampling scenarios. The default for ρ within the WRTDS_Kalman function is 0.9 [19, 20]. This was utilized for selenium and sulfate after exploration of larger and smaller ρ values (0.85 and 0.95) did not generate estimates that were substantially different (<5%), ρ = 0.95 was used for nitrate for WRTDS_Kalman[19] estimates for both rivers following results presented in Zhang and Hirsch[19].

Exceedance probability

One way of describing the trends in Se concentrations over the period of record is to use estimates of the expected number days in each year when Se concentrations exceeded the water quality criteria. These calculations are made by using the WRTDS model of Se for the Elk River. For each day of the 38-year long record[6] the WRTDS model provides an estimate of conditional mean and standard deviation of the natural log of concentration for that day (conditioned on year, time of year, and discharge). Using the observed residuals from the fitted WRTDS model as a representation of the probability distribution of the standardized residual for each day, we estimate, for each day in the period of record, the probability of exceedance of the criteria. The

expected number of days that concentration exceeded the criteria is simply the sum of the probabilities for all days in the year.

The approach to calculating the expected number of days on which the Se concentrations exceed a specified criterion is based on the estimated WRTDS model and the discharge record.

Let x_i = the concentration on day i,

$y_i = \ln(x)_i$

$\bar{y}_i$ = the estimated conditional mean of y_i from the WRTDS model

s_i = the standard deviation of the distribution of y_i from the WRTDS model

where i is the index of all 14,004 days in sequence, starting with 1984-07-10 and ending with 2022-11-11.

We can express the value if y_i on any given day as:

$$y_i = \bar{y}_i + s_i \cdot e_i$$

The e_i values are standardized residuals, computed from the WRTDS model and the data set of 774 observed concentrations. They are computed as:

$$e_j = \frac{y_j - \bar{y}_j}{s_j} \quad \text{for } j = 1, 2, \ldots, 774$$

The subscript j here refers to the sequence number of sample values, (1 to 774).

Rather than using some theoretical distribution (such as gaussian) for these standardized residuals we use the population of observed standardized residuals from the data set. [6] In the case considered here, the Elk River Se data, we have 774 observations and hence 774 observed

standardized residuals (these are a set of jack-knife cross-validation estimates computed in the modelEstimation() function in EGRET).[18] It is assumed here that each of these 774 residuals is equally likely to have occurred on any given day in the 14,004-day record.

For purposes of estimation of the probability of exceedance of the water quality criterion, denoted here as x^*. The natural log of the criterion is denoted as $y^* = \ln(x^*)$.

For each day in the period of record ($i = 1, 2, \ldots 14004$) we compute 774 equally likely outcomes one for each sampled day ($j = 1, 2, \ldots 774$)). Those 10,839,096 ($= 14004 \cdot 774$) outcomes are denoted $V_{i,j}$ and they are computed as:

$$V_{i,j} = \begin{cases} 1 \; if \; \bar{y}_i + s_i \cdot e_j \geq y^* \\ 0 \; if \; \bar{y}_i + s_i \cdot e_j < y^* \end{cases}$$

Then the estimated probability that concentration on day i exceeds the criterion x^* is defined as

$$p_i = \frac{\sum_{j=1}^{774} V_{i,j}}{774} \qquad \text{for } i = 1, 2, \ldots, 14004$$

We can then compute the expected number of exceedances for each water year in the record as $z_k = \sum_{i \in U_k} p_i$

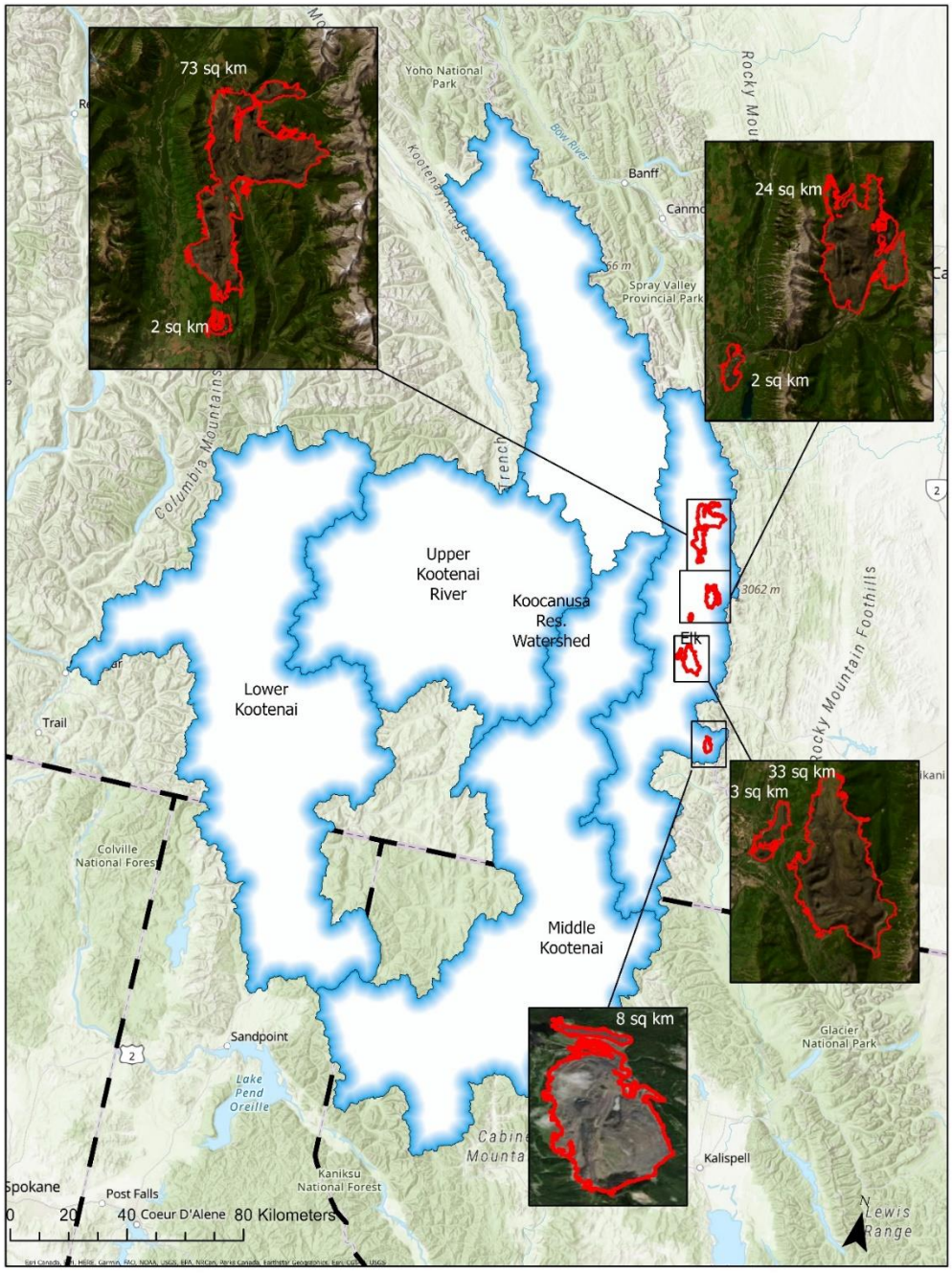
Where, $i \in U_k$ denotes the set of all days, i, in year k.

The z_k represent the expected number of days of exceedances in year k and can take on any value from 0 to 365 (or 366 in a leap year).

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

166 SI Figure 1

167 Total area of mine disturbance (waste rock deposits) based on GIS analysis of aerial imagery.



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SI Table 1a

Training classes and associated sample sizes from the GIS analysis of mine waste rock area

Sample size (n)	Class
228	Waste rock
89	Forest
68	Water
81	Open rock
60	Deforested

SI Table 1b

Final areas of waste rock from GIS analysis.

Mine	Area of disturbance, in square kilometers
Fording River Operations	75 km^2
Line Creek Operations	26 km^2
Elkview Operations	36 km^2
Coal Mountain Operations (in closure period)	8 km^2

SI Table 2

Elk River Watershed cumulative waste rock volumes by mine, reported by Teck Resources in their 2022 Implementation Plan Adjustment Document (Table 2.1). [21] Existing volumes are values at the end of 2020 in millions of bank (in situ) cubic meters.

Operation	Existing Volume (BCM x 10^6)	Permitted Volume (BCM x 106) at the end of mining
Fording River	3,036	4,787
Greenhills	808	1,186
Line Creek	797	1,445
Elkview	1,787	3,304
Coal Mountain[1]	311	311
Total	**6,739**	**11,033**

1. No longer operating and currently in a closure period.

181 SI Table 3

Se, NO_3^-, SO_4^{2-} annual estimates of load (optimized for accuracy) for the Elk River (Elk) and Kootenay River (Koot). The proportion that the Elk River contributed by year, from the combined estimates of the two tributaries is shown in percent for each constituent and discharge.[16]

Year	Koot Flow (m^3/sec)	Elk Flow (m^3/sec)	Elk proportion of total combined (%)	Koot Se (t/Yr)	Elk Se (t/Yr)	Elk proportion of total combined (%)	Koot NO_3^- (t/Yr)	Elk NO_3^- (t/Yr)	Elk proportion of total combined (%)	Koot SO_4^{2-} (t/Yr)	Elk SO_4^{2-} (t/Yr)	Elk proportion of total combined (%)
1979	128	58.8	31.50%				442.77	218	33%			
1980	173	67.3	28.00%				557.15	243	30%			
1981	214	90.3	29.70%				713.84	400	36%			
1982	189	72.5	27.70%				672.67	350	34%			
1983	173	67.8	28.20%				581.46	250	30%			
1984	154	60.5	28.20%				594.72	280	32%			
1985	142	61.6	30.30%		1.66		552.34	317	36%	103186	32077	24%
1986	188	75.8	28.70%		2.22		630.72	345	35%	123833	41149	25%
1987	154	61.1	28.40%		2.04		521.04	337	39%	115952	38303	25%
1988	139	52.1	27.30%		1.63		530.82	261	33%	104826	32286	24%
1989	166	63.1	27.50%		1.86		668.4	301	31%	114991	37871	25%
1990	204	89.8	30.60%		3.57		828.57	624	43%	126769	46310	27%
1991	238	100.4	29.70%		4.09		953.65	828	46%	141380	54032	28%
1992	143	53.6	27.30%		2.2		533.41	419	44%	104216	38947	27%
1993	159	67.9	29.90%		3.12		535.13	550	51%	112542	43943	28%
1994	158	61.6	28.10%		3.23		607.38	603	50%	119986	44531	27%
1995	171	76.5	30.90%		3.69		632.1	670	51%	122786	48108	28%
1996	228	101	30.70%		5.44		813.86	893	52%	153292	61970	29%
1997	181	80	30.70%		4.48		589.91	612	51%	132329	51921	28%
1998	175	75.9	30.30%		4.62		634.14	805	56%	136417	54786	29%
1999	220	78.7	26.30%		4.21			794			55958	
2000	185	70.8	27.70%		4.31			627			55155	
2001	108	39.6	26.80%		2.49			356			38955	
2002	169	84.3	33.30%		5.27			679			59197	
2003	133	64	32.50%	0.49	5.22	91.50%		672			58338	
2004	148	59.7	28.70%	0.36	4.22	92.10%		497			50190	
2005	168	77.6	31.60%	0.46	6.1	93.00%		811			62462	
2006	187	87.8	32.00%	0.49	7.83	94.20%		1214			73056	
2007	203	82.2	28.80%	0.56	7.78	93.30%		1115			69321	
2008	160	67.2	29.60%	0.4	6.88	94.50%		1153			63140	
2009	139	49.7	26.30%	0.4	5.54	93.30%	414.02	899	68%		50131	
2010	138	55.6	28.70%	0.38	6.05	94.10%	427.55	1104	72%		54625	
2011	204	80.8	28.40%	0.5	8.48	94.40%	596.62	1633	73%		66141	
2012	238	100.1	29.60%	0.58	11.53	95.20%	712.9	2139	75%		79029	
2013	212	98.9	31.80%	0.49	12.6	96.20%	657.21	2645	80%	135270	88804	40%
2014	192	86.6	31.10%	0.44	12.4	96.60%	589.34	2815	83%	128185	85490	40%
2015	152	63.6	29.50%	0.35	9.99	96.60%	494.69	2038	80%	117210	74140	39%
2016	162	60.9	27.30%	0.42	9.12	95.60%	539.4	2031	79%	127970	76617	37%
2017	210	80.5	27.70%	0.51	9.96	95.20%	848.21	2115	71%	146569	79502	35%
2018	179	70.3	28.20%	0.45	10.21	95.80%	595.46	2169	78%	131452	84313	39%
2019	144	51.6	26.40%	0.37	7.88	95.50%	460.4	1448	76%	124535	64884	34%
2020		74.6			10.5			2198			80595	
2021		74			11.36			2501			82322	
2022		87.8			12.27			2549			89262	
Mean	174	72	29.20%	0.45	5.77	94.50%	615	1034	58%	124938	4.5E+07	30%
Mean Post 2009	179	74	28.60%	0.44	9.85	95.30%	576	2020	76%	130170	74353	38%

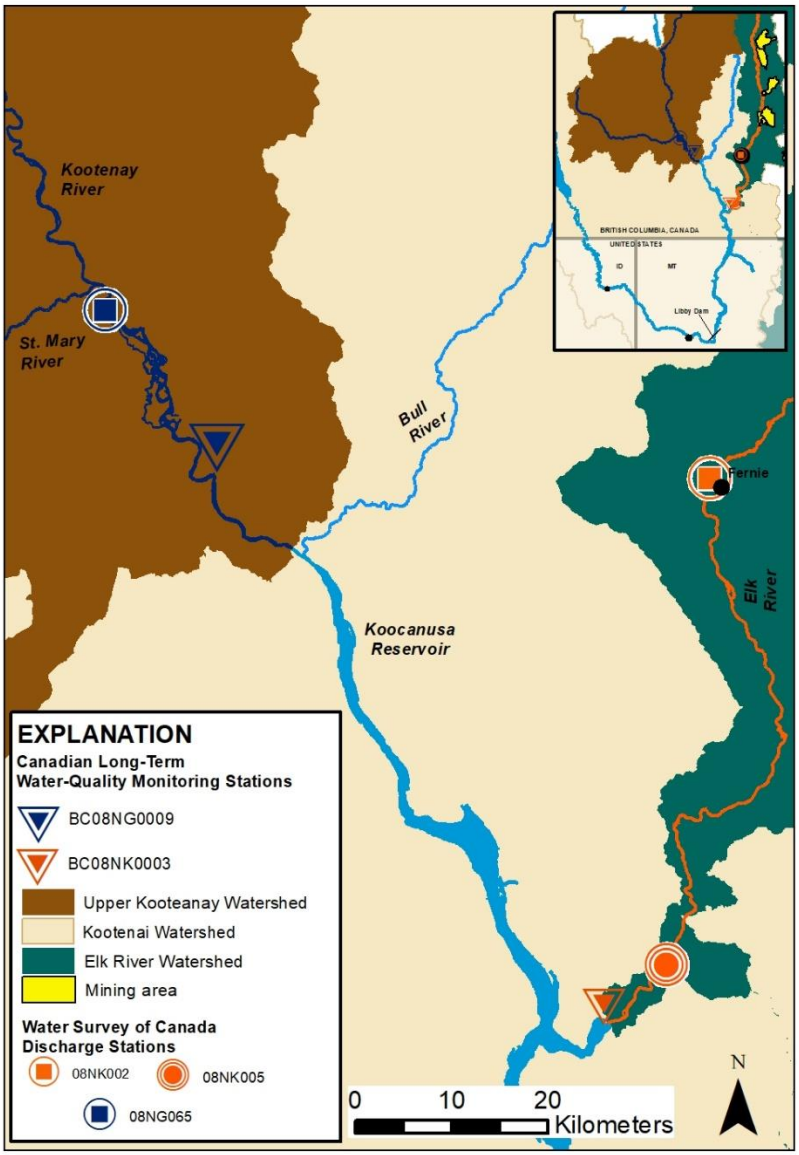
SI Table 4

Selenium and Nitrate treatment, locations, timing, and volumetric capacity as presented in the 2022 Teck Implementation Plan Adjustment.[21] Pilot phase start dates sourced from mass removal data provided by Teck.[6] Note, from pilot phase start to the operational date, treatment facilities did not operate continuously and additional treatment pilot projects occurred before 2015.

Water Treatment Facility	Facility Type	Pilot Phase Start	Operational Date	Hydraulic Capacity (m^3/day)
Line Creek Operation WLC Phase I	Active Water Treatment	October 25, 2015	December 31, 2018	6,000
Line Creek Operation WLC Phase II	Active Water Treatment	NA	January 1, 2020	1,500
Elkview Operation SRF Phase I	Saturated Rock Fill Treatment	January 1, 2018	September 1, 2021	20,000
Fording River Operation AWTF (FRO-South)	Active Water Treatment	December 22, 2021	September 1, 2022	20,000

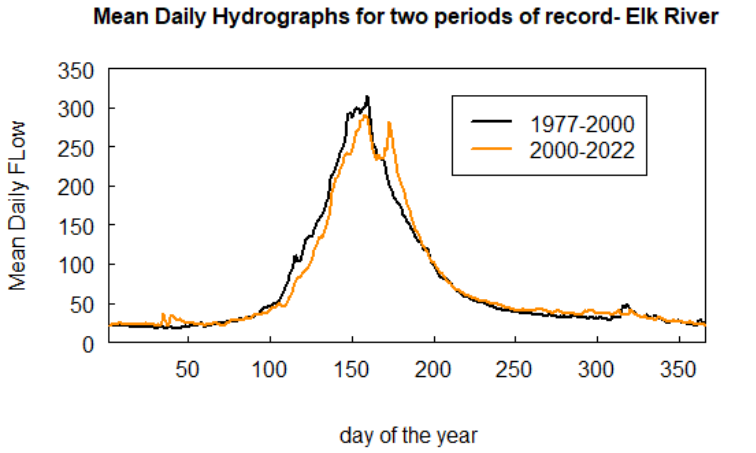
SI Figure 2

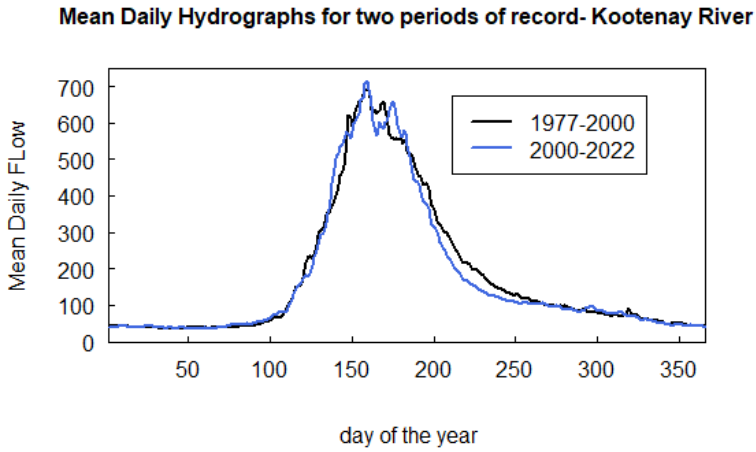
Map illustrating Canadian Water Survey of Canada discharge monitoring stations (halo symbols) and water quality sampling locations (triangles) for both the Elk (orange) and Kootenay (blue) Rivers.



SI Figure 3

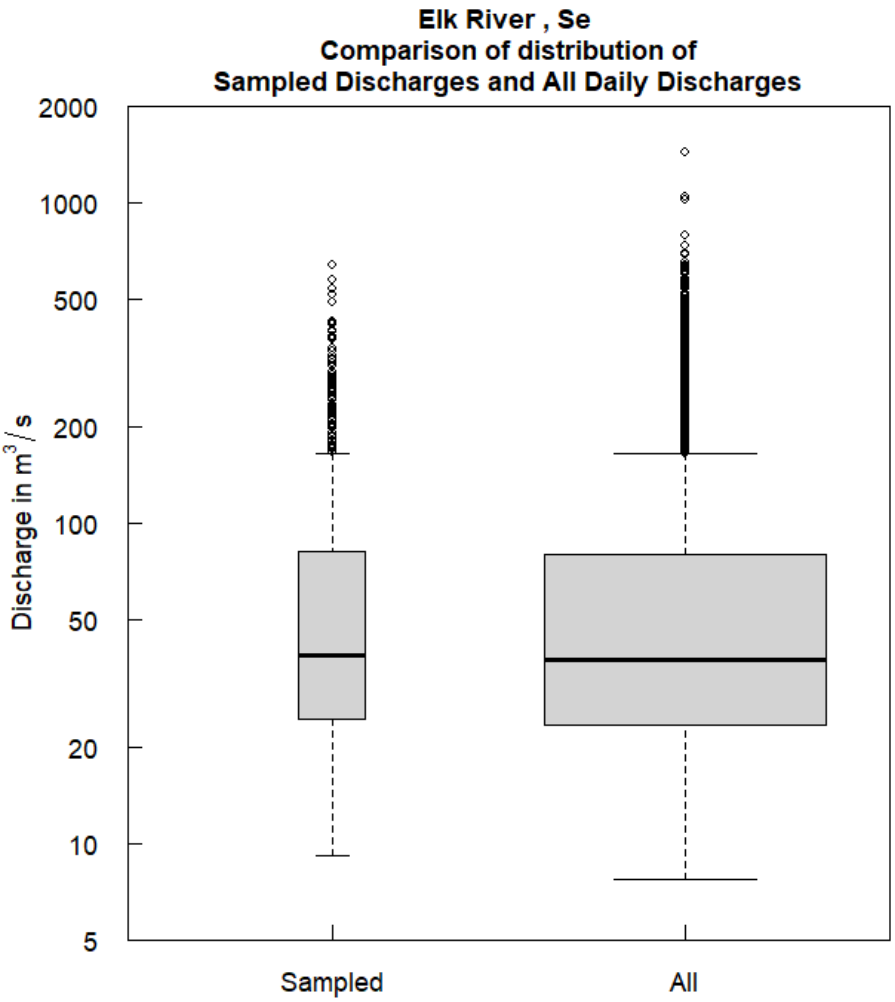
Mean daily hodographs for the Elk River at Hwy 93 (top) and Kootenay River at Fenwick (bottom) [22], discharge in m^3/sec. Two time periods are shown one from 1977-2000 and from 2000-2022.





199 SI Figure 4

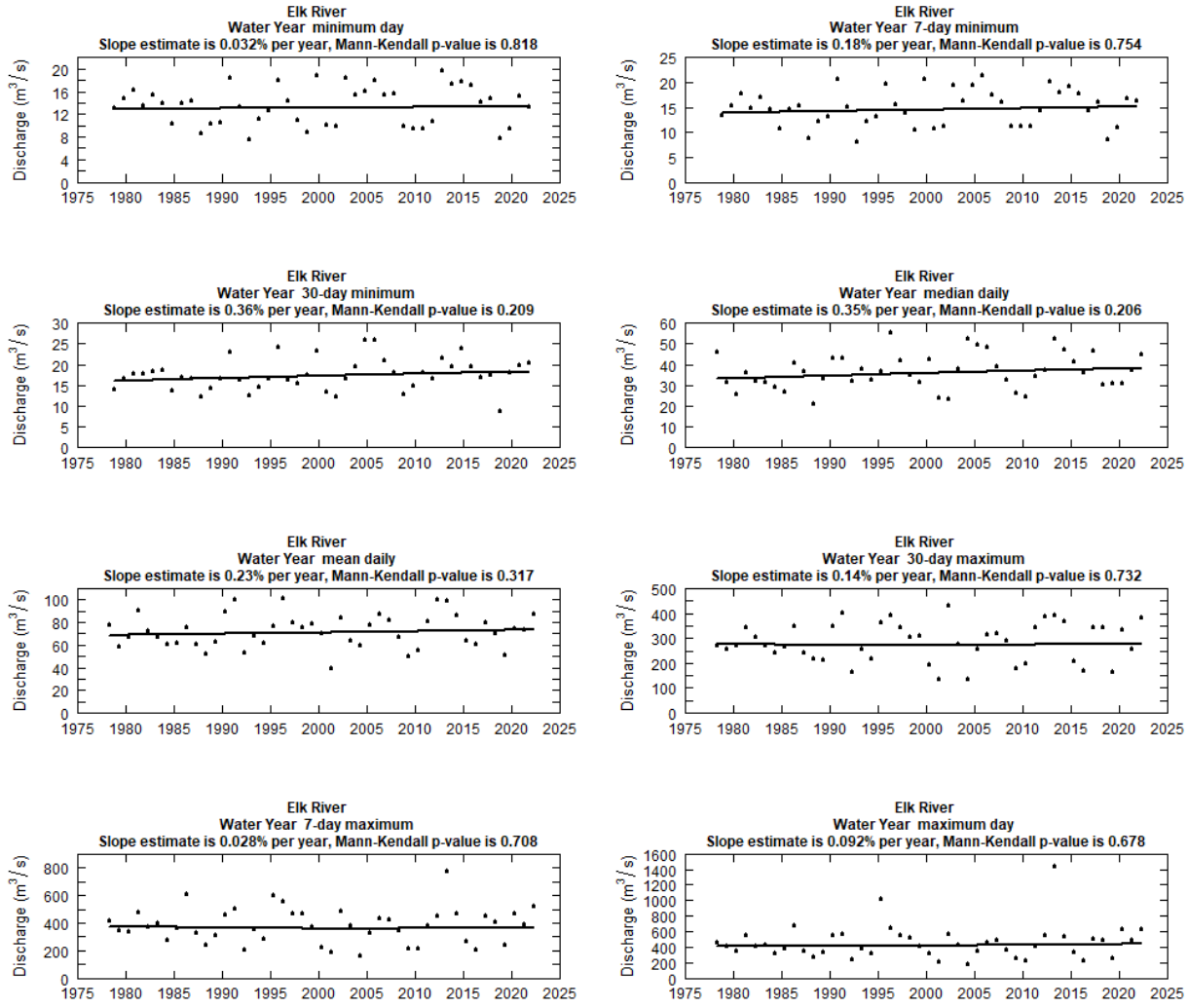
200 Box plot illustrating the range of discharge on days with water quality sampling vs those without water
201 quality samples for Se in the Elk River at Highway 93.



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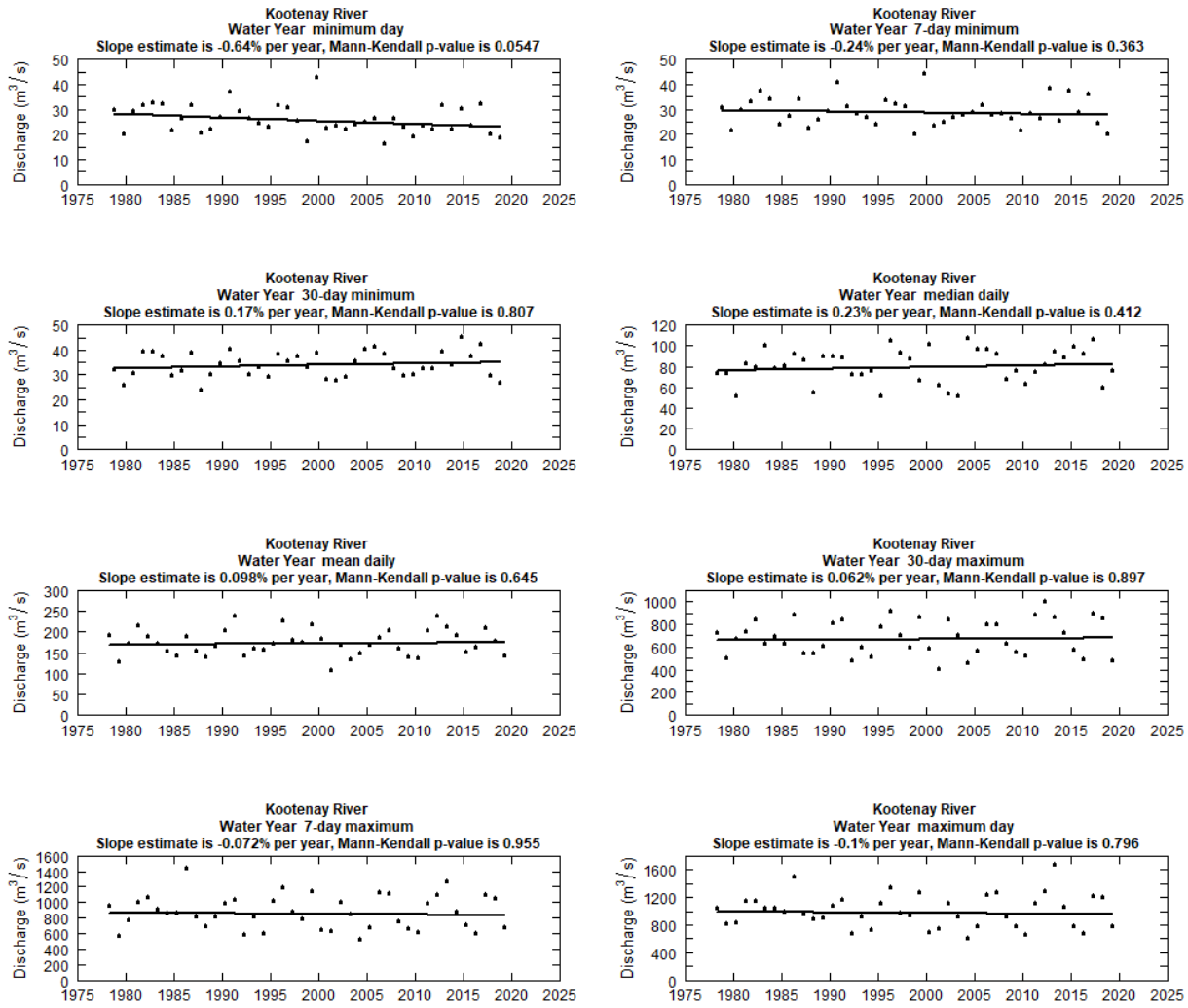
SI Figure 5

Elk River at Highway 93, flow record from 1979-2021. [6] Plots showing 8 different statistical flow metrics, with the Mann-Kendall trend test and corresponding Theil-Sen slope estimate to evaluate flow stationarity. The Theil-Sen slope provides an estimate of the direction and magnitude of the Mann-Kendall trend. [23]



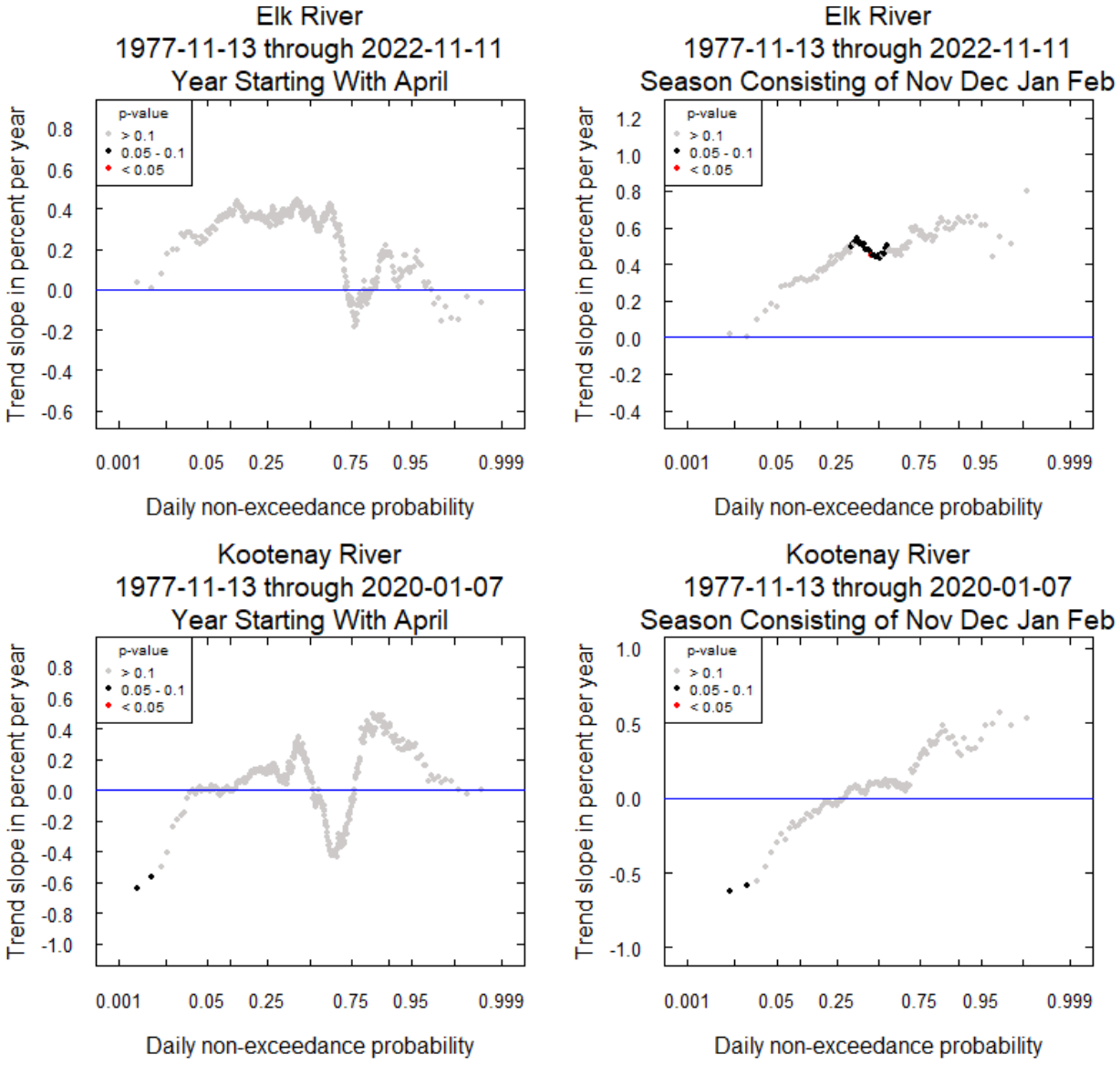
SI Figure 6

Kootenay River at Fenwick, flow record from 1979-2019. [6] Plots showing 8 different statistical flow metrics, and the Mann-Kendall trend test and corresponding Theil-Sen slope estimate to evaluate flow stationarity. The Theil-Sen slope provides an estimate of the direction and magnitude of the Mann-Kendall trend. [23]



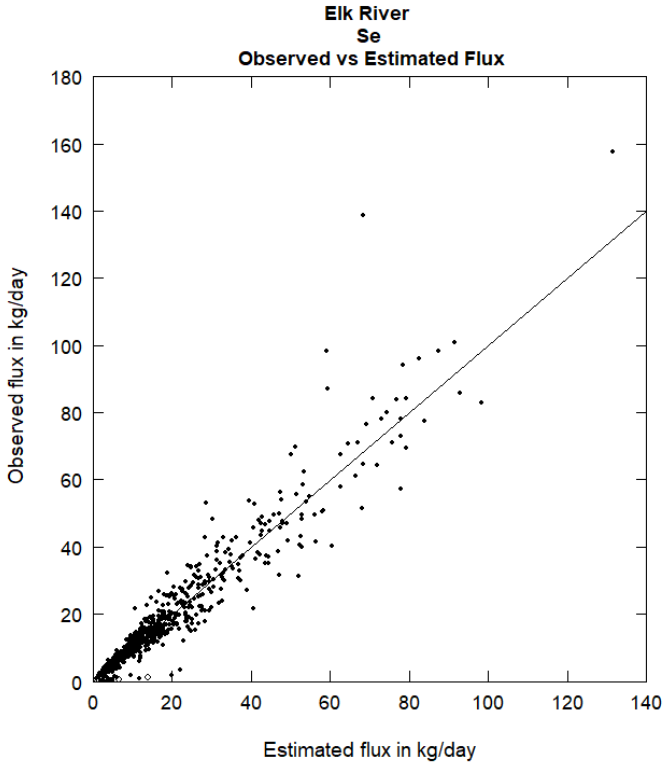
SI Figure 7

Streamflow trends for the Elk River at Highway 93 (top two panels) and the Kootenay River at Fenwick (bottom two panels). Left panels are Quantile-Kendall plots for the water year. Right are Quantile-Kendall plots for the low flow period (February-October). Quantile-Kendall plots are visualizations of trends in slope for ranked discharge, where each point is a trend slope for a given order statistic, the lowest daily discharge is on the left and the highest is on the right. [24]



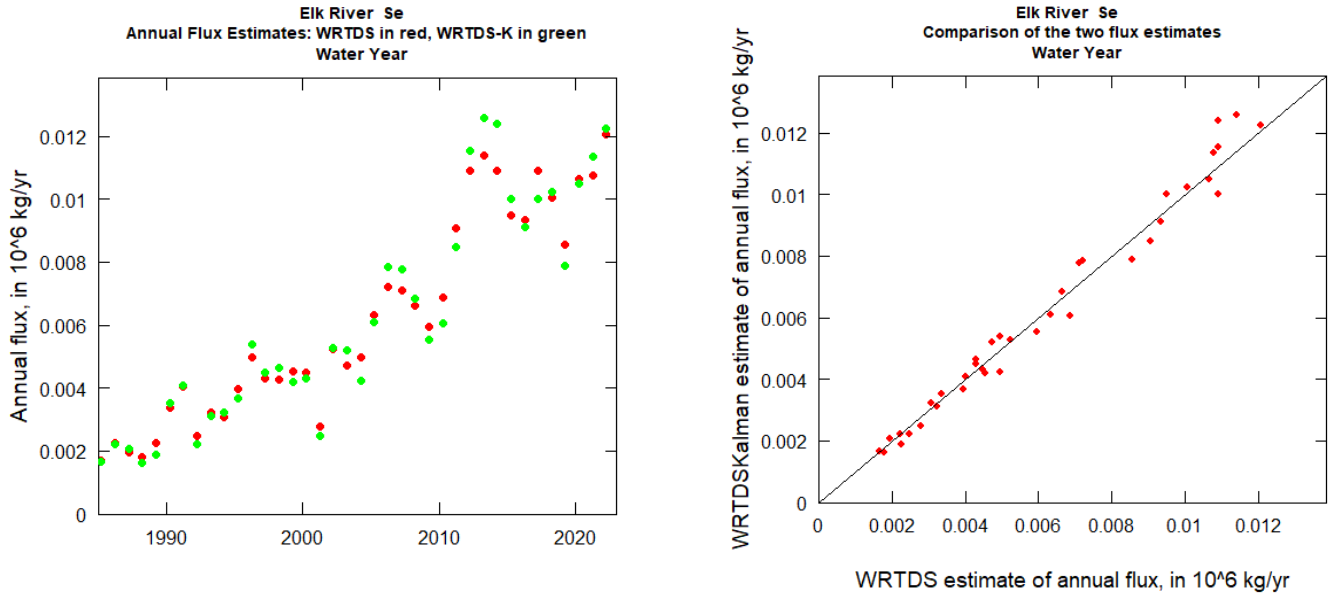
SI Figure 8

Plot illustrating the difference between observed daily loads on the days when samples were collected vs modeled daily load on the same day for Se in the Elk River at Highway 93. Open circles are generated values that represent censored (below detection limit) values. Equivalent R-squared value for this model (error in the estimates of log(Flux)) is 0.852. 20 of the 841 samples were below the detection limit. Models are available in Lange and Storb, 2023.[6]



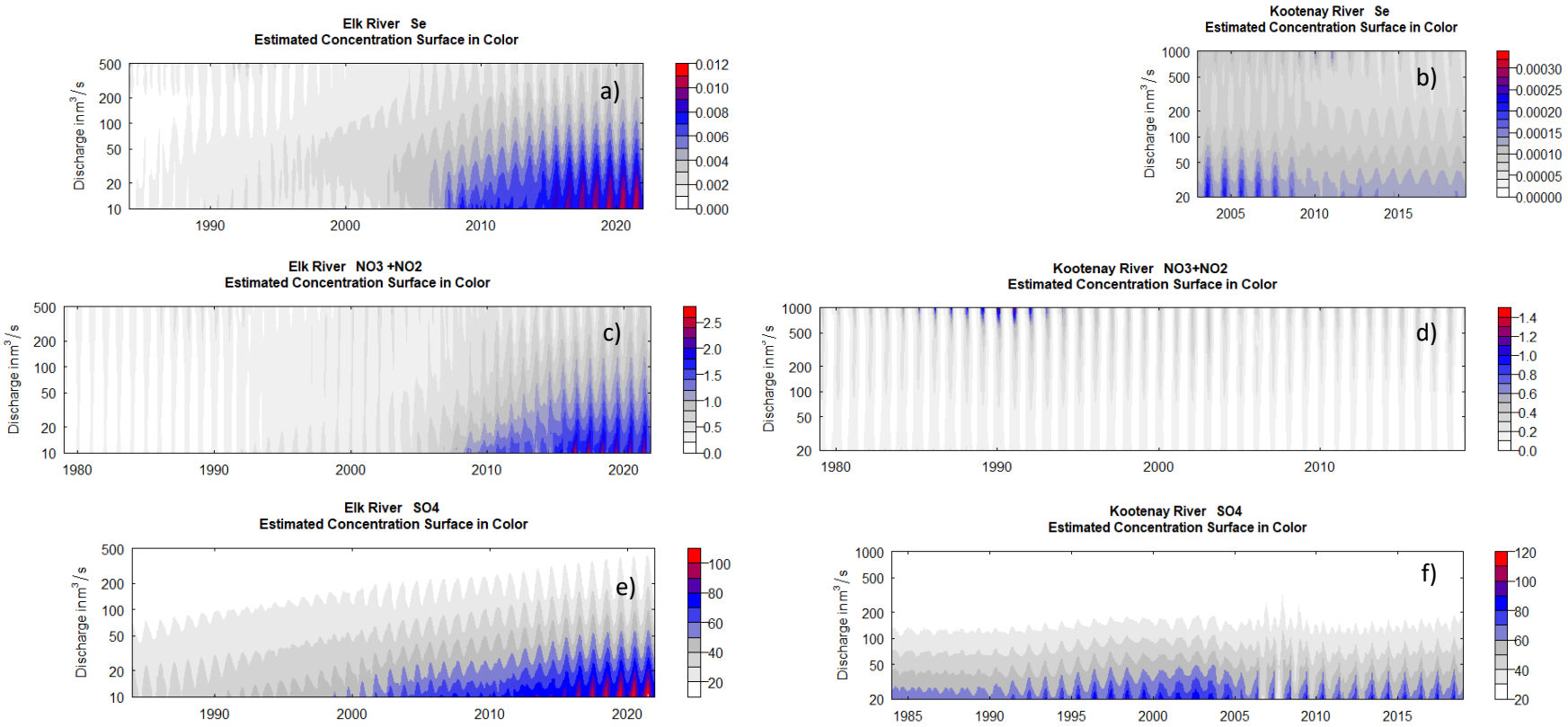
SI Figure 9

Two plots illustrating the difference between WRTDS and WRTDS_kalman models for Se in the Elk River at Highway 93. Models are available in Lange and Storb, 2023.[6]



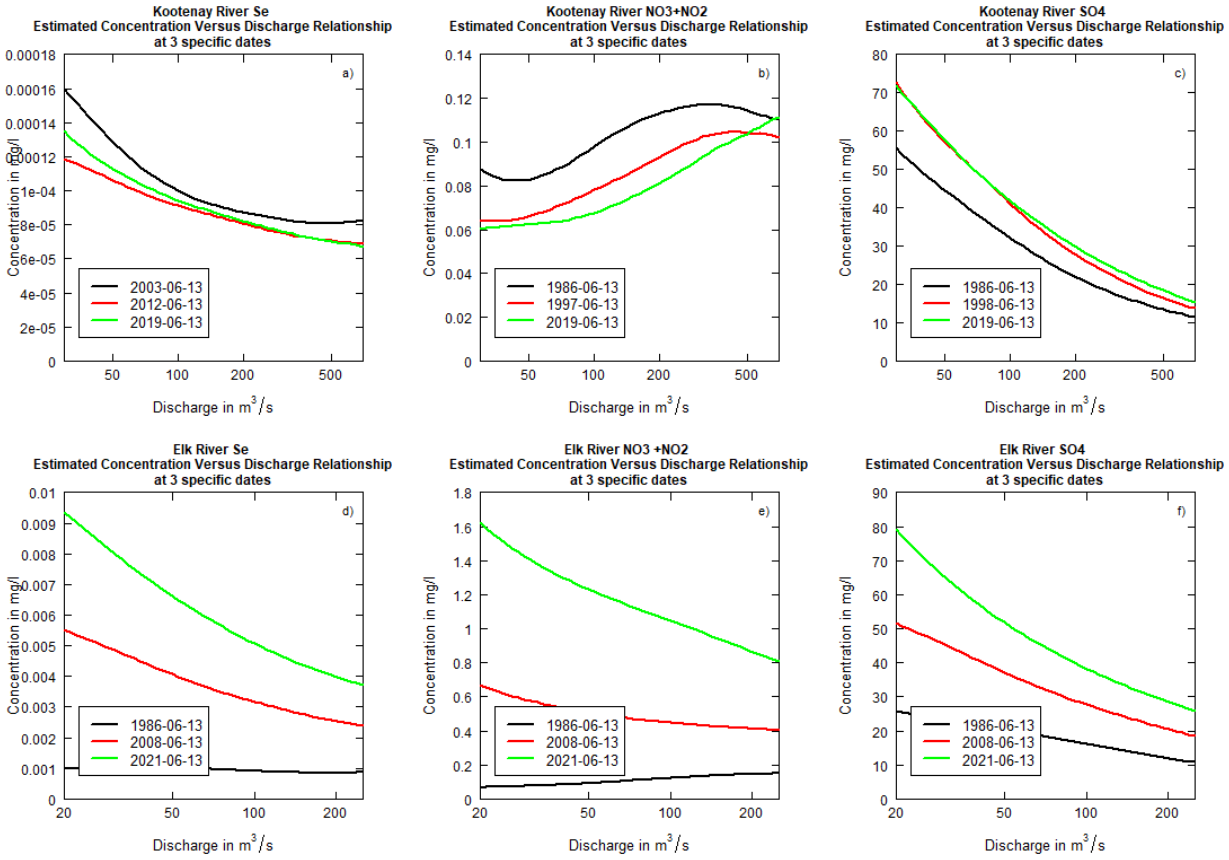
SI Figure 10

Weighted Regression based on Time Discharge and Season (WRTDS) modeled 3-D surfaces for Se, NO_3^-, SO_4^{2-} for the Elk River at Highway 93 and the Kootenay River at Fenwick. Plots illustrate and quantify the relationship between concentration and discharge over time for each WRTDS model. Left plots are the three solutes for the Elk River at Highway 93 and Right plots are the three solutes for the Kootenay River at Fenwick. Top row of plots is Se, middle is NO_3^-, bottom row is SO_4^{2-}. The color ramp represents concentration of the respective constituent in mg/l. Models are available in Lange and Storb, 2023.[6]



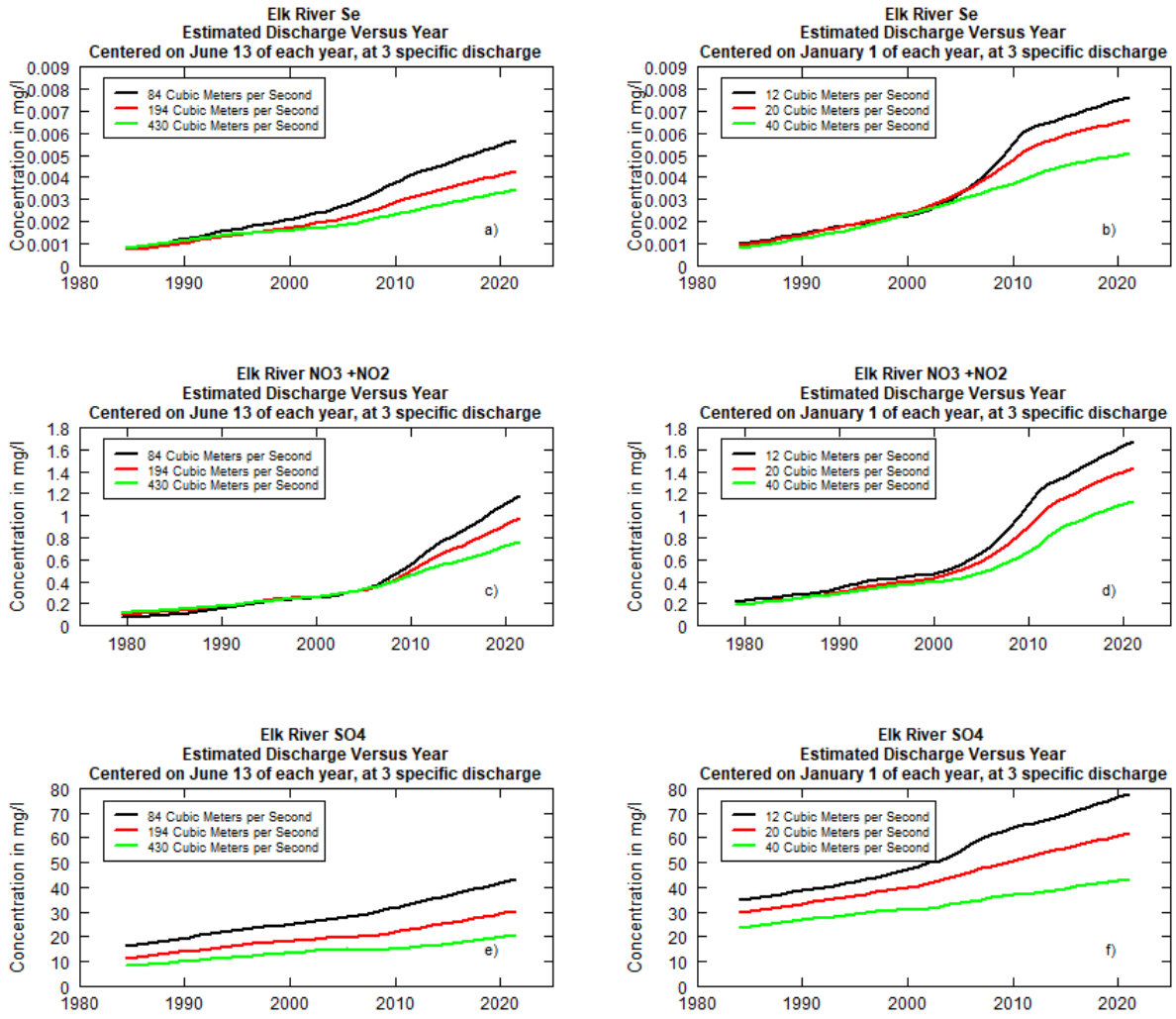
SI Figure 11

Modeled concentration vs discharge relationships. Top row (a-c) is the Kootenay River at Fenwick, the bottom row is the Elk River at Highway 93 (d-e). Each column is a solute, from left to right (Se; a & d, NO_3^-: b & e, SO_4^{2-}; c & f). Each line is a different date. These plots are focused on high flow times of the year (June 13) over time. Low flow times (ex. January 1) exhibit similar patterns but are not shown. Models are available in Lange and Storb, 2023.[6]



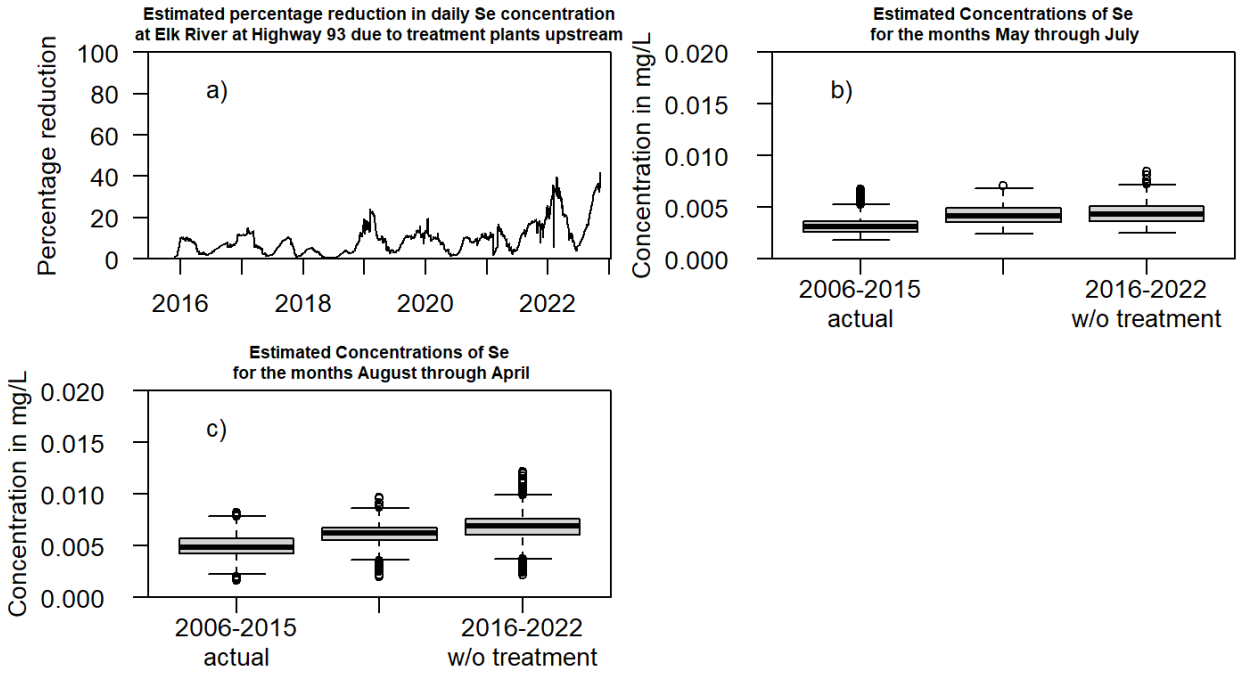
SI Figure 12

Changes in concentration over time at different discharges and times of the year for the Elk River at Highway 93. The left column (a-c-e.) is high discharge time of year (June 13) and the right column (b-d-f.) is a low discharge time of year (Jan 1). Discharge lines roughly represent the 5th, 50th, and 95th percentiles for flow duration curves for 60 days around (30 days before and after) each date. Models are available in Lange and Storb, 2023.[6]



SI Figure 13

Additional perspective on Elk River Mine water treatment. a.) Estimated percentage reduction in concentration due to treatment. Reductions range from 0% to 40%. b.) Boxplots of estimated daily concentrations of Se during the high discharge months of May, June, and July. First box is for water years 2006-2015, second is 2016 – 2022, and the third is also for 2016-2022 but simulated as if there had been no treatment upstream. [the medians of the three boxes shown are 0.0032, 0.0042, 0.0045]. c.) Boxplots of estimated daily concentrations of Se during the low discharge months of August through April. First box is for water years 2006-2015, second is 2016 – 2022, and the third is also for 2016-2022 but simulated as if there had been no treatment upstream [the medians of the three boxes shown are 0.0049, 0.0062, 0.0075].



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Appendix J

United States Environmental Protection Agency

Office of Water
EPA 820-R-24-001
April 2024

Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards

Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards

While this document cites statutes and regulations that contain requirements applicable to water quality standards, it does not impose legally binding requirements on the EPA, states, authorized Tribes, other regulatory authorities, or the regulated community, and may not apply to a particular situation based upon the circumstances. The EPA, state, Tribal and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those provided in this technical support document as appropriate and consistent with statutory and regulatory requirements. The EPA may update this document as new information becomes available. In addition to this document, the EPA has related documents that provide considerations and recommendations on implementing criteria based on the national Clean Water Act (CWA) section 304(a) recommended selenium criterion for freshwater, which are available at the EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

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List of Acronyms and Abbreviations

BAF	Bioaccumulation factor
CFR	Code of Federal Regulations
CWA	Clean Water Act
FR	Federal Register
GLI	Great Lakes Initiative
HAC	Highest attainable condition
NPDES	National Pollutant Discharge Elimination System
SSD	Species sensitivity distribution
TMDL	Total maximum daily load
UAA	Use attainability analysis
USEPA	U.S. Environmental Protection Agency
WQBELs	Water quality-based effluent limits
WQC	Water quality criteria
WQS	Water quality standards

Definitions[1]

Bioaccumulation
The uptake and retention of a chemical by an aquatic organism from all surrounding media (e.g., water, food, sediment).[2]

Critical species
In the context of the recalculation procedure, a resident species that (a) is commercially or recreationally important at the site, or (b) is listed as threatened or endangered under section 4 of the Endangered Species Act, or (c) is a species for which there is firm evidence that its loss would yield an unacceptable impact on the site's commercially or recreationally important species, endangered species, abundances of a variety of other species, or structure or function.[3]

Dissolved total selenium
All species of selenium that are dissolved into the water column and that would be measured in a water sample after it has been passed through a 0.45 µm membrane filter.[4]

Performance-based approach
A water quality standard that is a transparent process, rather than a specific outcome (such as the concentration portion of a criterion for a pollutant). This process or methodology is sufficiently detailed and has suitable safeguards that ensure predictable, repeatable outcomes. Approval of this process or methodology by the EPA would also serve for CWA purposes as the approval under CWA section 303(c)[5] of each outcome generated from following that process or method.[6]

Site-specific criterion/site-specific criterion element
Water quality criterion/criterion element that is modified to reflect site-specific conditions. For selenium, a criterion/criterion element that is developed to protect aquatic life at a particular

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[1] This glossary is meant to provide plain language definitions for key terms used in this document. Individuals should consult the Clean Water Act and the EPA's implementing regulations to identify whether there are legal definitions of these terms.
[2] USEPA. 2003. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Volume 2: Development of National Bioaccumulation Factors.* EPA-882-R-03-030. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005EZQ.PDF?Dockey=P1005EZQ.PDF.
[3] USEPA. 2013. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC, www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf.
[4] Protho, M.G. USEPA. 1993. Memorandum: Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria. Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2019-03/documents/metals-criteria-interpret-aqlife-memo.pdf.
[5] Approval by the EPA includes federal WQS promulgated by the EPA.
[6] *EPA Review and Approval of State and Tribal Water Quality Standards*, 65 Fed. Reg. 24641 (Apr. 27, 2000).

site, usually by taking into account a site's physical, chemical, and/or biological conditions (i.e., water quality characteristics or species composition).[7]

Steady-state

An organism is in steady-state when the rates of chemical uptake and depuration are equal and tissue concentrations remain constant over time.[8] For the purposes of the national CWA section 304(a) recommended selenium criterion, steady-state refers to conditions where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing.

Water quality criterion element

A magnitude, frequency, and duration for a particular media type (e.g., fish tissue, water column), which are individual components of the overall water quality criterion. The water quality criterion elements for selenium are related through a hierarchy, with fish tissue criterion elements having primacy over water column criterion elements, and the egg-ovary fish tissue criterion element having primacy over all other criterion elements.

[7] USEPA. 2017. *Water Quality Standards Handbook. Chapter 3: Water Quality Criteria.* EPA-823-B-17-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/default/files/2014-10/documents/handbook-chapter3.pdf.

[8] USEPA. 2003. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Volume 2: Development of National Bioaccumulation Factors.* EPA-882-R-03-030. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005EZQ.PDF?Dockey=P1005EZQ.PDF.

1.0 Introduction

1.1 The EPA's National CWA Section 304(a) Recommended Chronic Aquatic Life Selenium Criterion in Freshwater

In 2016, the U.S. Environmental Protection Agency updated its national Clean Water Act (CWA) section 304(a) recommended chronic aquatic life criterion for selenium in freshwater systems to reflect the latest scientific information and, in 2021, issued an erratum, *2021 Revision to: Final Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*.[9] The EPA's national CWA section 304(a) recommended selenium criterion still reflects the latest science and the EPA is not aware of any updated scientific information that would change this recommendation. The latest scientific information indicates that selenium toxicity to aquatic life is driven by dietary exposures and that the reproductive life-stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium. The recommended criterion has four criterion elements: (1) a fish egg-ovary criterion element; (2) a fish whole-body and/or muscle criterion element; (3) a water column criterion element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent criterion element (to account for potential chronic effects from short-term exposures to high concentrations in lentic and lotic aquatic systems) (see Table 1). Under the EPA's 2016 national CWA section 304(a) recommended selenium criterion, the fish tissue criterion elements have primacy over water column elements, except where there are no fish, where fish tissue data are not adequate, or for waterbodies with new or increased discharges where selenium concentrations in fish tissue might not have stabilized and reached steady-state. The EPA also recommends that the egg-ovary tissue criterion element has primacy over whole-body and muscle tissue criterion elements.

Toxicity data indicate that the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse reproductive effects in aquatic organisms. Toxicity to developing embryos and larvae is directly linked to egg selenium concentration.[10] The EPA derived the whole-body, muscle tissue, and water column elements from the egg-ovary element so that states and authorized Tribes could more readily implement their water quality criteria (WQC) based on the EPA's national CWA section 304(a)

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[9] In 2021, the EPA identified that the following text was missing from the second sentence in footnote 4 in the selenium criterion table: "When selenium inputs are increasing" and issued an erratum. The EPA corrected footnote 4 to state: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data." USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[10] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

recommended selenium criterion. The assessment of the available data on chronic selenium exposure for fish, invertebrates, and amphibians indicates that a criterion element derived from fish is expected to be protective of the aquatic community in a waterbody, since other taxa appear to be less sensitive to selenium than fish. The EPA did not develop an acute criterion for selenium when it updated the chronic criterion. Although selenium may cause acute toxicity at high concentrations, the most deleterious effects on aquatic organisms are due to selenium's bioaccumulative properties.

In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from chronic exposure to chemicals via the diet (through the food web pathway). Such studies also do not account for the accumulation kinetics of many bioaccumulative compounds, such as selenium, and may underestimate effects from long-term accumulation in some types of aquatic systems. Therefore, since acute studies do not address the primary exposure pathway for organisms to selenium and because chronic toxicity occurs at lower concentrations, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion. As described in the EPA's *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (hereafter referred to as *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*), the EPA also included an intermittent exposure criterion element to provide protection from the most significant chronic effects of selenium toxicity, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high concentration exposure events.[11]

The EPA recommends, as stated in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, that states and authorized Tribes[12] adopt into their water quality standards (WQS) a selenium criterion that includes all four criterion elements.[13] For more information see the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* which can be found at https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[11] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[12] Throughout this document and in the CWA, the term "states" means the fifty states, the District of Columbia, the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer a CWA WQS program.

[13] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg-ovary[2]**	**Fish Whole-body or Muscle[3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dry weight	8.5 mg/kg dry weight whole-body or 11.3 mg/kg dry weight muscle (skinless, boneless fillet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int}}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where $WQC_{30\text{-}day}$ is the water column monthly element for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

1.2 Selenium Technical Support Materials

The EPA has prepared a four-volume set of documents to provide recommendations to states, authorized Tribes, and other agencies for implementing their WQC based on the national CWA section 304(a) recommended selenium criterion for aquatic life.[14] These four documents constitute the Technical Support Materials for the EPA's *Aquatic Life Ambient Water Quality*

[14] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

Criterion for Selenium–Freshwater 2016.[15] Each document of the set focuses on a specific aspect of implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. Together, these four EPA documents provide information to assist states and authorized Tribes with adopting WQC based on the EPA's national CWA section 304(a) recommended selenium criterion and implementing them in various CWA programs.

1) *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*: Provides recommendations for the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools.
2) *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*: Provides an overview of how to establish or enhance existing fish tissue monitoring programs to facilitate implementation of fish tissue criterion elements based on the national CWA section 304(a) recommended selenium criterion.
3) *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*: Provides information to help National Pollutant Discharge Elimination System (NPDES) permit writers understand what permitting guidance (i.e., state or tribal implementation procedures) may be appropriate to implement state and authorized tribal WQS based on the EPA's national CWA section 304(a) recommended selenium criterion. This set of FAQs also provides recommendations on how to establish water quality-based effluent limits (WQBELs) in NPDES permits.
4) *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*: Provides information on how to complete assessments, list impaired waters, and develop total maximum daily loads (TMDLs) to implement EPA-approved[16] WQS that are based on the EPA's national CWA section 304(a) recommended selenium criterion.

[15] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[16] EPA-approved includes both WQS adopted by states, territories or authorized Tribes and approved by the EPA and WQS promulgated by the EPA.

2.0 Adopting the EPA's National CWA section 304(a) Recommended Selenium Criterion

2.1 The Four-Part Criterion

As described in the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*,[17] the EPA recommends that states and authorized Tribes adopt into their WQS one selenium criterion composed of four criterion elements: two fish tissue criterion elements (i.e., egg-ovary and whole-body and/or muscle tissue) and two water column criterion elements (i.e., 30-day average and intermittent exposure). The EPA recommends that states and authorized Tribes clearly express all four criterion elements as part of a single criterion. Including all four recommended criterion elements of the selenium criterion in state or Tribal WQS ensures protection from excessive selenium when egg-ovary data are unavailable by allowing application of the criterion using other fish tissue or water column data. Along with the four criterion magnitude elements, the EPA also recommends adopting the corresponding durations and frequencies for each of the criterion elements.

States and authorized Tribes should clearly indicate in their WQS that fish tissue criterion elements supersede the water column criterion elements (except when selenium inputs are increasing and steady-state condition fish tissue data are not available), and that the egg-ovary criterion element supersedes all other criterion elements. Under the EPA's national CWA section 304(a) recommended selenium criterion, this hierarchy applies to all CWA implementation activities. The egg-ovary criterion element supersedes all other criterion elements because it was derived directly from toxicity data and served as the basis for deriving all the other criterion elements. This hierarchy should be described in a state or authorized Tribe's WQS, in footnotes accompanying the criterion. The EPA recommends using the footnotes provided with the national CWA section 304(a) recommended selenium criterion (Table 1) to clearly and accurately describe the hierarchical structure.

The fish tissue criterion elements of this criterion are expressed in dry weight concentrations. This is because many of the toxicity values used to derive the criterion were expressed as dry weights. Instructions for converting between dry weight and wet weight concentrations are provided in Appendix C of *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*.[18]

The two monthly average water column values included in the national CWA section 304(a) recommended selenium criterion were derived for the protection of two different types of

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[17] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*. EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[18] USEPA. 2024. *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*. EPA-820-R-24-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC.https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf

waterbodies, lentic and lotic waters. Lentic waters are waterbodies that consist of standing water, including waterbody types such as lakes and ponds. Lotic waters are waterbodies that consist of flowing waters, including waterbody types such as rivers and streams. The application of the appropriate water column value is based on best professional judgement of the state or authorized Tribe. Although states and authorized Tribes do not need to classify their waters based on these terms, they may find it useful to consider incorporating these criterion elements into any existing classifications for different types of waterbodies (e.g., lakes). One resource that may be helpful for states and authorized Tribes is a geospatial dataset the United States Geological Survey (USGS) developed on lotic and lentic waters, available at https://www.sciencebase.gov/catalog/item/5d420fbde4b01d82ce8da8e9.

The national CWA section 304(a) recommended selenium criterion is a chronic criterion. The EPA no longer recommends an acute criterion for short-term ambient exposures because aquatic organisms are exposed to selenium primarily through their diet. Since acute studies are conducted as water-only exposures, they do not address this primary exposure pathway for organisms and do not account for the slow accumulation kinetics of selenium. Additionally, chronic toxicity occurs at lower concentrations than acute toxicity, so protection from acute toxicity is provided by the chronic criterion. Given these properties of selenium, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion.

Selenium has the potential to bioaccumulate in aquatic food webs, where it can continue to pose a risk even after short-term elevations of the selenium concentration in the water column return to ambient levels. Therefore, the EPA's national CWA section 304(a) recommended selenium criterion includes an intermittent exposure water column criterion element to provide protection from long-term chronic effects that may be caused by short-term elevations above ambient background concentrations of selenium water column concentrations in aquatic systems.

If data for a particular criterion element are not available, WQC based on the national CWA section 304(a) recommended selenium criterion should be implemented with one of the other criterion elements, according to the hierarchy of the criterion. For example, if fish tissue data are not available, but water column data are, the criterion should be implemented using the water column criterion element. This situation may occur for fishless waters or when fish tissue data are not available because they were not collected or were not adequate. Fishless waters are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (see the executive summary of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*).[19]

[19] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

As noted in the introduction of this document, the national CWA section 304(a) recommended selenium criterion is designed for the protection of aquatic life. As a result, when the national CWA section 304(a) recommended selenium criterion is adopted into state or Tribal WQS, it should be applied to all waterbodies with an aquatic life designated use and, by extension, all species within those waterbodies that are protected by that use. The assessment of the available data for fish, invertebrates, and amphibians indicates that fish are the most sensitive taxa to the impacts from selenium. As such, the criterion elements were derived from fish toxicity values to be protective of the entire aquatic community, not solely fish species. When all four criterion elements are applied together according to the hierarchy, they protect aquatic life from the chronic effects of exposure to selenium in waters that are both inhabited by fish and not inhabited by fish (i.e., fishless).

Table 1, reproduced from the USEPA 2021, provides an example of how the four-part criterion could be adopted into a state or authorized Tribe's WQS. The EPA recommends adopting this table, including the frequency and duration components, with all accompanying footnotes.

Footnote 1 in Table 1 indicates that the fish tissue concentrations of the criterion are expressed as steady-state. An organism is in steady-state when the rates of chemical uptake and depuration are equal and tissue concentrations remain generally constant over time.[20]

Footnotes 2 and 3 in Table 1 describe the structure of the criterion. They explain the primacy of the whole-body and/or muscle tissue criterion element over the water column criterion elements, and the egg-ovary criterion element over any other criterion element. This means when assessing a waterbody in steady-state conditions against WQC based on the national CWA section 304(a) recommended selenium criterion, egg-ovary data take precedence for assessment decisions followed by whole-body and/or muscle tissue data, and lastly by water column data (see *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*).[21]

Footnote 4 in Table 1 states that the water column criterion elements of the national CWA section 304(a) recommended selenium criterion were derived from the egg-ovary criterion element via mechanistic bioaccumulation modeling.[22]

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[20] USEPA. 2003. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Volume 2: Development of National Bioaccumulation Factors.* EPA-882-R-03-030. U.S. Environmental Protection Agency, Office of Water, Washington, DC. pp. 1-4. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005EZQ.PDF?Dockey=P1005EZQ.PDF.

[21] USEPA. 2024. *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*. EPA-820-R-24-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-faq-cwa303.pdf

[22] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

Footnote 4 also indicates that when both steady-state fish tissue data are absent and selenium inputs to the waterbody (e.g., receiving stream) are increasing, the water column criterion element is the applicable criterion element. For the purposes of the EPA's national CWA section 304(a) recommended selenium criterion, steady-state refers to conditions where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing.[23] New or increased inputs of selenium will likely result in higher selenium concentrations in the water column and food web; however, the increase in selenium concentrations in fish tissue is likely to occur at a slower rate than in the water column. When fish tissue data are not in steady-state and do not represent the total potential bioaccumulative effects of selenium in the aquatic ecosystem, the water column criterion element is more appropriate to protect the entire aquatic ecosystem. Fish tissue data should not be considered for CWA implementation of the criterion (e.g., NPDES permits program or CWA section 303(d) listing of impaired waters) until after selenium concentrations in fish tissue have stopped increasing due to new or increased selenium inputs to a waterbody or watershed of a waterbody. However, any fish tissue data, whether steady-state or not, that are higher than the fish tissue criterion elements indicate a potential impact on the aquatic community. Therefore, in the situation where the water column criterion element is met, but the fish tissue criterion element is exceeded, the EPA recommends developing a site-specific water column criterion element that more appropriately reflects a protective water column concentration for that site (see section 2.2 for more information on the development of site-specific water column criterion elements).

In the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, "new inputs" are defined as new activities resulting in the release of selenium into a lentic or lotic aquatic system. These "new inputs" are both new and increased inputs of selenium and are referring to the release of a substantial amount of additional selenium from either anthropogenic point or nonpoint sources into a waterbody or watershed. New or increased selenium inputs do not refer to seasonal variability of selenium that occurs naturally within a system (e.g., spring run-off events or precipitation-driven pulses) or *de minimis* discharges. Examples of new or increased inputs include releases of additional selenium into the waterbody or watershed from the opening or expansion of petroleum refining facilities, the opening or expansion of mining operations where increased selenium is likely to be discharged, or other anthropogenic activities (such as irrigation of selenium-rich soils) that would substantially increase selenium loadings to a waterbody or watershed of a waterbody over time. Any available appropriate data, including data collected by the state or authorized Tribe, by permittees, and by other entities may be evaluated to determine if there are new or increasing selenium inputs.

[23] This definition of steady-state applies to the national CWA section 304(a) recommended selenium criterion. It does not apply to the discussion of steady-state modeling or conditions in the "Technical Support Document for Water Quality-based Toxics Control" (EPA/505/2-90-001, March 1991).

A watershed is defined as "an area of land that drains water, sediment, and dissolved materials to a common receiving body or outlet" (i.e., waterbody).[24] For large waterbodies, a state or authorized Tribe may want to evaluate new or increased inputs of selenium within a portion of the watershed that is at or up to a hydrologic unit code level of eight digits in size or consider whether a different watershed size is warranted.

For implementation purposes, states and authorized Tribes may assume that the selenium concentration in fish tissue is in steady-state unless a substantial new or increased input of selenium has recently occurred in the waterbody's watershed. In general, the EPA estimates that once a new or increased input of selenium commences the concentration of selenium in fish tissue will reach steady-state after several months in lotic systems and longer time periods (e.g., two to three years) in lentic systems.[25] These time frames can be used as a general estimate of what is considered a recent input of selenium into a watershed. However, these values are general estimates and the time needed for fish tissue to achieve steady-state after a new or increased input of selenium to a waterbody has occurred is site-specific. Potential factors affecting steady-state conditions in an aquatic system can include: (1) the hydrodynamics of the aquatic system (e.g., particularly reservoirs with multiple riverine inputs and controlled releases of water into downstream waterbodies); (2) the location of the selenium input; and (3) the site-specific food web. Therefore, if states and authorized Tribes make the assumption that fish tissue is in steady-state, the EPA recommends documenting the rationale behind those assumptions to demonstrate that any resulting CWA decisions are protective and have a sound scientific rationale.

Alternatively, fish tissue data can be used to evaluate whether fish tissue concentrations are in steady-state. The EPA recommends monitoring fish tissue in the affected waterbody, starting when new or increased inputs of selenium begin (or before if possible) and continuing until fish tissue data demonstrate that selenium concentrations in fish tissue are no longer increasing. With sufficient and appropriate monitoring data, collected over several sequential sampling periods, the state or authorized Tribe can use appropriate statistical methods (one example is the Mann-Kendall statistical approach, but other statistical approaches may be used) to determine when the fish tissue concentrations are no longer increasing.[26]

When implementing state and Tribal criteria based on the national CWA section 304(a) recommended selenium criterion, if selenium inputs are decreasing in a waterbody, the

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[24] USEPA. *Watershed Academy website*, *Introduction to Watershed Ecology*. https://cfpub.epa.gov/watertrain/moduleFrame.cfm?parent_object_id=518

[25] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[26] The following documents may be referenced for guidance on statistical analyses: *Statistical Methods in Water Resources* (https://pubs.usgs.gov/tm/04/a03/tm4a3.pdf); *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (https://www.epa.gov/sites/default/files/2015-06/documents/volume1.pdf); *Statistical Analysis for Monotonic Trends* (https://www.epa.gov/sites/default/files/2016-05/documents/tech_notes_6_dec2013_trend.pdf); *Statistical Methods for Environmental Pollution Monitoring* (https://www.osti.gov/servlets/purl/7037501).

hierarchy of the criterion should apply, and fish tissue criterion elements should take precedence for CWA implementation. Just as the concentration of selenium increases in fish tissue at a slower rate than it does in the water column, selenium concentration also decreases at a slower rate in fish tissue compared to the selenium concentration in the water column. Similarly, selenium can persist in an aquatic ecosystem, and it will take more time to decrease in sediment and biota than in the water column. When water column concentrations of selenium are decreasing, those concentrations may meet the water column criterion element before the fish tissue concentrations meet the fish tissue criterion elements. That is, there is a lag between decreasing water column concentrations of selenium and decreasing selenium concentrations in fish tissue. While the fish tissue selenium concentrations are elevated, effects on aquatic life are still possible. Therefore, the EPA recommends using the fish tissue criterion elements for the CWA implementation of WQC based on the national CWA section 304(a) recommended criterion when selenium inputs to a waterbody or the watershed of a waterbody are decreasing.

In footnote 4, the EPA intends for "dissolved total selenium," to have a defined meaning. Within that term, "total selenium" refers to the combination of all species of selenium that are dissolved into the water column and does not refer to the total recoverable form of selenium. Dissolved total selenium is the selenium that would be measured in a water sample after it has been passed through a 0.45 µm membrane filter.[27] In other words, the criterion is for dissolved selenium in total, not the individual species of selenium (e.g., selenite or selenate, individually).

Footnote 5 of Table 1 describes the equation and input parameters for the intermittent criterion element. The intermittent criterion element is a rearrangement of the 30-day average water column criterion element and was designed to help with permitting where discharges into waterbodies occur only intermittently, rather than continuously. CWA section 303(d) assessments of waterbodies should still be made using the 30-day average water column criterion element, but for waterbodies that have intermittent discharges, this criterion element can be used to determine proper limits for permits. For more information, see section 3.3 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*,[28] questions 11 and 15 of *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs,*[29] and questions 2-2, 3-1, and 3-4 of *Frequently Asked*

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[27] Protho, M.G. USEPA. 1993. Memorandum: Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria. Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2019-03/documents/metals-criteria-interpret-aqlife-memo.pdf.

[28] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[29] USEPA. 2024. *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*. EPA-820-R-24-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits.[30]

Footnote 6 of Table 1 explains the duration component of the fish tissue criterion elements. These criterion elements were developed to protect aquatic populations from negative impacts caused by selenium. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in the fish at a given site.[31] Thus, the recommended duration for the selenium criterion is an instantaneous measurement. States and authorized Tribes have flexibility in how they interpret a discrete fish sample to represent a given species' population at a site at a given time. Generally, fish tissue samples collected to calculate the central tendency of tissue concentrations for a species at a site are collected either in one sampling event, as a composite, or over a short time interval (less than a week) due to the logistical constraints and costs for obtaining samples. The EPA recommends defining a single sampling event as a week or less and does not recommend averaging fish concentrations across sampling events, but instead evaluating each sampling event separately for implementation purposes. The *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion* contains additional information on sampling fish populations.[32]

The frequency component of the fish tissue criterion elements of the national CWA section 304(a) recommended selenium criterion differs from the "once-in-three years on average" frequency of most other national CWA section 304(a) recommended aquatic life water column criteria. Selenium is a bioaccumulative pollutant; therefore, elevated levels of selenium in various ecological compartments (e.g., biota, surficial sediments) require a long period of time to decrease. As a result, the associated aquatic community requires a long time to recover following the reduction or removal of an elevated selenium exposure to a given system. The time frame to recover is variable and will depend on the biogeochemical processes of the system, including residence time and food web dynamics. Thus, the "once-in-three years on average" frequency component is not appropriate for selenium in fish tissue, as this could lead to sustained ecological impacts. Therefore, the recommended frequency for the fish tissue criterion elements of the national CWA section 304(a) recommended selenium criterion is "not

[30] USEPA. 2024. *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination Systems Permits.* EPA-820-R-24-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

[31] See the *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion* for information on selection of sampling location and target species to assure appropriate representation of the waterbody. Mobility and home range of a species should be considered when selecting a target species to assure fish tissue is representative of selenium exposure at the sampling location.

[32] USEPA. 2024. *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion*. EPA-820-R-24-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf.

to be exceeded." For additional information regarding duration and frequency, see sections 2.7.6 and 2.7.7 of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*.[33]

In addition to the four-part criterion, states and authorized Tribes have the option of adopting a method to derive site-specific water column criterion elements, referred to by the EPA as a "performance-based approach." The EPA discusses a performance-based approach for a WQS in the preamble of the rule *EPA Review and Approval of State and Tribal Water Quality Standards*.[34] Here the EPA considers a performance-based approach to be a WQS that is a transparent process rather than a specific outcome. [35] The state or authorized Tribe can adopt a process, such as a criterion derivation methodology, rather than a specific outcome, such as a concentration of a pollutant. If a state or authorized Tribe adopts a process or methodology that is sufficiently detailed and has suitable safeguards that ensure predictable, repeatable outcomes, the EPA can approve that process as a WQS. Approval of this process or methodology would also serve for CWA purposes as the approval of each outcome generated from following that process or method. More information about this approach and what information should be included in the method can be found in section 2.2.1 of this document.

The EPA recommends the adoption of the entire national CWA section 304(a) recommended selenium criterion, including all of the criterion elements, footnotes, frequency, and duration. In addition, states and authorized Tribes have the option to develop, adopt, and submit for the EPA approval, site-specific criteria or criterion elements for individual sites (see sections 2.2 and 3.1 for information on options for developing and adopting site-specific criteria). While the EPA presents some methods for developing site-specific criteria in this document and in the national CWA section 304(a) recommended selenium criterion document, any method that is scientifically defensible and protective of the designated uses may be used to develop site-specific criteria. States and authorized Tribes also have the option of developing a methodology for deriving site-specific water column criterion elements for individual sites, which can be adopted as part of a WQS in addition to the four-part criterion and submitted to the EPA for review. If approved by the EPA, this process can be used to develop site-specific water column criterion elements for state and Tribal waters (see section 2.2.1 of this document).

2.2 Options for Site-specific Water Column Criterion Elements

The EPA recommends that when states and authorized Tribes adopt the national CWA section 304(a) recommended selenium criterion, they adopt all four criterion elements, including the 30-day average water column criterion element. States and authorized Tribes also have the

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[33] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[34] *EPA Review and Approval of State and Tribal Water Quality Standards.* 65 Fed. Reg. 24641 (Apr. 27, 2000). A performance-based approach can be included in state or tribal WQS or as a part of a federal WQS promulgated by the EPA in accordance with the CWA section 303(c) and the EPA's implementing regulations.

[35] *EPA Review and Approval of State and Tribal Water Quality Standards* 65 Fed. Reg. 24641 (Apr. 27, 2000).

additional option of adopting site-specific water column criterion elements, if desired (either through individual adoption or a performance-based approach). The relationship between the concentration of selenium in the tissues of fish and the concentration of selenium in the water column can differ substantially between different aquatic systems. The chemical form of selenium, species of fish, the species and proportion of prey, and a variety of site-specific biogeochemical factors affect selenium bioaccumulation and thus determine the allowable concentration of selenium in ambient water that is protective of aquatic life. Because of the site-specific nature of this relationship, the EPA provides two methodologies for deriving site-specific water column criterion elements:

1) The mechanistic modeling approach; and
2) The empirical bioaccumulation factor (BAF) approach.

The mechanistic modeling approach uses scientific knowledge of the physical and chemical processes underlying bioaccumulation to specifically model bioaccumulation and trophic transfer through the food web to establish a relationship between the concentrations of selenium in the water column and the concentration of selenium in the tissue of aquatic organisms. The EPA used this approach to derive the default water column criterion elements.[36] This approach generally requires the collection of particulate material and water samples and knowledge of the site's food web. The empirical bioaccumulation factor approach, on the other hand, establishes a site-specific relationship between water column selenium concentrations and fish tissue selenium concentrations by measuring both matrices directly and using the ratio between them to determine a site-specific water column criterion element. Both approaches are described in detail in Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*.[37] Both methodologies provide acceptable approaches for translating the recommended fish tissue criterion elements into site-specific water column criterion elements. States and authorized Tribes may use either approach to develop a site-specific water column criterion element. The decision of which approach to use will likely be driven by which data is easiest to collect for a particular site.

In addition, a state or authorized Tribe can develop site-specific criteria using other methodologies if the methodologies are scientifically defensible and the criteria are based on a sound scientific rationale and protective of the applicable designated uses, pursuant to the CWA section 303(c) and the EPA's implementing regulations, including 40 Code of Federal Regulations (CFR) section 131.11. Where a state or authorized Tribe adopts the 304(a) recommended fish tissue criterion elements and the hierarchy of the criterion, and then adopts a site-specific water column criterion element, the hierarchy of the criterion still applies; fish tissue criterion elements take precedence over the site-specific water column criterion

[36] Presser, T.S. and S.N. Luoma. 2010. A methodology for ecosystem-scale modeling of selenium. *Integrated Environmental Assessment and Management* 6:685-710; Presser, T.S. and S.N. Luoma. 2010. *Ecosystem-Scale Selenium Modeling in Support of Fish and Wildlife Criteria Development for the San Francisco Bay-Delta Estuary, California*. U.S. Geological Survey, Reston, Virginia.
[37] Ibid.

elements in the implementation of the criterion, unless selenium inputs are increasing in a waterbody.

One instance where a state or authorized Tribe may want to develop a site-specific water column criterion element for selenium is for fishless waters. Fishless waters are defined as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (see the executive summary of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*).[38] Even though the national CWA section 304(a) recommended selenium criterion tissue elements are for fish tissue, the criterion is intended to protect an aquatic life designated use that would include other aquatic life, including invertebrates. In this instance, a site-specific water column criterion element for a fishless waterbody would need to be protective of the entire aquatic community present in that water and potentially downstream waters (40 CFR 131.10(b)). When deriving a site-specific water column criterion element for fishless waters, it may be appropriate to use the mechanistic model[39] to translate from either a fish tissue criterion element or an invertebrate toxicity threshold. Fish tissue criterion elements should be used to protect the whole aquatic community if fish populations were once supported in that waterbody or for protection of fish downstream, depending on the type of waterbody downstream and the fish present in that downstream waterbody. Invertebrate toxicity thresholds may be appropriate if fish currently and historically have not used the waterbody. Currently (April 2024), the EPA is not aware of sufficient toxicity data on invertebrates for an invertebrate toxicity threshold for selenium. However, as new toxicity data become available, this may be an appropriate taxa to use for deriving site-specific water column criterion elements for fishless waters. The appropriateness of that toxicity data will be evaluated on a case-by-case basis but should generally account for dietary exposure and evaluate survival, growth, and potentially reproductive effects. Potential downstream impacts should also be accounted for by evaluating trophic transfer factors and evaluating what impacts the selenium concentrations of the invertebrates may have on downstream fish species.

There is also one instance where the EPA specifically recommends developing a site-specific water column criterion element. This is when the ambient water column selenium concentrations in a waterbody/waterbody segment are meeting the national CWA section 304(a) recommended water column criterion elements, but the fish tissue concentrations are greater than the national recommended fish tissue criterion elements. In this situation, the elevated fish tissue concentrations may indicate an impact on the aquatic system and indicate

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[38] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[39] See Appendix K of USEPA 2021 for a description of the mechanistic model. USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

that a water column criterion element lower than the national CWA section 304(a) recommended water column criterion element is needed to protect aquatic life.

There are two approaches to adopting and submitting site-specific selenium water column criterion elements to the EPA for review under section 303(c) of the CWA: 1) states and authorized Tribes may adopt and submit individual water column criterion elements each time they are derived; or 2) they may adopt and submit a performance-based approach for deriving site-specific water column criterion elements (see section 2.2.1). States and authorized Tribes should carefully consider the relative tradeoffs of each approach. The adoption and the EPA approval of individual site-specific water column criterion elements could potentially be simpler to apply in assessment, CWA section 303(d) listing and TMDL development, as well as in NPDES permit development since the implementation can immediately proceed with the established criterion element value rather than having the additional step of calculating the criterion element value using the criterion derivation methodology. On the other hand, the performance-based approach could provide states and authorized Tribes the flexibility to adaptively derive site-specific water column criterion elements to account for the most up-to-date data and information for the site without making it necessary for them to submit the site-specific water column criterion element to the EPA for review each time those criterion elements are derived. Under the performance-based approach, stakeholders would have the opportunity to provide comments on the derivation process (e.g., translation mechanisms and associated sampling plans, input parameters, and data analysis methods) during the WQS adoption process under CWA section 303(c) and the EPA's implementing regulations at 40 CFR Part 131. However, comments on the resulting site-specific water column criterion elements derived through the performance-based approach would be submitted as part of individual actions through each of the implementing programs, such as during a public notice for an NPDES permit. The performance-based approach could involve more coordination among the implementation programs to ensure that they are aiming to achieve the same desired condition in the waterbody. For more information on the performance-based approach, see *EPA Review and Approval of State and Tribal Water Quality Standards*.[40]

When developing site-specific water column criterion elements either through the adoption of individual site-specific water column criterion elements or a performance-based approach, states and authorized Tribes must ensure that the site-specific water column criterion elements are protective of the designated use and based on a sound scientific rationale (40 CFR 131.11(a)) and developed using scientifically defensible methods (40 CFR 131.11(b)(1)(iii)). To help ensure that the designated uses are protected, states and authorized Tribes may want to engage with local community stakeholders to evaluate how the waterbody is used and what species are present in the waterbody. If threatened or endangered fish species are present, states and authorized Tribes may need to derive alternative water column elements with a refined protection goal that account for site-specific bioaccumulation characteristics (both when individually adopting a site-specific water column criterion element or using a performance-based approach). The EPA recommends consulting with its regional offices early

[40] *EPA Review and Approval of State and Tribal Water Quality Standards*. 65 Fed. Reg. 24641 (Apr. 27, 2000).

and often during the process. The EPA also recommends states and authorized Tribes periodically review the adopted criterion elements, including during the triennial WQS review process (33 USC 1313(c)(1); 40 CFR 131.20), to ensure that those water column criterion elements continue to protect the designated use over time.

2.2.1 Adoption of Site-specific Water Column Criterion Elements through a Performance-Based Approach

As previously mentioned, states and authorized Tribes can choose to adopt into their WQS and submit to the EPA a set of procedures to translate the fish tissue criterion elements into site-specific water column criterion elements. The EPA considers this a performance-based approach to developing site-specific water column criterion elements. Any translation procedure should be transparent, sufficiently detailed, and include suitable safeguards to ensure repeatable, predictable outcomes. In addition, the resulting water column criterion elements must protect the applicable designated use (including federally listed species, if present). If the EPA approves the procedures of the performance-based approach consistent with CWA section 303(c) and the EPA's implementing regulations at 40 CFR Part 131, each resulting site-specific water column criterion element generated consistent with that approach does not need to be individually adopted into the state or authorized Tribe's WQS or individually approved by the EPA under CWA section 303(c).

Importantly, for public transparency, the EPA strongly recommends states and authorized Tribes maintain a list of the resulting site-specific water column criterion elements and underlying data used for their respective derivations on their publicly accessible website. The EPA also strongly encourages states and authorized Tribes to coordinate closely with the EPA when developing a performance-based approach and when conducting the first few studies to develop site-specific water column criterion elements.

In *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* the EPA provides recommendations for site-specific water column translation procedures in Appendix K.[41] States and authorized Tribes may consider the procedures described in this appendix as a starting point for developing a performance-based approach to derive site-specific water column criterion elements. Appendix K presents the general methods for conducting a site-specific water column translation by either the mechanistic model approach or the BAF approach. In addition, it presents a number of options for determining the values to use as input parameters in both of these methods. Interested states or authorized Tribes should decide which of those options they will use for their performance-based approach or describe in their WQS under which circumstances each option will be used.

[41] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

In addition to the general methods provided in Appendix K, states and authorized Tribes should include additional methods in a performance-based approach that can help to make the procedure more transparent and repeatable. These could include methods for: 1) selecting sites and sampling locations within a site; 2) determining what aquatic species (including threatened and endangered species) are present at the site; 3) selecting target species; 4) selecting tissue type for sampling; 5) sampling plans, including spatial and temporal considerations at a site, whether individual or composite samples will be collected, the total number of samples that will be collected per replicate, and the number of replicates that will be collected; 6) extrapolating trophic transfer factors or conversion factors from existing data; and 7) specifying how data will be processed and analyzed. States and authorized Tribes should consider what level of protection will appropriately protect the designated aquatic life use of the waterbody, which will influence what percentile of the resulting data set should be used for the final criterion value. States and authorized Tribes should also account for spatial and temporal variability and assure that the resulting criterion element is protective of the entire site.

States and authorized Tribes' methods for their performance-based approaches should be clear and definitively state which methods will be followed in particular situations. For example, a state or authorized Tribe's performance-based approach should specifically address fishless waters by either including a method specifically designed to derive a site-specific water column criterion element for a fishless water (potentially translating from an invertebrate threshold when data for that are available) or specifying that the performance-based approach cannot be used to derive a site-specific water column criterion element for a fishless water. An example of a detailed performance-based approach is the *Draft Translation of Selenium Tissue Criterion Elements to Site-specific Water Column Criterion Elements for California Version 1, August 8, 2018*.[42]

If a state or authorized Tribe chooses to adopt a performance-based approach as part of a WQS, one method for incorporating the performance-based approach could be to adopt the national CWA section 304(a) recommended selenium criterion table (Table 1) into its WQS with the addition of a footnote that references the document that describes the state or authorized Tribe's translation methodology, sampling plans, and data analysis. By adopting such a footnote into the WQS, the state or authorized Tribe will have incorporated by reference their translation procedure that is scientifically defensible, produces repeatable, predictable outcomes and results in criterion elements that protect the applicable designated use. Any revisions to this document that has been incorporated by reference would be a revision to a WQS that would be subject to the EPA review under CWA section 303(c) and the EPA's implementing regulations at 40 CFR Part 131.

[42] USEPA. 2018. *Draft Translation of Selenium Tissue Criterion Elements to Site-specific Water Column Criterion Elements for California Version 1, August 8, 2018.* U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2018-12/documents/california_selenium_2040-af79_pba_20181121_508c_.pdf.

2.2.2 Adoption of Site-specific Water Column Criterion Elements Individually

States and authorized Tribes can choose to adopt and submit to the EPA for review site-specific water column criterion elements each time they are derived and adopted. These site-specific water column criterion elements can be derived using one of the methodologies described in Appendix K or another scientifically defensible approach. These site-specific water column criterion elements must protect the designated use and must be reviewed and approved by the EPA under CWA section 303(c) and the EPA's implementing regulations at 40 CFR Part 131 before they are applicable for CWA purposes, such as NPDES permitting, waterbody assessment and listing, and TMDL development.

2.3 Relationship of the EPA's National CWA section 304(a) Recommended Selenium Criterion to the Great Lakes Initiative

The EPA's national CWA section 304(a) recommended selenium criterion does not supersede the requirements applicable to the Great Lakes at 40 CFR Part 132. Those requirements, known as the *Great Lakes Water Quality Guidance* (also known as the *Great Lakes Initiative* or *GLI*), apply to all streams, rivers, lakes, and other bodies of water within the U.S. portion of the Great Lakes drainage basin. For those waters, states and authorized Tribes must adopt WQS, including criteria, that are *consistent with (as protective as)* the WQS regulations the EPA promulgated on March 23, 1995 (see 60 FR 15366, March 23, 1995, and 40 CFR 132.1(b) and 132.4).

Under the Great Lakes system-specific regulations, if a state or authorized Tribe adopts a revised criterion for selenium, the EPA in its review must determine if the new criterion is as protective as the selenium chronic criterion (5 µg/L) promulgated in 40 CFR 132.6, Table 2, and whether all binding implementation procedures are as protective as the GLI procedures (see 40 CFR 132.5(g)). The EPA makes its approval/disapproval decisions about new and revised WQS on a case-by-case basis depending upon the supporting information. For waters within the Great Lakes Basin, states and authorized Tribes should evaluate whether the national CWA section 304(a) recommended selenium criterion meets the requirements at 40 CFR Part 132. The EPA would evaluate any proposed revised selenium criterion submitted as part of a state or authorized Tribe's new or revised WQS on a case-by-case basis, in accordance with the CWA and the EPA's implementing regulations at 40 CFR Parts 131 and 132.

3.0 Options for Implementing the EPA's Selenium Criterion

States and authorized Tribes have some flexibility when adopting the national CWA section 304(a) recommended selenium criterion. Options include, but are not limited to, deriving site-specific fish tissue criterion elements, deriving site-specific water column criterion elements (see section 2.2 of this document), adopting WQS variances, revising designated uses, and using

compliance schedules. This section describes how each option could fit within a state or authorized Tribe's WQS adoption and implementation processes.

3.1 Site-specific Fish Tissue Criterion Elements

3.1.1 Site-specific Fish Tissue Criterion Elements Using the Species Recalculation Procedure[43]

The EPA's WQS regulation at 40 CFR 131.11(b)(1)(ii) provides states and authorized Tribes with the opportunity to adopt criteria that are "…modified to reflect site-specific conditions." As with any criterion, a site-specific criterion must protect the designated use, must be based on a sound scientific rationale, and is subject to the EPA review and approval or disapproval (40 CFR 131.11(a)).

One process that can be used to modify a criterion to reflect site-specific conditions is the recalculation procedure. The recalculation procedure is a process used to modify the taxonomic composition of the toxicity data set used for the species sensitivity distribution (SSD)[44] upon which a site-specific criterion is based, in order to better match the species assemblage that is expected to occur at the site. The recalculation procedure creates a site-specific toxicity data set (and corresponding SSD) that is appropriate for deriving a site-specific aquatic life criterion by correcting, adding, and/or deleting test results from the national toxicity data set for the pollutant of concern based on the resident species of the site. For site-specific recalculations at sites with limited diversity, "the underlying concept of having tests for a diversity of species is more fundamental than having tests for particular taxonomic groups that may be irrelevant to the site."[45] Therefore, recalculations can be performed, even if all of the minimum data requirements are not met. This procedure is intended to provide flexibility to states and authorized Tribes to derive site-specific criteria that best reflect the expected resident species at a site. The species recalculation procedure may result in site-specific criteria that differ from national CWA section 304(a) criteria recommendations (i.e., concentrations that are higher or lower than national recommendations) when there are demonstrated differences in sensitivity

[43] USEPA. 2013. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf.

[44] For additional description of the derivation of the fish tissue criterion elements and the distribution of species sensitivities, see section 3.1.3 of USEPA 2021. USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[45] USEPA. 2013. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf.

between the expected resident aquatic species and those that were used to derive the national criteria recommendations.

Critical species should also be taken into consideration when using the recalculation procedure. A critical species is a resident species that (a) is commercially or recreationally important at the site; or (b) is listed as threatened or endangered under section 4 of the Endangered Species Act (states and authorized Tribes may also want to consider species that are candidates for protection under the Endangered Species Act as critical species); or (c) is a species for which there is firm evidence that its loss would yield an unacceptable impact on the site's commercially or recreationally important species, endangered species, abundances of a variety of other species, or structure or function.[46] The deletion process of the recalculation procedure should not be undertaken unless toxicity data are available for at least one species in each *class* of aquatic plants or animals that contains a *critical species*. For example, if the site has a salmonid that fits the designation of a critical species, the deletion process should not be undertaken unless toxicity data for a species in class teleostei[47] are available (possibly via new testing). It is important to note that species not present at the site, but included in the national criterion database, may be surrogates for other species that are present at the site, but not included in the national criterion database.

The EPA developed the national CWA section 304(a) recommended selenium criterion by first deriving an egg-ovary criterion element from egg-ovary toxicity data then deriving all other criterion elements from that egg-ovary criterion element. A state or authorized Tribe developing site-specific fish tissue criterion elements using the recalculation procedure could similarly start with deriving the egg-ovary criterion element and then deriving other fish-tissue criterion elements. States and authorized Tribes should also consider corresponding changes to the applicable water column criterion elements. See section 2.2 of this document for more information. Note that a state or authorized Tribe that is considering adopting the EPA's national CWA section 304(a) selenium criterion recommendations for the fish tissue criterion elements as recommended but is interested in developing site-specific water column values can proceed as described in section 2.2 of this document.

As with any criteria, states and authorized Tribes using the recalculation procedure should ensure that their site-specific criteria "…provide for the attainment and maintenance of the water quality standards of downstream waters" (40 CFR 131.10(b)). This requirement can be met in a number of ways, including using a combination of water quality standards, such as criteria (numeric criteria and/or narrative criteria) and general policies. Additional information

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[46] USEPA. 2013. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC, www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf.

[47] Teleostei is currently listed as a class by the Integrated Taxonomic Information System (itis.gov). Osteichthyes was previously classified as a superclass that contained the classes Sarcopterygii and Actinopterygii, but those two taxa have now been elevated to superclasses and Teleostei has been elevated to class (https://www.itis.gov/servlet/SingleRpt/SingleRpt).

about this requirement can be found in *Protection of Downstream Waters in Water Quality Standards: Frequently Asked Questions*.[48] In addition, states and authorized Tribes should consider how they will demonstrate that a species that is contained in the national data set but is being removed from that data set in order to derive the new site-specific criterion is not present at the site (or a surrogate for another species present at the site) before using the recalculation approach. For additional information on the procedure, see the EPA's *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*.[49] States and authorized Tribes interested in developing site-specific fish tissue criterion elements based on the revised deletion process should engage their EPA Regional office early in the process to ensure the development of sound scientific analyses. The EPA cautions that the deletion process for site-specific criterion development is not appropriate for a performance-based approach because different survey techniques are needed at different sites to determine which species are present.

When developing site-specific criterion elements, consideration should be given to what area is defined as the site. In the general context of site-specific criteria, a "site" may be a state, region, watershed, waterbody, or segment of a waterbody. The site-specific criterion must provide adequate protection for the entire site, however the site is defined. For the recalculation procedure, all species that occur at the site need to be considered when deciding what species, if any, are to be deleted (or added) from (to) the data set. Unique populations or less sensitive uses within sites may justify a designation as a distinct site. If the site contains endangered or threatened species and/or their critical habitat, and the EPA's approval of the state or authorized Tribe's proposed site-specific criterion may affect these species or habitat, the EPA must engage in interagency consultation under the Endangered Species Act section 7(a)(2) with either or both the United States Fish and Wildlife Service and National Marine Fisheries Service, depending on the species, to ensure that the EPA's approval is not likely to jeopardize the continued existence of any threatened or endangered species or result in the destruction or adverse modification of critical habitat. During consultation, the EPA would consider the potential effect of its approval on the species and on the physical and biological features of the critical habitat as well as how the EPA's approval of the site-specific criterion may affect water quality required for the species within the critical habitat.

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[48] USEPA. 2014. *Protection of Downstream Waters in Water Quality Standards: Frequently Asked Questions*. EPA 820-F-14-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. www.epa.gov/sites/production/files/2018-10/documents/protection-downstream-wqs-faqs.pdf.

[49] USEPA. 2013. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf.

For additional information on site-specific WQC, see chapter 3 of the EPA's *Water Quality Standards Handbook*.[50]

3.1.2 Site-specific Criteria for Sites with Naturally Elevated Selenium Levels

Natural conditions are generally associated with the condition of ecological integrity, defined as the structure, composition, function, or diversity of a habitat in the absence of human disturbance or alteration.[51] For some areas in the U.S., selenium may be elevated in soils and waterbodies under natural conditions. States and authorized Tribes have two options when addressing areas that have naturally elevated selenium concentrations: (1) they may develop site-specific criteria for waterbodies in these locations (e.g., where the current aquatic life designated use is appropriate); or (2) they may refine or subcategorize the aquatic life designated use for waterbodies in these areas and set new criteria to be protective of the modified use (See section 3.3 of this document for more information on revising designated uses). These are two separate pathways for accomplishing the goal of setting appropriate criteria for these waterbodies.

To set a site-specific criterion using a natural conditions justification, states and authorized Tribes should demonstrate that the elevated levels of selenium are due to natural conditions and that no anthropogenic activities (e.g., agriculture, mining, irrigation, coal, or oil combustion) are responsible for elevating selenium concentrations in the waterbody. In many areas where soils are elevated in selenium, anthropogenic activities either release selenium or expose soils or rock to weathering, which results in the release of additional selenium into the water column. When an anthropogenic activity leads to a greater release of selenium than natural weathering, the state or authorized Tribe should not develop a site-specific criterion using the natural conditions justification. However, if the anthropogenic impacts can be separated from the natural impacts in these situations where both occur, then the state or authorized Tribe could develop a site-specific criterion for the portion of the elevated selenium that is due solely to those natural causes.

When developing a site-specific criterion, the state or authorized Tribe should identify the site boundaries and temporal dynamics to which a natural background (concentration of a parameter due only to non-anthropogenic sources) criterion applies (i.e., it is important that such criteria are developed to maintain and protect the spatial and temporal variation in

[50] USEPA. 2017. Chapter 3: Water Quality Criteria in *Water Quality Standards Handbook*. EPA 823-B-17-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2014-10/documents/handbook-chapter3.pdf.

[51] Landres, P.B., P. Morgan, and F.J. Swanson. 1999. Overview of the use of natural variability concepts in managing ecological systems. *Ecological Applications* 9(4): pp. 1179–1188; Davies, S.P. and S.K. Jackson. 2006. The biological condition gradient: A descriptive model for interpreting change in aquatic ecosystems. *Ecological Applications* 16(4): pp. 1251–1266.

selenium concentrations under natural conditions[52]). If concentrations vary seasonally, adoption of seasonal criteria may be appropriate. Where concentrations vary spatially, additional waterbody segmentation may be appropriate. In cases where selenium is elevated due to both natural conditions and anthropogenic activities, methods such as modeling (e.g., historical or hydrologic modeling), an examination of past paleolimnological studies, comparison to reference sites with no anthropogenic impacts, or other scientifically defensible techniques may be considered as an option for defining natural conditions.[53] Using these or other tools, a state or authorized Tribe may be able to define what proportion of elevated selenium is due to natural versus anthropogenic impacts. A site-specific criterion could then be derived for the portion of the concentration that is elevated due to natural conditions.

When deriving site-specific fish tissue criterion elements, states and authorized Tribes should consider corresponding changes to the applicable water column criterion elements. If selenium at the site is entirely from natural sources, then the water column criterion element could be set at ambient water column concentrations. However, if some of the selenium is from anthropogenic sources, then the water column concentration should be derived using modeling methods. See section 2.2 for more information on options for adopting site-specific water column criterion elements.

As articulated in the 1997 memorandum *Establishing Site-Specific Aquatic Life Criteria Equal to Natural Background,*[54] the EPA recommends that interested states and authorized Tribes establish site-specific numeric aquatic life criteria by setting the criteria values equal to natural background, where natural background is defined as due *only* to non-anthropogenic sources (i.e., non-human-induced sources). The EPA cautions against using the terminology "ambient" interchangeably with "natural." Ambient conditions refer to current conditions at a site and can include both impacts from anthropogenic and natural sources.

3.2 Water Quality Standards Variances

A state or authorized Tribe could consider a WQS variance if it determines that it is not feasible at present to attain a designated use and an associated criterion, such as a newly adopted

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[52]See the *Technical Support for Fish Tissue Monitoring for Implementing of the EPA's 2016 Selenium Criterion* for information on sampling fish and spatial and temporal variability. https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf.

[53] Swetnam, T.W., C.D. Allen, and J.L. Betancourt. 1999. Applied historical ecology: Using the past to manage for the future. *Ecological Applications* 9(4): pp. 1189–1206; Hughes, R.M., S.G. Paulsen, and J.L. Stoddard. 2000. EMAP-Surface waters: A multiassemblage, probability survey of ecological integrity in the U.S.A. *Hydrobiologia* 422/423: pp. 429–443; Kilgour, B.W., and L.W. Stanfield. 2006. Hindcasting reference conditions in streams. *American Fisheries Society Symposium* 48: pp. 623–639; Herlihy, A.T., S.G. Paulsen, J. Van Sickle, J.L. Stoddard, C.P. Hawkins, and L.L. Yuan. 2008. Striving for consistency in a national assessment: The challenges of applying a reference-condition approach at a continental scale. *Journal of the North American Benthological Society* 27(4): pp. 860–877.

[54] Davies, T. USEPA. 1997. Memorandum: Establishing Site Specific Aquatic Life Criteria Equal to Natural Background. Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2014-08/documents/naturalbackground-memo.pdf.

selenium criterion, but it may be attainable in the future. A WQS variance may also be considered if there is uncertainty about the feasibility of meeting the designated use and criterion, but progress can be made by implementing known controls and tracking environmental improvements. The WQS regulations at 40 CFR 131.3(o) define a *WQS variance* as "a time-limited designated use and criterion for a specific pollutant(s) or water quality parameter(s) that reflect the highest attainable condition (HAC) during the term of the WQS variance." The WQS variance regulation at 40 CFR 131.14 ensures that incremental progress is made toward attainment of the applicable designated use and associated criterion, while not allowing any lowering of the currently attained ambient water quality. This is accomplished by establishing an HAC, which serves as the basis for deriving less-stringent NPDES permit limits and requirements throughout the term of the WQS variance.[55] Although less stringent than the adopted criterion, the HAC still provides a target for incrementally improving water quality until the applicable designated use and associated criterion become attainable. It is important to note that these permit limits and requirements are applicable only to the discharger(s), pollutant(s)/parameter(s), and waterbody or waterbody segment(s) identified in the WQS variance. All other applicable standards not specifically addressed by the EPA-approved WQS variance would continue to apply for CWA purposes.

A state or authorized Tribe can choose to adopt a WQS variance that is applicable to a single discharger, multiple dischargers, or an entire waterbody/waterbody segment. Because the national CWA section 304(a) recommended selenium criterion is intended to protect aquatic life (CWA section 101(a)(2)), in its WQS variance submission the state or authorized Tribe must provide supporting documentation demonstrating that attaining the designated use and criterion is not feasible throughout the term of the WQS variance due to one of the factors listed at 40 CFR 131.14(b)(2)(i)(A). Such a demonstration, when combined with the other regulatory requirements of 40 CFR 131.14, provides the framework for WQS variances as a water quality improvement tool. The regulations at 40 CFR Part 132 apply to states and authorized Tribes that regulate waters in or contributing to waters of the Great Lakes System. If 40 CFR 131.14 and 40 CFR Part 132 overlap, the more stringent regulation applies. WQS variances are considered new or revised WQS and, therefore, must be reviewed and approved by the EPA in order to be in effect for CWA purposes.

To help states and authorized Tribes in the process of adopting WQS variances, the EPA has developed various resources, including a WQS variances website,[56] the *WQS Variance Building Tool*,[57] *Checklist for Evaluating State Submission of Discharger-Specific Water Quality Standards*

[55] WQS variances with a term greater than five years must be reevaluated at least every five years to determine whether the HAC is more stringent than originally adopted. See 40 CFR 131.14 (b)(1)(v).
[56] USEPA. *Water Quality Standards Variance website.* https://www.epa.gov/wqs-tech/water-quality-standards-variances.
[57] USEPA. *Water Quality Standards Variance Building Tool*. https://www.epa.gov/wqs-tech/water-quality-standards-variance-building-tool.

Variances,[58] and several other resources available on the "Resources" tab of the *WQS Variance Building Tool*. States and authorized Tribes are encouraged to coordinate with the EPA throughout the WQS variance adoption process to develop their WQS variance submissions.

3.3 Revision to Designated Uses

Prior to adopting a revised selenium criterion, a state or authorized Tribe may want to evaluate potential revisions to the relevant designated uses for certain waterbodies as discussed below. The WQS regulation at 40 CFR 131.10(g) provides that states or authorized Tribes may remove a CWA section 101(a)(2) designated use that is *not* an existing use, as defined in 40 CFR 131.3(e), or establish subcategories of such a use that requires less stringent criteria if the state or authorized Tribe can demonstrate that attaining the designated use is not feasible because of one or more of the six factors listed in 40 CFR 131.10(g). Those factors are:

1) Naturally occurring pollutant concentrations prevent the attainment of the use; or

2) Natural, ephemeral, intermittent or low flow conditions or water levels prevent the attainment of the use, unless these conditions may be compensated for by the discharge of sufficient volume of effluent discharges without violating state water conservation requirements to enable uses to be met; or

3) Human caused conditions or sources of pollution prevent the attainment of the use and cannot be remedied or would cause more environmental damage to correct than to leave in place; or

4) Dams, diversions or other types of hydrologic modifications preclude the attainment of the use, and it is not feasible to restore the waterbody to its original condition or to operate such modification in a way that would result in the attainment of the use; or

5) Physical conditions related to the natural features of the waterbody, such as the lack of a proper substrate, cover, flow, depth, pools, riffles, and the like, unrelated to water quality, preclude attainment of aquatic life protection uses; or

6) Controls more stringent than those required by sections 301(b) and 306 of the Act would result in substantial and widespread economic and social impact.

Where required under the EPA's WQS regulations, the state or authorized Tribe must make such a demonstration through a *use attainability analysis* (UAA), which is defined under 40 CFR 131.3(g) as "…a structured scientific assessment of the factors affecting the attainment of the use which may include physical, chemical, biological, and economic factors as described in 40 CFR 131.10(g)." A state or authorized Tribe must conduct a UAA whenever (1) the state or

[58] USEPA. 2016. *Checklist for Evaluating State Submission of Discharger-Specific Water Quality Standards Variances*. U.S. Environmental Protection Agency, Office of Water. Washington, DC. https://www.epa.gov/sites/production/files/2016-03/documents/checklist-evaluating-discharger-specific.pdf.

authorized Tribe designates for the first time, or has previously designated for a waterbody, uses that do not include the uses specified in section 101(a)(2) of the CWA; or (2) the state or authorized Tribe wishes to remove a designated use that is specified in section 101(a)(2) of the Act, to remove a subcategory of such a use, or to designate a subcategory of such a use that requires a criterion less stringent than previously applicable (40 CFR 131.10(j)). If a state or authorized Tribe adopts a new or revised WQS based on a required use attainability analysis, the state or authorized Tribe shall also adopt the highest attainable use, as defined in 40 CFR 131.3(m).[59] The WQS regulations also do not allow states and authorized Tribes to remove any uses that are existing uses. In addition, uses cannot be removed if they can be attained by implementing effluent limits required under sections 301(b) and 306 of the Act and by implementing cost-effective and reasonable best management practices for nonpoint source control (40 CFR 131.10(h)).

For selenium, there are at least two potential circumstances where a use change may be appropriate for a waterbody. The first is fishless waters. States or authorized Tribes may want to recognize the difference in aquatic communities in these waters by creating a subcategory of the aquatic life designated use. When doing so, the state or authorized Tribe should be sure that protections for other forms of aquatic life, such as macroinvertebrates, are protected by the revised designated use and associated criterion. If aquatic-dependent wildlife use the waterbody, then the state or authorized Tribe may also want to set a selenium criterion for the protection of these species as well. The second is waterbodies located in areas where the selenium is naturally elevated in the underlying geology and, as a result, are particularly vulnerable to elevated selenium concentrations in the water. Ambient selenium concentrations in these waterbodies can be exacerbated by anthropogenic activities. If the conditions in this circumstance are precluding attainment of the designated use and cannot be remedied, a limited or modified aquatic life use may be more appropriate for these waterbodies. The EPA's regulations at 40 CFR 131.10(g) provide factors related to natural and anthropogenic conditions for states and authorized Tribes to employ in a UAA to justify revising the aquatic life use (see 40 CFR 131.10(j) for when a UAA is required). If the state or authorized Tribe can demonstrate the full aquatic life use is not feasible for a waterbody using at least one of the factors at 40 CFR 131.10(g), then the designated use the state or authorized Tribe must adopt in place of the full aquatic life use must be the highest attainable use for the waterbody, as required by 40 CFR 131.10(g).

[59] 40 CFR 131.3(m) defines "highest attainable use" as the "…modified aquatic life, wildlife, or recreation use that is both closest to the uses specified in section 101(a)(2) of the Act and attainable, based on the evaluation of the factor(s) in §131.10(g) that preclude(s) attainment of the use and any other information or analyses that were used to evaluate attainability."

For additional information on use changes and UAAs, see the EPA's *Water Quality Standards Handbook.*[60] Additional information also is available at http://water.epa.gov/scitech/swguidance/standards/uses/uaa/.

3.4 Compliance Schedules

If a state or authorized Tribe's applicable WQS[61] or implementing regulations include a provision authorizing the use of permit compliance schedules, a compliance schedule that is consistent with 40 CFR 122.47 can be included in an NPDES permit with WQBELs for selenium. The NPDES permit regulations at 40 CFR 122.47 allow compliance schedules if (1) the discharger cannot immediately meet the new WQBEL, and requires time to install treatment technology or implement other controls necessary to come into compliance with a new WQBEL; (2) the permitting authority (the state or the EPA) determines that a compliance schedule is *appropriate* in light of all the circumstances; and (3) the discharger can meet its final WQBEL at a date determined to be as-*soon-as-possible*. See section 502(17) of the CWA for a definition of *schedules of compliance* and 40 CFR 122.47 for more information about the schedules. For additional information on compliance schedules, see section 5 in *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*.[62]

Compliance schedules in NPDES permits can allow additional time to achieve compliance with WQBELs based on WQS adopted after July 1, 1977, if the state or authorized Tribe (i.e., a Tribe with TAS for WQS) has indicated clearly in its WQS or implementing regulations that it intends to allow the use of permit compliance schedules. As defined in the NPDES regulations, "a schedule of compliance" is "a schedule of remedial measures included in a 'permit', including an enforceable sequence of interim requirements (for example, actions, operations, or milestone events) leading to compliance with the CWA and regulations" (40 CFR 122.2). If the schedule is longer than one year, it must include annual interim requirements and dates for their achievement (40 CFR 122.47(a)(3)). Additionally, the permit must include a final WQBEL and a date for its achievement. The decision to include a compliance schedule as well as the dates and interim requirements must be supported by the administrative record. The permit fact sheet should include a justification for the compliance schedule being determined to be *appropriate* and why the chosen time frame was deemed to be *as soon as possible*. For

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[60] USEPA. 2012. Chapter 2: Designation of Uses in *Water Quality Standards Handbook*. EPA 823-B-12-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2014-10/documents/handbook-chapter2.pdf.

[61] 40 CFR 131.15 states that: "If a State intends to authorize the use of schedules of compliance for water quality-based effluent limits in NPDES permits, the State must adopt a permit compliance schedule authorizing provision. Such authorizing provision is a water quality standard subject to EPA review and approval under section 303 of the Act and must be consistent with sections 502(17) and 301(b)(1)(C) of the Act."

[62] USEPA. 2024. *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits.* EPA-820-R-24-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-faq-cwa402.pdf.

additional information on compliance schedules, see section 9.1.3 of the EPA's *NPDES Permit Writers' Manual*[63] and the May 10, 2007, memorandum from the EPA's Office of Wastewater Management on compliance schedules for water quality-based effluent limitations in NPDES permits.[64]

[63] USEPA. 2010. *NPDES Permit Writers' Manual*. EPA 833-K-10-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2015-09/documents/pwm_2010.pdf.

[64] Hanlon, J.A. USEPA. 2007. Memorandum: Compliance Schedules for Water Quality-Based Effluent Limitations in NPDES Permits. Office of Water, Washington DC. https://www3.epa.gov/npdes/pubs/memo_complianceschedules_may07.pdf.

Appendix K

United States Environmental Protection Agency

Office of Water
EPA-820-R-24-004
April 2024

Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs

Frequently Asked Questions: Implementing EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs

While this document cites statutes and regulations that contain requirements applicable to water quality standards, it does not impose legally binding requirements on the EPA, states, authorized Tribes, other regulatory authorities, or the regulated community, and may not apply to a particular situation based upon the circumstances. The EPA, state, Tribal, and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those provided in this technical support document as appropriate and consistent with statutory and regulatory requirements. The EPA may update this document as new information becomes available. In addition to this document, the EPA has related documents that provide considerations and recommendations on implementing criteria based on the national CWA section 304(a) recommended selenium criterion for freshwater, which are available at the EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

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List of Acronyms and Abbreviations

µg/L	micrograms per liter
CFR	Code of Federal Regulations
CWA	Clean Water Act
FAQ	Frequently Asked Question
mg/kg	milligrams per kilogram
NPDES	National Pollutant Discharge Elimination System
TMDL	Total Maximum Daily Load
USEPA	U.S. Environmental Protection Agency
WQBEL	Water Quality-based Effluent Limit
WQC	Water Quality Criterion
WQS	Water Quality Standard

Definitions[1]

Bioaccumulation
The uptake and retention of a chemical by an aquatic organism from all surrounding media (e.g., water, food, sediment).[2]

Dissolved total selenium
All species of selenium that are dissolved into the water column and that would be measured in a water sample after it has been passed through a 0.45 µm membrane filter.[3]

Performance-based approach
A water quality standard that is a transparent process, rather than a specific outcome (such as the concentration portion of a criterion for a pollutant). This process or methodology is sufficiently detailed and has suitable safeguards that ensure predictable, repeatable outcomes. Approval of this process or methodology by the EPA would also serve for CWA purposes as the approval under CWA section 303(c)[4] of each outcome generated from following that process or method.[5]

Steady-state
An organism is in steady-state when the rates of chemical uptake and depuration are equal and tissue concentrations remain constant over time.[6] For the purposes of the national CWA section 304(a) recommended selenium criterion, steady-state refers to conditions where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing.

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[1] This glossary is meant to provide plain language definitions for key terms used in this document. Individuals should consult the Clean Water Act and the EPA's implementing regulations to identify whether there are legal definitions of these terms.

[2] USEPA. 2003. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Volume 2: Development of National Bioaccumulation Factors.* EPA-882-R-03-030. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005EZQ.PDF?Dockey=P1005EZQ.PDF

[3] Protho, M.G. USEPA. 1993. Memorandum: Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria. Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2019-03/documents/metals-criteria-interpret-aqlife-memo.pdf

[4] Approval by the EPA includes WQS promulgated by the EPA.

[5] *EPA Review and Approval of State and Tribal Water Quality Standards* 65 Fed. Reg. 24641 (Apr. 27, 2000).

[6] USEPA. 2003. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Volume 2: Development of National Bioaccumulation Factors.* EPA-882-R-03-030. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005EZQ.PDF?Dockey=P1005EZQ.PDF

Water quality criterion element
A magnitude, frequency, and duration for a particular media type (e.g., fish tissue, water column), which are individual components of the overall water quality criterion. The water quality criterion elements for selenium are related through a hierarchy; with fish tissue criterion elements having primacy over water column criterion elements, and the egg-ovary fish tissue criterion element having primacy over all other criterion elements.

1.0 Introduction

1.1 The EPA's National CWA Section 304(a) Recommended Chronic Aquatic Life Selenium Criterion in Freshwater

In 2016, the U.S. Environmental Protection Agency updated its national Clean Water Act (CWA) section 304(a) recommended chronic aquatic life criterion for selenium in freshwater systems to reflect the latest scientific information and, in 2021, issued an erratum, *2021 Revision to: Final Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* US EPA, Office of Water, EPA 822-R-21-006, August 2021.[7] The EPA's national CWA section 304(a) recommended selenium criterion still reflects the latest science and the EPA is not aware of any updated scientific information that would change this recommendation. The latest scientific information indicates that selenium toxicity to aquatic life is driven by dietary exposures and that the reproductive life-stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium. The recommended criterion has four criterion elements: (1) a fish egg-ovary criterion element; (2) a fish whole-body and/or muscle criterion element; (3) a water column criterion element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent criterion element (to account for potential chronic effects from short-term exposures to high concentrations in lentic and lotic aquatic systems) (see Table 1). Under the EPA's 2016 national CWA section 304(a) recommended selenium criterion, the fish tissue criterion elements have primacy over water column elements, except where there are no fish, where fish tissue data are not adequate, or for waterbodies with new or increased discharges where selenium concentrations in fish tissue might not have stabilized and reached steady-state. The EPA also recommends that the egg-ovary tissue criterion element has primacy over whole-body and muscle tissue criterion elements.

Toxicity data indicate that the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse reproductive effects in aquatic organisms. Toxicity to developing embryos and larvae is directly linked to egg selenium concentration.[8] The EPA derived the whole-body, muscle tissue, and water column elements from the egg-ovary element so that states and authorized Tribes could more readily implement their water quality criteria (WQC) based on the EPA's national CWA section 304(a) recommended selenium criterion. The assessment of the available data on chronic selenium

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[7] In 2021, the EPA identified that the following text was missing from the second sentence in footnote 4 in the selenium criterion table: "When selenium inputs are increasing" and issued an erratum. The EPA corrected footnote 4 to state: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data."

[8] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

exposure for fish, invertebrates, and amphibians indicates that a criterion element derived from fish is expected to be protective of the aquatic community in a waterbody, since other taxa appear to be less sensitive to selenium than fish. The EPA did not develop an acute criterion for selenium when it updated the chronic criterion. Although selenium may cause acute toxicity at high concentrations, the most deleterious effects on aquatic organisms are due to selenium's bioaccumulative properties.

In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from chronic exposure to chemicals via the diet (through the food web pathway). Such studies also do not account for the accumulation kinetics of many bioaccumulative compounds, such as selenium, and may underestimate effects from long-term accumulation in some types of aquatic systems. Therefore, since acute studies do not address the primary exposure pathway for organisms to selenium and because chronic toxicity occurs at lower concentrations, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion. As described in the EPA's *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (hereafter referred to as *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*), the EPA also included an intermittent exposure criterion element to provide protection from the most significant chronic effects of selenium toxicity, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high concentration exposure events.[9]

The EPA recommends, as stated in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, that states and authorized Tribes[10] adopt into their water quality standards (WQS) a selenium criterion that includes all four criterion elements.[11] For more information see the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–*

[9] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[10] Throughout this document and in the CWA, the term "states" means the fifty states, the District of Columbia, the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer a CWA WQS program.

[11] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Freshwater 2016, which can be found at https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg-ovary[2]**	**Fish Whole-body or Muscle[3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dry weight	8.5 mg/kg dry weight whole-body or 11.3 mg/kg dry weight muscle (skinless, boneless fillet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where $WQC_{30\text{-}day}$ is the water column monthly element for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

1.2 Selenium Technical Support Materials

The EPA has prepared a four-volume set of documents to provide recommendations to states, authorized Tribes, and other agencies for implementing their WQC based on the national CWA section 304(a) recommended selenium criterion for aquatic life.[12] These four documents

[12] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

constitute the Technical Support Materials for the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*.[13] Each document of the set focuses on a specific aspect of implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. Together, these four EPA documents provide information to assist states and authorized Tribes with adopting WQC based on the EPA's national CWA section 304(a) recommended selenium criterion and implementing it in various CWA programs.

1) *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*: Provides recommendations for the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools.
2) *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*: Provides an overview of how to establish or enhance existing fish tissue monitoring programs to facilitate implementation of fish tissue criterion elements based on the national CWA section 304(a) recommended selenium criterion.
3) *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*: Provides information to help National Pollutant Discharge Elimination System (NPDES) permit writers understand what permitting guidance (i.e., state or Tribal implementation procedures) may be appropriate to implement state and authorized Tribal WQS based on the EPA's national CWA section 304(a) recommended selenium criterion. This set of FAQs also provides recommendations on how to establish water quality-based effluent limits (WQBELs) in NPDES permits.
4) *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*: Provides information on how to complete assessments, list impaired waters, and develop total maximum daily loads (TMDLs) to implement the EPA-approved[14] WQS that are based on the EPA's national CWA section 304(a) recommended selenium criterion.

[13] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[14] EPA-approved includes both WQS adopted by states, territories, or authorized Tribes and approved by the EPA and WQS promulgated by the EPA.

Q1: How should states and authorized Tribes implement criteria based on the national CWA section 304(a) recommended selenium criterion in their assessment and CWA section 303(d) listing programs?

A1: The national CWA section 304(a) recommended selenium criterion is structured to enable assessment of waterbodies using existing and readily available water quality-related data and information for any element of the criterion. Table 2 provides an assessment matrix indicating whether available data and information support a CWA section 303(d) listing determination for WQS approved by the EPA that are based on the national CWA 304(a) recommended selenium criterion. Nine aquatic life use assessment scenarios are presented in Table 2 and further explained below.

The assessment matrix does not address waterbody conditions when selenium inputs are new or increasing and steady-state fish tissue data are absent. Information on how to perform assessments for this scenario are provided in Q10. Information on performing assessments for fishless waters is provided in Q6.

Assessment Scenario Descriptions

Assessment scenarios 1 through 6 describe water quality situations for which data and information (hereafter referred to as data) are available to assess the fish tissue criterion elements and data are either available, not available, or insufficient to assess the water column criterion elements.

> Scenarios 1, 2, and 3: Available data indicate that the fish tissue concentrations in the waterbody do not exceed the fish tissue criterion elements. The recommended criterion is structured such that the fish tissue criterion elements supersede the water column criterion elements. Hence, if the results for the fish tissue and water column criterion elements do not agree or if water column data are insufficient or not available, the assessment decision should be based on results for the fish tissue criterion elements. The national CWA section 304(a) recommended selenium criterion is met in all three scenarios.
>
> Scenarios 4, 5, and 6: Available data indicate that the fish tissue concentrations in the waterbody exceed the fish tissue criterion elements. The recommended criterion is structured such that the fish tissue criterion elements supersede the water column criterion elements. Hence, if the results for the fish tissue and water column elements do not agree or if water column data are insufficient or not available, the assessment decision should be based on the results for the fish tissue criterion elements. The national CWA section 304(a) recommended selenium criterion is not met in all three scenarios and the waterbody-pollutant combination is impaired.

Table 2. Matrix of Assessment Scenarios for WQS Approved by the EPA Based on the National CWA Section 304(a) Recommended Selenium Criterion.[1]

		Water Column Elements[5]		
		Not Exceeded	**Exceeded**	**Insufficient or Not Available**
Fish Tissue Elements[2, 3, 4]	**Not Exceeded**	(Scenario 1) Criterion Met	(Scenario 2) Criterion Met	(Scenario 3) Criterion Met
	Exceeded	(Scenario 4) Criterion Not Met	(Scenario 5) Criterion Not Met	(Scenario 6) Criterion Not Met
	Insufficient or Not Available	(Scenario 7) Criterion Met	(Scenario 8) Criterion Not Met	(Scenario 9) Not Assessed

[1] The assessment matrix does not address waterbody conditions when selenium inputs are new or increasing and steady-state fish tissue data are absent.

[2] Fish tissue criterion elements include the following: (a) fish egg-ovary element and (b) fish whole-body and/or muscle tissue element.

[3] There is no primacy between fish whole-body and muscle criterion elements.

[4] The fish egg-ovary criterion element supersedes the fish whole-body and/or muscle criterion element when both types of data are available.

[5] Water column criterion elements include the following: (a) monthly average exposure criterion element and (b) intermittent exposure criterion element. The duration component of both of these elements applies to any 30-day period.

Note that the national CWA section 304(a) recommended selenium criterion is structured such that the fish egg-ovary criterion element has primacy over the fish whole-body and/or muscle element. Hence, if the results for the fish egg-ovary criterion element and the fish whole-body and/or muscle criterion elements do not agree, the assessment should be based on the results for the fish egg-ovary criterion element. There is no primacy between the fish whole-body and the muscle criterion elements. Hence, if either the fish whole-body and or muscle criterion elements are exceeded, the criterion would not be met unless fish egg-ovary criterion element is met.

Assessment scenarios 7 through 9 describe water quality assessment situations for which data are insufficient or not available to assess the fish tissue criterion elements and data are either available, not available, or insufficient to assess the water column criterion elements.

> Scenario 7: Available data indicate that the water column concentrations of the waterbody do not exceed the water column criterion elements and data are insufficient or not available to assess the fish tissue criterion elements. The recommended criterion is structured such that water quality assessments can be made in the absence of fish tissue data using the water column criterion elements. The national CWA section 304(a) recommended selenium criterion is met in this scenario.

Scenario 8: Available data indicate that the water column criterion elements are exceeded, and data are insufficient or not available to assess the fish tissue criterion elements. The national CWA section 304(a) recommended selenium criterion is not met in this scenario and the waterbody-pollutant combination is impaired.

Scenario 9: Data are insufficient or not available to assess the water column and the fish tissue criterion elements. Therefore, the national CWA section 304(a) recommended selenium criterion cannot be assessed. The EPA recommends that states and authorized Tribes collect fish tissue and/or water column data so that an assessment decision can be made.

Q2: What data should states and authorized Tribes assemble and evaluate to complete water quality assessments with criteria based on the national CWA section 304(a) recommended selenium criterion?

A2: States and authorized Tribes are required under Title 40 of the *Code of Federal Regulations* (CFR) section 130.7(b)(5) to assemble and evaluate all existing and readily available water quality-related data and information when conducting water quality assessments to determine which waters belong on their CWA section 303(d) lists. Existing and readily available data and information relevant for conducting assessment determinations for criteria based on the national CWA section 304(a) recommended selenium criterion includes water column and fish tissue selenium data and information relevant to the jurisdictions, including such data and information collected by stakeholders. States and authorized Tribes must use such data and information in making listing decisions unless they provide a rationale for not doing so. 40 CFR 130.7(b)(6)(iii). The EPA will evaluate whether a state or authorized Tribe provides a technical, science-based rationale for decisions not to use data or information.[15]

Q3: What should states and authorized Tribes include in their assessment methodology for evaluating selenium data?

A3: States and authorized Tribes are required under 40 CFR 130.7(b)(6)(i) to submit to the EPA Regional Administrator a "description of the methodology used to develop the list" as part of their CWA section 303(d) list submissions. An assessment methodology constitutes the decision-making process that a state or authorized Tribe plans to use to determine the water quality attainment status of waters within their jurisdiction. The methodology should describe how data are evaluated and used to make water quality attainment determinations, including data quality, quantity, and representativeness considerations. Additional information regarding data quality, quantity, and representativeness considerations are available in the EPA's

[15] USEPA. 2023. Information Concerning 2024 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions. https://www.epa.gov/system/files/documents/2023-03/2024IRmemo_032923.pdf

Integrated Reporting memo for the 2006 reporting cycle.[16] States and authorized Tribes must use available data and information in making listing decisions unless they provide a rationale for not doing so (40 CFR 130.7(b)(6)(iii)). The EPA will evaluate whether a state or authorized Tribe provides a technical, science-based rationale for decisions not to use data or information.[17] The EPA encourages states and authorized Tribes to make the assessment methodology available to the public for review and comment prior to, or along with, solicitations for data. Such engagement helps facilitate stakeholder input to the state's or authorized Tribe's assessment of water quality status, including assessments for selenium. In reviewing state lists submitted under section 303(d), the EPA will evaluate whether the state's approach for use of fish and water column data is reasonable, including issues of data quality, quantity, and representativeness.

Q4: Should states and authorized Tribes include the collection of fish tissue and water column data in their monitoring programs to assess for selenium?

A4: The national CWA section 304(a) recommended selenium criterion includes a flexible approach that states and authorized Tribes may want to follow in designing monitoring plans for selenium. This flexibility stems from the fact that both the fish tissue and water column criterion elements are designed to protect against chronic selenium effects. States and authorized Tribes should consider a variety of factors when deciding how to monitor for selenium, including assessment and implementation needs, representativeness of the results, available resources, and public input.

When possible, the EPA recommends that states and authorized Tribes collect fish tissue data to support assessments of the recommended selenium criterion. Fish tissue data reflect an integrative accumulation of selenium over time and space (see section 2.7.6 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*).[18] Measurements of selenium in fish tissue are most closely linked to the chronic adverse effects of selenium, since chronic selenium toxicity is based on the food-chain bioaccumulation route, not a direct waterborne route.

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[16] USEPA. 2005. Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act. https://www.epa.gov/sites/production/files/2015-10/documents/2006irg-report.pdf

[17] USEPA. 2024. Information Concerning 2024 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions. https://www.epa.gov/system/files/documents/2023-03/2024IRmemo_032923.pdf

[18] USEPA. 2016. *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-16-006. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC, https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf.

States and authorized Tribes that want to collect fish tissue data should take into consideration species specific factors such as selenium sensitivity, bioaccumulation, mobility (including anadromous and potamodromous species), spawning period, and fish stocking rates when designing their monitoring plan. The document *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*[19] provides additional information to support the collection, evaluation, and assessment of fish tissue data for selenium. Section 3.1 of the document discusses that many states and authorized Tribes have existing fish tissue monitoring programs that can be leveraged to collect fish tissue data to assess against the fish tissue criterion elements of the recommended selenium criterion.

Q5: How should states and authorized Tribes complete assessments and CWA section 303(d) listings for waterbodies that only have selenium water column data?

A5: The national CWA section 304(a) recommended selenium criterion is structured to enable the assessment of waterbodies with data related to any element of the criterion. Hence, states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion should complete water quality assessments for waterbodies that only have selenium water column data (and no selenium fish tissue data) by comparing that data to the water column criterion element. Additional information about completing assessments for these scenarios is available in Q1 (see Scenarios 7 and 8). After a waterbody is added to a CWA section 303(d) list based on water column data alone, states and authorized Tribes may consider collecting fish tissue data to confirm the assessment determination before developing a restoration plan (e.g., TMDL).

Q6: How should states and authorized Tribes complete selenium assessments and CWA section 303(d) listings for fishless waters?

A6: The national CWA section 304(a) recommended selenium criterion is structured to enable assessment of waterbodies with data related to any element of the criterion. Hence, states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion should complete water quality assessments using the water column criterion elements for fishless waters.[20]

Note that the national CWA section 304(a) recommended selenium criterion is designed to protect all freshwater aquatic life, including populations of fish, amphibians, aquatic

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[19] USEPA. 2024. *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion.* EPA 820-R-24-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf.

[20] The EPA describes *fishless waters* as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (e.g., extirpation) due to temporary or permanent changes in water quality (e.g., selenium pollution), flow, or instream habitat.

invertebrates, and plants. The criterion elements were derived from fish toxicity values to be protective of the entire aquatic community, not solely fish species. The criterion elements, including the water column criterion elements, are designed to protect aquatic life from the chronic effects of exposure to selenium in waters both inhabited by fish and not inhabited by fish.

Q7: How should states and authorized Tribes complete selenium assessments and CWA section 303(d) listings when there are data for multiple fish species?

A7: The national CWA section 304(a) recommended selenium criterion is designed to protect all freshwater aquatic life. Hence, for states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion, if available data and information from one or more fish species for a waterbody exceeds the criterion, then the criterion is not met and the waterbody-pollutant combination is impaired. The criterion is not met even if one or more other fish species for a waterbody is meeting the criterion. States and authorized Tribes should not composite or average data from multiple species. Also, the hierarchy of the fish tissue criterion elements (i.e., egg-ovary data supersedes muscle/whole-body) apply within each fish species. States and authorized Tribes should not apply the hierarchy across species, as this would be inconsistent with the procedure used to derive the fish tissue criterion elements,[21] which used species-specific conversion factors to calculate toxicity values.

Q8: How should states and authorized Tribes complete selenium assessments and CWA section 303(d) listings when there are multiple samples for a single fish species?

A8: As discussed in section 2.0 of the EPA's *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion,*[22] the EPA's national CWA section 304(a) recommended selenium criterion was designed to protect "populations of fish, amphibians, aquatic invertebrates, and plants." Because the selenium criterion establishes population level protection, for states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion, the EPA generally recommends implementing these fish tissue criterion elements with a measure of central tendency (e.g., mean, median), as this will represent the level of exposure that the population is experiencing. Note that in section 2.0 of the EPA's *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016*

[21] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[22] USEPA. 2024. *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion.* EPA 820-R-24-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf.

Selenium Criterion, the EPA also recommends using a measure of central tendency only for characterizing a dataset that was collected during a single sampling event (identified location over a specified period of time (generally less than a week)). For implementation purposes, a measure of central tendency should not be calculated between different sampling events; instead, different sampling events should all be considered as discrete datasets. The EPA recommends that states describe in their assessment methodology how they plan to measure the population of fish species.

Additional information on approaches and factors to consider in collecting, evaluating, and assessing data from individual or composite fish tissue samples is provided in the document *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion.*[23]

Q9: How should the selenium fish tissue frequency of "not to be exceeded" be applied in the assessment and CWA section 303(d) listing programs?

A9: As discussed in the *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards,* the frequency of the fish tissue criterion elements (i.e., "not to be exceeded") differs from the typical "once-in-three years on average" frequency of most water column criteria for aquatic life criteria. Selenium is a bioaccumulative pollutant; therefore, elevated levels of selenium in various ecological compartments (e.g., biota, surficial sediments) require a long period of time to decrease and the associated aquatic community requires a long time to recover following reduction or removal of an elevated selenium exposure to a given system. The time frame to recovery is variable and will be dependent on the biogeochemical processes of the system, including residence time and food web dynamics. Thus, the typical frequency component is not appropriate for selenium in fish tissue, as this could lead to sustained ecological impacts. Therefore, the recommended frequency for the fish tissue criterion elements is "not to be exceeded." For additional information regarding frequency, see sections 2.7.6 and 2.7.7 of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*.

Although the frequency component is expressed as "not to be exceeded," not meeting the fish tissue criterion elements does not mean that fish populations cannot recover. As such, if a state or authorized Tribe determines that the fish tissue criterion elements are not met and identifies the water as impaired on their CWA section 303(d) list, the state or authorized Tribe may determine in the future that the selenium criterion is met and remove the waterbody-pollutant combination from the list. The EPA recommends that states include in their assessment methodology a discussion of how the fish tissue criterion elements will be implemented,

[23] USEPA. 2024. *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion.* EPA 820-R-24-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-fishtissue-tsd.pdf.

including information on how the criterion will be determined to be met after an exceedance of the fish tissue criterion elements.

Q10: How should states and authorized Tribes complete selenium assessments and CWA 303(d) listings when there are new or increased inputs of selenium?

A10: The national CWA section 304(a) recommended selenium criterion includes fish tissue and water column elements expressed as a single criterion. Within the criterion, the fish tissue elements take primacy over the water column elements, except when, as described in footnote 4 of the criteria recommendation, selenium inputs to the waterbody are increasing and steady-state fish tissue data are absent (see Table 1). For the purposes of the EPA's 2016 national CWA section 304(a) recommended selenium criterion, steady-state refers to conditions where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing.[24] New and increased inputs of selenium are referring to the release of a substantial amount of additional selenium from either anthropogenic point or nonpoint sources into a watershed. New or increased selenium inputs do not refer to seasonal variability of selenium that occurs naturally within a system (e.g., spring run-off events or precipitation-driven pulses) or *de minimus* man-made discharges.

Additional information on how to determine if the water column criterion elements should take primacy over the fish tissue criterion elements for assessment purposes is provided in the *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*. Section 2.1 includes examples of what may represent substantial new or increased inputs of selenium, as well as information on how much time it may take for fish tissue to reach steady-state in lentic and lotic systems after the introduction of a substantial new or increased input of selenium. The EPA recommends that states include in their assessment methodology a discussion of how they will determine whether the water column criterion elements take primacy over the fish tissue criterion elements, including information on how they will determine if there are substantial new or increased selenium inputs to the waterbody and if fish tissue has reached steady-state.

[24] This definition of steady-state applies to the national CWA section 304(a) recommended selenium criterion. It does not apply to the discussion of steady-state modeling or conditions in the "Technical Support Document for Water Quality-based Toxics Control" (EPA/505/2-90-001, March 1991).
https://www3.epa.gov/npdes/pubs/owm0264.pdf

Q11: How should states and authorized Tribes implement the selenium water column intermittent element in assessments and CWA section 303(d) listings?

A11: The national CWA section 304(a) recommended selenium criterion includes both an intermittent criterion element and a monthly average water column criterion element (i.e., 30-day average). The intermittent criterion element is a calculation based on the known ambient concentration, the monthly average exposure water column criterion element, and the fraction of any 30-day period during which elevated selenium concentrations occur. As explained in section 3.3 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*,[25] the intermittent criterion element is a re-expression of the chronic 30-day average water column criterion element and will yield the same level of protection as the monthly average exposure criterion element, provided that the equation uses (1) the average of the concentrations occurring for the fraction of time defined as being intermittently elevated and (2) the average of the concentrations occurring for the remaining time, defined as being background.

The intermittent criterion element can only be exceeded under the same conditions that would have caused the monthly average exposure criterion element to be exceeded. Thus, evaluating data against the intermittent element and the monthly average exposure criterion element will always result in the same assessment decision. As such, states and authorized Tribes may find it more straightforward to assess selenium water column data using the monthly average criterion element because it does not require knowledge of the background conditions or number of days with an intermittent spike.

Q12: How can states and authorized Tribes remove selenium impairments from their CWA section 303(d) lists?

A12: As with any applicable water quality standard, states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion will make assessment decisions based on an evaluation of existing and readily available water quality data against the criterion. The assessment decision informs whether a waterbody should be identified as impaired and included on the state or authorized Tribe's CWA section 303(d) list and assigned a priority ranking for TMDL development. Selenium impairments included on the CWA section 303(d) list may be excluded from subsequent lists (sometimes described as "delisting") for several reasons, including: (1) the water quality standard is met based on the criterion and available data, (2) there were flaws in the original listing, (3) a TMDL was established and approved by the EPA, or (4) other point source or nonpoint source control

[25] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

requirements are stringent enough to implement applicable water quality standards (40 CFR 130.7(b)(1)(iii)).

Q13: Can states and authorized Tribes develop a water column criterion element for selenium that considers site-specific conditions for assessment, 303(d) listing, and TMDL development?

A13: States and authorized Tribes can take one of two approaches for developing a water column criterion element for selenium that considers site-specific conditions. Under the first approach, if a state or authorized Tribe thinks that the default national recommended water column criterion element is not appropriate for a waterbody, that state or authorized Tribe can choose to adopt into its WQS and submit to the EPA for approval a site-specific water column criterion element developed using the procedures provided in Appendix K of the national CWA section 304(a) recommended selenium criterion[26] or another scientifically defensible method that is protective of the aquatic life use. Under the second approach, a state or authorized Tribe can choose to adopt into its WQS and submit to the EPA for approval a set of procedures to translate the fish tissue criterion elements into a site-specific water column criterion element. This is considered a performance-based approach to site-specific criteria development.[27] Information on both approaches is provided in sections 2.2.1 and 2.2.2 of *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards.*[28]

States and authorized Tribes should consider which of the two approaches they want to use when adopting site-specific water column criterion elements. For example, the first approach (i.e., a site-specific water column criterion element) is more straightforward to apply in assessment, 303(d) listing, and TMDL development and will likely reduce workload on those programs. Furthermore, the opportunity for public comment on the site-specific water column criterion element would occur in response to a single CWA section 303(c) action. The second approach (i.e., performance-based) facilitates an opportunity for states and authorized Tribes

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[26] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[27] In addition to the four-part criterion, states or authorized Tribes may want to adopt a method to derive site-specific water column criterion elements, referred to by the EPA in 2000 as a "performance-based approach." The EPA first formalized the concept of a performance-based approach for a WQS in the preamble of the rule *the EPA Review and Approval of State and Tribal Water Quality Standards*. In that document, the EPA describes this approach as a WQS that is a transparent process rather than a specific outcome. The state or authorized Tribe can adopt a process, such as a criterion derivation methodology, rather than a specific outcome, such as a concentration of a pollutant. If a state or authorized Tribe adopts a process or methodology that is sufficiently detailed and has suitable safeguards that ensure predictable, repeatable outcomes, the EPA can approve that process as a WQS and this approval would also serve for CWA purposes as the approval of each outcome generated from following that process or method.

[28] USEPA. 2024. *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards*. EPA 820-R-24-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-adopting-tsd.pdf.

to adaptively derive a site-specific water column criterion element to account for the most up-to-date data for the waterbody using the procedures in the EPA-approved criterion. When a performance-based approach is used, however, more coordination will likely be needed between the CWA implementing programs to consistently employ such water column criterion elements, and the opportunity for public comment on the translated site-specific water column criterion element would occur in response to different and multiple program actions (e.g., assessment decisions, or development of TMDLs or permit limits).

Q14: Which criterion elements should be used to develop TMDLs?

A14: States and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion should consider developing TMDLs for selenium based on the monthly average exposure water column criterion element or a site-specific water column criterion element developed through one of the two approaches described in Q13. As discussed in Q1, four scenarios (i.e., 4, 5, 6, and 8 in Table 2) exist for which a waterbody can be assessed as impaired. For three of the scenarios (i.e., 5, 6, and 8 in Table 2), states and authorized Tribes should consider using the monthly average exposure water column criterion element to develop TMDLs. The EPA recommends the approach of using the monthly average exposure water column criterion element for these scenarios because it was derived to ensure adequate protection of the fish tissue criterion elements. However, for scenario 4 (wherein available data indicate that the fish tissue criterion element is exceeded and the water column criterion element is not exceeded), states and authorized Tribes should consider developing a site-specific water column criterion element and use it for developing the TMDL. Using the monthly average exposure water column criterion element under scenario 4 to develop the TMDL could result in a loading capacity for selenium that exceeds the existing load, which is already resulting in an exceedance of the fish tissue criterion elements in the waterbody. For this scenario, states and authorized Tribes should consider developing a site-specific water column criterion element that would provide adequate protection of the fish tissue criterion elements. The approaches described above are suggestions. Regardless of what approach states and authorized Tribes choose to pursue, they should work closely with the EPA for technical support and to help ensure timely review and action by the federal government.

Q15: How should TMDLs account for the intermittent criterion element?

A15: For states and authorized Tribes that adopt criteria based on the national CWA section 304(a) recommended selenium criterion, developing TMDLs based on the monthly average exposure water column criterion element would also address the intermittent water column criterion element. Section 3.3 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* explains that the intermittent criterion element is a re-expression of

the chronic 30-day average water column criterion element.[29] As such, the monthly average exposure water column criterion element and the intermittent criterion element are intended to provide the same level of protection of the fish tissue criterion elements.

Q16: Does the EPA have recommendations that address whether a receiving waterbody should be considered lentic or lotic for assessments and 303(d) listings?

A16: The national CWA section 304(a) recommended selenium criterion includes water column criterion element values for both lotic and lentic freshwater systems. Lentic waters are waterbodies that consist of standing water, including waterbody types such as lakes and ponds. Lotic waters are water bodies that consist of flowing waters, including waterbody types such as rivers and streams. Generally, classifications of fresh waters into lotic or lentic categories are made by the state or authorized Tribe, based on site-specific information. Although the distinction between lentic and lotic systems is often straightforward, some aquatic systems may possess both lentic and lotic characteristics. For assessment purposes, states and authorized Tribes should use a waterbody segmentation approach that is consistent with water quality standards and other appropriate waterbody-related factors, and capable of providing a spatial scale that is adequate to characterize attainment status. Additional information on lentic and lotic waters is provided in Section 3.2.4 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* and Section 2.1 of *the EPA's Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards,*.

[29] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Appendix L

United States Environmental Protection Agency

Office of Water
EPA-820-R-24-002
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Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits

Frequently Asked Questions (FAQs): Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits

While this document cites statutes and regulations that contain requirements applicable to water quality standards and National Pollutant Discharge Elimination System (NPDES) permitting, it does not impose legally binding requirements on the EPA, states, authorized Tribes, other regulatory authorities, or the regulated community and may not apply to a particular situation based upon the circumstances. The EPA, state, Tribal, and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those provided in this technical support document as appropriate and consistent with statutory and regulatory requirements. The EPA may update this document as new information becomes available. In addition to this document, the EPA has related documents that provide considerations and recommendations for implementing criteria based on the national Clean Water Act (CWA) section 304(a) recommended selenium criterion for freshwater, which are available at the EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

Note: For applicable state or authorized Tribal water quality standards that are not based on the national CWA section 304(a) recommended selenium criterion, the EPA will assess the need for alternative state or authorized Tribal specific implementation permitting guidance (i.e., state or Tribal implementation procedures) to ensure consistency with the NPDES permitting requirements at Title 40 of the Code of Federal Regulations (CFR) Part 122.

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List of Acronyms and Abbreviations

µg/L	Micrograms per liter
AML	Average monthly limit
BMP	Best management practice
BPJ	Best professional judgement
CFR	Code of Federal Regulations
CWA	Clean Water Act
FAQ	Frequently asked question
FT	Fish tissue
FTE	Fish tissue element
LTA	Long-term average
MDL	Maximum daily limit
mg/kg	milligrams per kilogram
NPDES	National Pollutant Discharge Elimination System
POTW	Publicly owned treatment works
RP	Reasonable potential
TMDL	Total maximum daily load
TSD	Technical support document
USEPA	U.S. Environmental Protection Agency
WC	Water column
WCE	Water column element
WLA	Wasteload allocation
WQBELs	Water quality-based effluent limits
WQC	Water quality criterion
WQS	Water quality standard

Definitions[1]

Best Management Practice (BMP)
Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of *waters of the United States*. Best management practices also include treatment requirements, operating procedures, and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

Best Professional Judgement (BPJ)
The method used by NPDES permit writers to develop technology-based NPDES permit conditions on a case-by-case basis using all reasonably available and relevant data (USEPA 2010).

Bioaccumulation
The uptake and retention of a chemical by an aquatic organism from all surrounding media (e.g., water, food, sediment) (USEPA 2003).

Chronic
A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of a chronic effect can be growth, reduced reproduction, etc., in addition to lethality (USEPA 1991).

Criteria
Per 40 CFR 131.3(b), criteria are elements of State water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular use. When criteria are met, water quality will generally protect the designated use.

Effluent
A wastewater treated or untreated, that flows out of a treatment plant, sewer, or industrial outfall. Generally, refers to wastes discharged into surface waters.

Frequency
How often criteria can be exceeded without unacceptably affecting the community (USEPA 1991).

Lentic
Characterized by largely standing water, such as lakes and ponds (Jones 1997).

[1] This glossary is meant to provide plain language definitions for key terms used in this document. Individuals should consult the Clean Water Act and the EPA's implementing regulations to identify whether there are legal definitions of these terms.

Lotic
Characterized by flowing water, such as rivers and streams (Jones 1997).

Magnitude
How much of a pollutant (or pollutant parameter such as toxicity), expressed as a concentration or toxic unit is allowable (USEPA 1991).

Reasonable Potential (RP)
Where a pollutant may be discharged at a level that will cause, have the reasonable potential to cause, or contributes to an in-stream excursion above a water quality standard based on a number of factors including, as a minimum, the factors listed in 40 CFR 122.44(d)(1)(ii).

Steady-state
An organism is in steady-state when the rates of chemical uptake and depuration are equal and tissue concentrations remain constant over time (USEPA 2003). For the purposes of the national CWA section 304(a) recommended selenium criterion, steady-state refers to conditions where sufficient time has passed after the introduction of a new or increased input of selenium into a waterbody or watershed of a waterbody such that that fish tissue concentrations of selenium are no longer increasing (USEPA 2024b).

Total Maximum Daily Load (TMDL)
The sum of the individual wasteload allocations and load allocations. A margin-of-safety is included with the two types of allocations so that any additional loading, regardless of source, would not produce a violation of water quality standards (USEPA 1991).

Wasteload Allocation (WLA)
The portion of a receiving water's total maximum daily load that is allocated to one of its existing or future point sources of pollution (USEPA 1991).

Water Quality Standard (WQS)
A law or regulation that consists of beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody and an antidegradation policy (USEPA 1991).

Introduction

EPA's National CWA Section 304(a) Recommended Chronic Aquatic Life Selenium Criterion in Freshwater

In 2016, the U.S. Environmental Protection Agency updated its national Clean Water Act (CWA) section 304(a) recommended chronic aquatic life criterion for selenium in freshwater systems to reflect the latest scientific information and, in 2021, issued an erratum, *2021 Revision to: Final Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016,* USEPA, Office of Water, EPA 822-R-21-006, August 2021.[2] The EPA's national CWA section 304(a) recommended selenium criterion still reflects the latest science and the EPA is not aware of any updated scientific information that would change this recommendation. The latest scientific information indicates that selenium toxicity to aquatic life is driven by dietary exposures and that the reproductive life-stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium. The recommended criterion has four criterion elements: (1) a fish egg-ovary criterion element; (2) a fish whole-body and/or muscle criterion element; (3) a water column criterion element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent criterion element (to account for potential chronic effects from short-term exposures to high concentrations in lentic and lotic aquatic systems) (see Table 1). Under the EPA's 2016 national CWA section 304(a) recommended selenium criterion, the fish tissue criterion elements have primacy over water column elements, except where there are no fish, where fish tissue data are not adequate, or for waterbodies with new or increased discharges where selenium concentrations in fish tissue might not have stabilized and reached steady-state. The EPA also recommends that the egg-ovary tissue criterion element has primacy over whole-body and muscle tissue criterion elements.

Toxicity data indicate that the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse reproductive effects in aquatic organisms. Toxicity to developing embryos and larvae is directly linked to egg selenium concentration (USEPA 2021). The EPA derived the whole-body, muscle tissue, and water column elements from the egg-ovary element so that states and authorized Tribes could more readily implement their water quality criteria based on the EPA's national CWA section 304(a) recommended selenium criterion. The assessment of the available data on chronic selenium exposure for fish, invertebrates, and amphibians indicates that a criterion element derived from fish is expected to be protective of the aquatic community in a waterbody, since other taxa appear to be less sensitive to selenium than fish. The EPA did not develop an acute criterion for selenium when it updated the chronic criterion. Although selenium may cause acute toxicity at

[2] In 2021, the EPA identified that the following text was missing from the second sentence in footnote 4 in the selenium criterion table: "When selenium inputs are increasing" and issued an erratum. The EPA corrected footnote 4 to state: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data." https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

high concentrations, the most deleterious effects on aquatic organisms are due to selenium's bioaccumulative properties.

In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from chronic exposure to chemicals via the diet (through the food web pathway). Such studies also do not account for the accumulation kinetics of many bioaccumulative compounds, such as selenium, and may underestimate effects from long-term accumulation in some types of aquatic systems. Therefore, since acute studies do not address the primary exposure pathway for organisms to selenium and because chronic toxicity occurs at lower concentrations, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion. As described in the EPA's *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (hereafter referred to as *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*), the EPA also included an intermittent exposure criterion element to provide protection from the most significant chronic effects of selenium toxicity, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high concentration exposure events (USEPA 2021).

The EPA recommends, as stated in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, that states and authorized Tribes[3] adopt into their water quality standards (WQS) a selenium criterion that includes all four criterion elements (USEPA 2021). For more information see the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* which can be found at https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[3] Throughout this document and in the CWA, the term "states" means the fifty states, the District of Columbia, the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands (CWA section 502(3)). The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer a CWA WQS program.

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg-ovary [2]**	**Fish Whole-body or Muscle [3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dry weight	8.5 mg/kg dry weight whole-body or 11.3 mg/kg dry weight muscle (skinless, boneless fillet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int}}{f_{int}}$$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1 Fish tissue elements are expressed as steady-state.
2 Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in note 4 below.
3 Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in note 4 below.
4 Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5 Where $WQC_{30\text{-}day}$ is the water column monthly element, for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6 Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

Selenium Technical Support Materials

The EPA has prepared a four-volume set of documents to provide recommendations to states, authorized Tribes, and other agencies for implementing their water quality criteria based on the national CWA section 304(a) recommended selenium criterion for aquatic life (USEPA 2021). These four documents constitute the Technical Support Materials for EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). Each document of the set focuses on a specific aspect of implementation of criteria based on the national CWA section 304(a) recommended selenium criterion. Together, these four EPA documents provide information to assist states and authorized Tribes with adopting water quality criteria based on the EPA's CWA section 304(a) recommended selenium criterion and implementing them in various CWA programs.

1) *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*: Provides recommendations for the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools.
2) *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*: Provides an overview on how to establish or enhance existing fish tissue monitoring programs to facilitate implementation of fish tissue-based criterion elements based on the national CWA section 304(a) recommended selenium criterion.
3) *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*: Provides information to help National Pollutant Discharge Elimination System (NPDES) permit writers understand what permitting guidance (i.e., state or Tribal implementation procedures) may be appropriate to implement state and authorized Tribal WQS based on the EPA's CWA section 304(a) recommended selenium criterion. This set of FAQs also provides recommendations on how to establish water quality-based effluent limits (WQBELs) in NPDES permits.
4) *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*: Provides information on how to complete assessments, list impaired waters, and develop total maximum daily loads (TMDLs) to implement on EPA-approved[4] WQS that are based on the EPA's national CWA section 304(a) recommended selenium criterion.

This document supports CWA regulators in states and authorized Tribes to implement WQS based on the EPA's CWA section 304(a) recommended four-part selenium water quality criterion (WQC)[5] in the EPA's 2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016 (USEPA 2021) as it relates to CWA section 402 NPDES permits. These NPDES FAQs are intended to support NPDES permit writers in understanding what permitting guidance (i.e., state or Tribal implementation procedures) may be appropriate to implement EPA-approved state and authorized Tribal WQS based on the EPA's CWA section 304(a) recommended selenium criterion (USEPA 2021).

[4] EPA-approved includes both WQS adopted by states, territories or authorized Tribes and approved by the EPA and WQS promulgated by the EPA.

[5] These FAQs apply specifically with respect to implementation of EPA-approved state and Tribal water quality criteria for selenium that are based on the EPA's national CWA section 304(a) recommended selenium criterion – that is, criteria that include elements that are substantially identical to the recommended selenium criterion. Where the applicable state or Tribal water quality criterion for selenium differs from the EPA's recommended selenium criterion, these NPDES FAQs may not be relevant or applicable. As required by CWA section 301(b)(1)(C), permit writers must include permit limits necessary to meet the applicable state or Tribal water quality criterion.

1. Identifying the Applicable Selenium WQC to Implement in the NPDES Permit

Q1-1: What is the applicable selenium criterion to use in implementing the NPDES permit program?

A1-1: The applicable selenium criterion for CWA purposes is a state or authorized Tribal criterion that has been approved by the EPA as protective of the waterbody's designated uses or a WQC promulgated by the EPA on behalf of a state or authorized Tribe. The applicable selenium criterion may be site-specific, or applicable throughout the state or authorized Tribe's jurisdiction. NPDES permits must include permit limits necessary to meet the applicable state or Tribal WQS, as required by CWA section 301(b)(1)(C). Accordingly, where selenium may be discharged at a level that causes, has the reasonable potential to cause, or contributes to an in-stream excursion above the state or authorized Tribe's applicable selenium criterion, permit writers must develop and include WQBELs that will meet the applicable selenium criterion (see CWA section 301(b)(1)(C) and NPDES regulations at 40 CFR 122.44(d)(1)(i)). In addition, a state or authorized Tribal permit writer may have authority under a state statute or regulation or Tribal law to include WQBELs necessary to meet a lower state or authorized Tribal WQC for selenium that has not yet been approved by the EPA. Permit writers must also include more stringent conditions necessary to meet other appropriate water-quality related requirements under state or Tribal law if a state or authorized Tribe specified such conditions in a CWA section 401 certification.

As a component of their EPA-approved WQS, a state or authorized Tribe's selenium aquatic life criterion, may be based on: (1) the 2016 national CWA section 304(a) recommended selenium criterion; (2) the 2016 national CWA section 304(a) recommended selenium criterion modified to reflect site-specific conditions; (3) Water Quality Guidance for the Great Lakes System, 40 CFR 132.6 (60 FR 15387, March 23, 1995); or (4) other scientifically defensible methods for developing selenium aquatic life criterion. Some states or authorized Tribes' selenium aquatic life criterion may be based on the EPA's 1987 recommended selenium criteria, if previously approved by the EPA. The EPA's 2016 national CWA section 304(a) recommended selenium criterion, as summarized in Table 1, replaces the EPA's 1987 recommended selenium acute and chronic water quality criteria.

States and authorized Tribes have the option to develop, adopt, and submit for approval by the, site-specific criteria. See the EPA's *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* for more information on the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools (USEPA 2024b).

Q1-2: Which criterion elements of EPA's national CWA section 304(a) recommended selenium criterion should be used when implementing the criterion through the NPDES permit program?

A1-2: Where a state or authorized Tribe has adopted a selenium aquatic life criterion based on the EPA's national CWA section 304(a) recommended selenium criterion, the NPDES permitting authority can use any of the criterion elements to assess the need for and to establish effluent limits as stringent as necessary to meet the applicable selenium criterion element. NPDES permits must include permit limits necessary to meet the applicable state or Tribal WQS, as required by CWA section 301(b)(1)(C). The permitting authority may make the determination of which criterion elements to use to evaluate reasonable potential (RP) and develop permit limits based on their best professional judgement (BPJ). Additionally, while all four elements of the EPA's national CWA section 304(a) recommended selenium criterion could be used to evaluate RP and develop permit limits, under the recommended criterion, the fish tissue criterion elements have primacy over water column elements.[6] Because the selenium criterion was designed to be protective of the most sensitive aquatic species and biological effect (i.e., fish and fish reproduction effects), the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) notes that the criterion is also protective of other aquatic life, including amphibians and invertebrates. Therefore, in fishless waters,[7] the water column criterion elements could be used as the basis for RP determinations and effluent limits because they would be protective of the aquatic life uses designated by the state or authorized Tribe. For further information on fishless waters, see *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b).

The flowcharts provided in Attachment 1 present a recommended approach for implementing selenium WQC based on the recommended criterion in NPDES permits that considers both the fish tissue and water column elements of the criterion. Specific questions about using the four-part criterion to assess RP and develop WQBELs are discussed in sections two through four of this document.

[6] Fish tissue values may include egg/ovary, whole-body, or fillet. Footnotes 2, 3, and 4 in Table 1 explain that the criterion affirms the primacy of the egg-ovary criterion element over any other criterion element and the whole-body and/or muscle tissue criterion element over the water column criterion elements.

[7] For purposes of EPA's 2016 recommended four-part selenium criterion, EPA defines "fishless waters" as waters with insufficient instream habitat and/or flow to support a population of any fish species on a continuing basis, or waters that once supported populations of one or more fish species but no longer support fish (e.g., extirpation) due to temporary or permanent changes in water quality (e.g., selenium pollution), flow or instream habitat (USEPA 2024b).

Q1-3: What are some considerations when using WQS based on EPA's national CWA section 304(a) recommended selenium water column criterion elements in NPDES permitting?

A1-3: Several factors should be considered when using WQS based on the EPA's national CWA section 304(a) recommended selenium water column criterion elements in NPDES permitting:

1) The relationship between the concentration of selenium in the fish tissue and the concentration of selenium in the water column can vary substantially across aquatic systems.
2) The chemical form of selenium, species of fish, species and proportion of prey, and a variety of site-specific biogeochemical factors affect selenium bioaccumulation. These factors affect the allowable concentration of selenium in ambient water that is protective of aquatic life.
3) The water column elements are the recommended criterion elements to use in fishless waters, for new or increased discharges of selenium, or other scenarios where fish tissue is not at steady-state in terms of selenium bioaccumulation.

The EPA's national CWA section 304(a) recommended selenium criterion includes two fish tissue criterion elements and two water column criterion elements (a 30-day average exposure criterion element and an intermittent exposure criterion element). However, due to the site-specific nature of the relationship between water column and fish tissue concentrations of selenium, the EPA provides two recommended approaches for translating the recommended fish tissue criterion elements into site-specific water column criterion elements. The first approach is the mechanistic model used by the EPA to derive its default water column criterion elements. The second approach uses an empirical bioaccumulation factor approach. Both approaches are described further in Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium– Freshwater 2016* (USEPA 2021). The two approaches can be used to develop site-specific values for the water column criterion elements. Site-specific criteria values can either be individually developed and adopted into a state or Tribe's WQS and submitted to the EPA for review and approval, or these values can be developed by using a performance-based approach to derive site-specific criteria values if that performance-based approach has been adopted into a state or Tribe's WQS and reviewed and approved by the EPA. See the EPA's *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* for more information on the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including information related to the development and adoption of site-specific criteria (USEPA 2024b). If the EPA has approved a performance-based approach adopted by a state or authorized Tribe, NPDES permit writers can use that methodology to develop site-specific water column values to implement fish tissue based WQC in NPDES permits. An exception is fishless waters, where a performance-based approach may not be appropriate unless the state or authorized Tribe developed an alternative scientifically defensible approach such as potentially translating from an invertebrate threshold when data for invertebrates are available (USEPA 2024b).

Within this NPDES FAQ document, references to the "water column criterion element" means the EPA's national CWA section 304(a) recommended default water column criterion value, a state- or site-specific water column value approved by the EPA, including a value based on an invertebrate toxicity threshold,[8] or a site-specific water column value developed using a performance-based approach where appropriate and approved by the EPA. Please see *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b) for additional discussion regarding the flexibilities available to states in the adoption of water column criterion elements.

Q1-4: Does a state or authorized Tribal adoption of a site-specific value for either egg-ovary or whole-body/muscle elements require approval by the EPA under CWA section 303(c) before using the site-specific value in NPDES permitting?

A1-4: Site-specific values for the fish tissue criterion elements require approval by the EPA under CWA section 303(c) before such criterion elements are applicable for CWA purposes, including NPDES permitting (40 CFR 131.21(c)). However, as indicated in FAQ 1-1, a state or authorized Tribal permit writer may have authority under a state statute or regulation or Tribal law to include a WQBEL necessary to meet a lower state or authorized Tribal criterion that has not yet been approved by the EPA, or to include such conditions as part of their CWA section 401 certification.

The whole-body fish and fish muscle element as well as the water column elements in the EPA's national CWA section 304(a) recommended selenium criterion are derived from the egg-ovary criterion element. As a result, any state or authorized Tribe that adopts a site-specific value for the egg-ovary criterion element and intends to also adopt other CWA section 304(a) selenium criterion elements in their WQS, would need to derive the related whole-body/muscle criterion element and the water column criterion elements, all of which are based on protection of the egg-ovary criterion element. The state or authorized Tribe would submit the site-specific criterion elements to the EPA for review and approval under CWA section 303(c) (unless the site-specific water column criterion elements were derived by following performance-based approach approved by the EPA). See EPA's *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* for additional discussion on the development of site-specific criterion values for the elements of the four-part criterion and performance-based approaches (USEPA 2024b).

[8] Invertebrate toxicity thresholds may be appropriate if fish currently and historically have not used the water body. If using an invertebrate toxicity threshold, states and authorized Tribes would need to ensure that the resulting water column criterion element is protective of the uses in downstream water bodies. As of now the EPA is not aware of sufficient toxicity data on invertebrates for an invertebrate toxicity threshold for selenium. However, as new toxicity data becomes available, invertebrates may be an appropriate taxa to use for deriving site-specific water column criterion values for fishless waters (USEPA 2024b).

Q1-5: When using criterion elements based on the EPA's national CWA section 304(a) recommended selenium water column criterion elements, can permit writers use mixing zones, initial zones of dilution, or dilution factors in NPDES permits?

A1-5: States and authorized Tribes may, at their discretion, include in their water quality standards, policies generally affecting their application and implementation, such as mixing zones (40 CFR 131.13). These general policies are subject to review and approval by the EPA and must be consistent with the CWA. Even if a state or authorized Tribe chooses to allow mixing zones generally, it may also choose to include in its policy circumstances under which mixing zones are prohibited entirely (e.g., for particular pollutants, waterbodies, and/or waterbody types).

The EPA recommends in section 5.1.2 of the Water Quality Standards Handbook (USEPA 2014) that states and authorized Tribes should carefully consider whether mixing zones are appropriate where a discharge contains bioaccumulative pollutants. Because fish tissue contamination tends to be a far-field problem affecting entire or downstream waterbodies rather than a near-field problem being confined to the area within a mixing zone, where allowed, mixing zones should be applied carefully so that they do not result in impairment of the designated use of the waterbody as a whole or impede progress toward the CWA goals of restoring and maintaining the physical, chemical, and biological integrity of the Nation's waters.

If applicable state or authorized Tribal WQS allow mixing zones or consideration of dilution for bioaccumulative pollutants, a state or authorized Tribe may find it appropriate to restrict or eliminate mixing zones for bioaccumulative pollutants in certain situations such as the following:

- Where mixing zones may encroach on areas often used for fish harvesting, particularly for stationary species such as shellfish; or
- Where there are uncertainties in the protectiveness of the water quality criteria or the assimilative capacity of the waterbody, or evidence indicates a lack of assimilative capacity.

Where a state or authorized Tribal WQS allows mixing zones for bioaccumulative pollutants, the permit writer may use mixing zones or dilution allowances in assessing the need for and deriving WQBELs.

In such cases, WQBELs should be established that provide for WQS to be met during critical conditions after accounting for allowable dilution or at the edge of the regulatory mixing zone. The permit or fact sheet should describe the geographic extent of the mixing zone or dilution volume allowed under the permit and describe the evaluation conducted to determine the appropriateness of the mixing zone. The NPDES permitting authority should make any allowance for dilution or mixing on a case-by-case basis that accounts for site-specific conditions, including but not limited to effluent flow, stream flow, ambient pollutant

concentrations, as well as other physical, chemical, and biological characteristics of the receiving water.

When a CWA-effective state or authorized Tribal WQS prohibits mixing zones or consideration of dilution for bioaccumulative pollutants, WQBELs for bioaccumulative pollutants must require meeting the WQS at the point of discharge.

Q1-6: When implementing the EPA's national CWA section 304(a) recommended selenium fish tissue criterion elements in NPDES permits, how is steady-state defined and demonstrated?

A1-6: For the purposes of the EPA's national CWA section 304(a) recommended selenium criterion, steady-state[9] refers to conditions where sufficient time has passed after the introduction of new inputs of selenium into a watershed of a waterbody such that fish tissue selenium concentrations are no longer increasing (see the description in section 2.1 of *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b) of footnote 4 in the criterion table of the *2021 Revision to the Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, (USEPA 2021). "New inputs" are both new and increased inputs of selenium and refers to the release of a substantial amount of additional selenium from either anthropogenic point or nonpoint sources into a waterbody or watershed.

In general, the EPA estimates that once a new or increased input of selenium commences, the concentration of selenium in fish tissue will reach steady-state after several months in lotic systems and longer time periods (e.g., two to three years) in lentic systems (USEPA 2021). These times frames can be used to provide a general estimate of steady-state after introduction of a new input of selenium into a watershed. However, these values are general estimates and the time needed for fish tissue to achieve steady-state after a new or increased input of selenium to the waterbody has occurred is site-specific. Potential factors affecting steady-state conditions in an aquatic system include: (1) the hydrodynamics of the aquatic system (e.g., particularly reservoirs with multiple riverine inputs and controlled releases of water into downstream waterbodies); (2) the location of the selenium input; and (3) the site-specific food web. Therefore, if states and authorized Tribes assume that fish tissue is in steady-state in terms of selenium bioaccumulation, the EPA recommends documenting the rationale supporting those assumptions to demonstrate that any resulting regulatory decisions are protective and have a sound scientific rationale. Any available data, including data collected by the state or authorized Tribe, by permittees, and by other sources may be evaluated to determine if there are new or increasing selenium inputs. Where the permitting authority has information that there are substantial new, known inputs or releases that would affect selenium concentrations in fish relative to environmental concentrations, the fish tissue should

[9] This definition of steady-state applies to the national CWA section 304(a) recommended selenium criterion. It does not apply to the discussion of steady-state modeling or conditions in USEPA 1991. For more information on the definition of steady-state, see USEPA 2024b.

not be considered as being in steady-state with respect to selenium and fish tissue criterion elements should not be used to assess permit compliance or as permit limits. As noted under FAQ 1-3, the water column criterion element should be used in these cases to assess RP and develop selenium WQBELs.

Note that the term "steady-state" should not be interpreted as "constancy." Waterbodies that normally experience a range of seasonal or yearly changes in flow for example, may still be in steady-state with respect to selenium fish tissue concentrations so long as there are no new inputs of selenium or increases in existing selenium inputs to the waterbody.

Fish tissue data can be used to evaluate whether fish tissue concentrations are in steady-state. The EPA recommends monitoring fish tissue in the affected waterbody, starting when new or increased inputs of selenium begin (or before if possible) and continuing until fish tissue data demonstrate that selenium concentrations in fish tissue are no longer increasing. With sufficient and appropriate monitoring data, collected over several sequential sampling periods, the state or authorized Tribe can use appropriate statistical methods (one example is the Mann-Kendall statistical approach, but other statistical approaches may be used) to determine when the fish tissue concentrations are no longer increasing.[10] However, see FAQ 3-2 for additional implementation considerations.

[10] The following documents may be referenced for guidance on statistical analyses: *Statistical Methods in Water Resources* (https://pubs.usgs.gov/tm/04/a03/tm4a3.pdf); *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (https://www.epa.gov/sites/default/files/2015-06/documents/volume1.pdf); *Statistical Analysis for Monotonic Trends* (https://www.epa.gov/sites/default/files/2016-05/documents/tech_notes_6_dec2013_trend.pdf); *Statistical Methods for Environmental Pollution Monitoring* (https://www.osti.gov/servlets/purl/7037501).

2. NPDES Reasonable Potential (RP) Determinations

Q2-1: Which elements of the EPA's national CWA section 304(a) recommended selenium criterion that are included in an EPA-approved WQS should be used for conducting RP analyses?

A2-1: Any of the four elements from the EPA's national CWA section 304(a) recommended selenium criterion that are incorporated into a CWA-effective WQS can be used by the NPDES permitting authority to determine whether the discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above the applicable WQS. If the NPDES permitting authority has site-specific, steady-state fish tissue data that were collected under and met appropriate quality-assurance procedures, those data may be used directly to assess RP.[11] In fishless waters or where steady-state condition fish tissue data are not available, the water column elements should be used to conduct RP analyses. Since the water column element was derived to be protective of fish (and other aquatic life), the NPDES permitting authority has the discretion to use the water column element to conduct RP analyses for all permitted discharges to freshwater receiving waterbodies. Additionally, the NPDES permitting authority may choose to use the water column element in RP analyses for selenium until such time as the EPA approves in 40 CFR Part 136 an analytical method for measuring selenium in fish tissue. In any case, such determinations should ensure that state and authorized Tribal WQS are met.

Q2-2: How should the NPDES permitting authority determine whether a 30-day chronic water column criterion element or an intermittent water column criterion element is appropriate for making RP determinations?

A2-2: Under the NPDES program, continuous discharges are discharges that occur without interruption throughout the operating hours of the discharging facility, except for infrequent shutdowns for maintenance, process changes, or other similar activities (40 CFR 122.2). For a continuous discharge, when using a water column criterion element to evaluate the RP of the discharge, the NPDES permitting authority should use the 30-day chronic element. The 30-day chronic element is based on a monthly average exposure, which is well suited for conducting RP analyses and developing WQBELs for continuous discharges (see also chapter 5 of the EPA's Technical Support Document (TSD) for Water Quality-based Toxics Control (USEPA 1991)).

[11] USEPA 2024c addresses fish tissue monitoring for assessment of the fish tissue elements of the selenium criterion and for the development of site-specific water column criterion elements. Elements of this document will be useful for consideration when sampling fish for RP determinations, such as target species and tissue types, however some considerations may differ such as temporal and spatial considerations (e.g., distance from the facility, age of data) that may be specific to RP determinations.

For non-continuous discharges, the intermittent water column criterion element provides an appropriate measure of aquatic life protection. The intermittent water column element can be calculated using ambient spikes of selenium in the receiving water, with the equations provided in the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* (Table 1) (USEPA 2021). Note that both the continuous (30-day) and the intermittent water column elements are designed to be as protective as the EPA's criterion elements for fish tissue over a 30-day period.

Q2-3: How should an NPDES permitting authority implement criteria based on the EPA's national CWA section 304(a) recommended selenium criterion in RP analyses?

A2-3: The information outlined below, and illustrated in Attachment 1, summarizes recommendations for conducting RP analyses, assuming the state or authorized Tribe has adopted all four elements of the EPA's national CWA section 304(a) recommended selenium criterion. If RP is demonstrated, the permit must contain a WQBEL to limit the selenium discharge such that the state's regulatory selenium criterion is met.

The flowchart in Attachment 1 titled *Permitting for Existing Dischargers*, specifies conditions under which the fish tissue or the water column criterion elements may be used in RP determinations (40 CFR 122.44(d)(1)(ii)). The other flowchart, titled *Permitting for New or Increased Dischargers*, is applicable for new dischargers or dischargers who have increased their selenium discharges. In situations where there will be new or increased discharges, selenium concentrations in fish tissue should not be assumed to be in steady-state, and the water column criterion element should be used in RP determinations, pending the collection of fish tissue data demonstrating steady-state conditions (see FAQ 1-6 for information on steady-state).

Below are four scenarios and recommendations for conducting RP analysis:

a) **Fish tissue data available (with criterion excursion in fish tissue):** If site-specific selenium concentrations in fish tissue indicate an excursion of the applicable fish tissue criterion elements and an NPDES facility discharges quantifiable concentrations of selenium to the waterbody, then the facility would have RP, regardless of whether the fish tissue concentrations are in steady-state. This is because the concentration of selenium in fish tissue indicates that an excursion of the criterion has already occurred. Whether or not fish tissue concentrations of selenium are in steady-state, RP has been demonstrated. See the *Permitting for Existing Dischargers* flowchart (Attachment 1).

b) **Fish tissue data available (with *no* criterion excursion in fish tissue at steady-state):** If the NPDES permitting authority has site-specific fish tissue data that represent steady-state (see FAQ 1-6), and the data do not indicate an excursion of the fish tissue criterion elements that are effective under the CWA, the NPDES permitting authority may determine if a discharge has RP by implementing one of several options:

i) If the state or authorized Tribe has also adopted the water column element as part of their selenium criterion in their EPA-approved WQS, the NPDES permitting authority may evaluate RP using the water column selenium criterion element or an EPA-approved site-specific water column selenium criterion element. Other environmental factors may also affect the use of fish tissue data when determining RP. See the *Permitting for Existing Dischargers* flowchart (Attachment 1) and FAQ 2-4.

ii) If the state or authorized Tribe has developed sufficient implementation procedures, and fish tissue data demonstrates steady-state conditions, the NPDES permitting authority may conclude that there is no RP because there is not an excursion of the fish tissue selenium criterion element. In this case, the NPDES permitting authority should consider requesting additional information as part of the application process and establishing ongoing fish tissue monitoring requirements in the permit as well as perhaps other permit requirements (see FAQ 3-2) to demonstrate that fish tissue selenium concentrations remain in steady-state and that there has not been an excursion of the fish tissue criterion element.

iii) The NPDES permitting authority may consider using a certain percentage of the fish tissue criterion element as a maximum threshold above which RP has been demonstrated. The NPDES permitting authority may also consider applying statistical principles, for example a margin-of-safety factor (see the EPA's TSD (USEPA 1991)), to account for uncertainty (due to a limited data set) around detecting the maximum fish tissue selenium value and, as a result, apply selenium concentrations in fish tissue that are lower than fish tissue selenium criterion values when evaluating RP.

c) **Fish tissue data available (with *no* criterion excursion in fish tissue, but the fish tissue data does *not* demonstrate steady-state):** If a state or authorized Tribe has site-specific fish tissue data that do not indicate an excursion of the fish tissue selenium criterion elements, but the NPDES permitting authority determines that the observed fish tissue concentrations are not reflective of steady-state, then the NPDES permitting authority should use the water column selenium criterion element to determine RP. See the flowchart *Permitting for New or Increased Dischargers* (Attachment 1) and FAQ 1-6. If the NPDES permitting authority determines that the selenium levels in fish tissue are not likely to be in steady-state, the NPDES permitting authority may consider requesting additional fish tissue data as part of the application process and establishing in the permit ongoing fish tissue monitoring to ensure that there is not an excursion of the fish tissue selenium criterion.

d) **No fish tissue data available:** If the NPDES permitting authority does not have site-specific fish tissue data or the receiving waterbody is fishless, the NPDES permitting authority should use the water column selenium criterion element for conducting RP analyses.

Q2-4: What data should be used by an NPDES permitting authority to assess RP using criteria based on the EPA's national CWA section 304(a) recommended selenium criterion?

A2-4: NPDES permitting authorities may use any available relevant and representative fish tissue or effluent data to assess RP. See sections 2.1 – 2.3 of the EPA's *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion* for a review of relevant and representative fish tissue monitoring strategies (USEPA 2024c).

For new dischargers, NPDES permitting authorities should use estimates of effluent characteristics (see sections 3.2 and 3.3 of the EPA's TSD (USEPA 1991)) or, preferably, measurements of effluent selenium concentrations to assess RP using the water column selenium criterion elements (see flowchart in Appendix 1, *Permitting for New or Increased Dischargers*). As noted in FAQ 2-3, for existing discharges and when fish tissue is in steady-state, RP analysis may be based on site-specific fish tissue data if the state or authorized Tribe has appropriate quality-assurance procedures for determining RP (USEPA 2021; see also FAQ 2-5). If the NPDES permitting authority has not yet developed NPDES implementation procedures to determine RP using fish tissue, the EPA recommends using the water column selenium criterion elements in RP determinations (USEPA 2021). For other situations where fish tissue is unlikely to be at or determined to not be at steady-state (e.g., recent new or increased inputs of selenium in the watershed), the water column selenium criterion elements should be used to determine RP.

Where states and authorized Tribes use the selenium water column criterion elements to conduct RP determinations, existing NPDES implementation procedures used for other acute and chronic aquatic life protection criteria may be appropriate (see section 3.3 of the EPA's TSD (USEPA 1991)). Additionally, information regarding effluent variation and, where appropriate, available dilution in the receiving stream, should be considered to determine whether the permittee's selenium discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion of the water column selenium elements.

The NPDES permitting authority may take a more conservative approach by presuming RP based on other information provided by the NPDES permit applicant, such as the known presence of other sources of selenium potentially contributing to the receiving water. The lack of other sources of selenium potentially contributing to the receiving water does not preclude a finding of RP and as recommended by the EPA an RP analysis should be assessed for each NPDES facility.

Determinations of RP should be based on analysis of receiving stream assimilative capacity. Because the selenium water column monthly average exposure criterion element is based on a 30-day average value and the effects of selenium on fish are due to bioaccumulation, the NPDES permitting authority may consider using a 30-day flow value for the receiving water when evaluating RP of a discharge.

Q2-5: Where the EPA's national CWA section 304(a) recommended selenium criterion has been incorporated into an EPA-approved WQS, how should a permitting authority evaluate fish tissue data for RP determinations and compliance with WQBELs?

A2-5: Implementation procedures for WQS developed by a state or authorized Tribe should specify the methods to collect fish and analyze fish tissue concentrations of selenium. The implementation procedures should also include procedures regarding sampling design, which fish species will be collected, timing of fish collection, the fish tissue criterion elements being examined, how fish are processed for selenium analyses, and the analytical method used to analyze selenium in fish tissue. The EPA's *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion* provides a review of relevant and representative fish tissue monitoring strategies (USEPA 2024c), which the state or authorized Tribe should consult.

NPDES regulations at 40 CFR 122.41(j) require permittees to perform monitoring representative of the monitored activity and to retain records of this monitoring. In addition, 40 CFR 122.48(b) requires that NPDES permits specify the type, intervals, and frequency of monitoring sufficient to yield data which are representative of the monitored activity. The regulations at 40 CFR 122.44(i)(2) also require reporting of monitoring results with a frequency dependent on the nature and effect of the discharge.

Regardless of the specific sample type (composite samples vs. individual samples), the maximum central tendency (e.g., mean, median, etc.) selenium data from fish tissue for each sampling event should be used in RP determinations and evaluating compliance with WQBELs (see the EPA's *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion* (2024b)). If different samples of fish from a single sampling event are used to determine RP, consistent with the EPA's RP procedures for other pollutants, the maximum fish tissue value (i.e., maximum composite value or maximum central tendency individual fish concentration) obtained from the different samples of fish should be used as the basis for an RP determination based on the fish tissue criterion element evaluated by the fish sampling and analysis (i.e., egg/ovary, whole fish, and/or fish muscle) (USEPA 1991). If there is more than one sampling event, the fish tissue concentration, either the maximum composite value or maximum central tendency individual fish concentration, observed over all sampling events should be used as the basis for RP determinations (see also FAQ 2-3 and section 2.0 of the EPA's *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion* (USEPA 2024c)).

The EPA's fish tissue criterion elements are expressed as instantaneous values, i.e., they are thresholds that are not to be exceeded (USEPA 2021). Therefore, the fish tissue concentration (i.e., maximum composite value or maximum central tendency individual fish concentration) observed over all sampling locations within a sampling event should be used to determine compliance with the WQBEL. Compliance with the WQBEL should be assessed for each sampling event specified in the NPDES permit.

Q2-6: In cases where a discharge occurs to a lotic (e.g., a flowing stream) water, but downstream waters are lentic (e.g., lakes, impoundments), which the EPA national CWA section 304(a) recommended selenium water column criterion elements incorporated into an EPA-approved WQS should be used in the RP analysis?

A2-6: The regulations at 40 CFR 122.44(d)(1) provides that NPDES permit limitations must control all pollutants or pollutant parameters which the NPDES permitting authority determines are or may be discharged at a level which will cause, have the reasonable potential to cause, or contributes to an in-stream excursion above any state water quality standard. This includes water quality standards for downstream waters. The national CWA section 304(a) recommended selenium criterion water column monthly average exposure element for lentic waters is lower than the criterion element for lotic waters. Therefore, if an NPDES discharge is located in lotic waters upstream of lentic waters, the permit writer should ensure that both the lotic WQC and the downstream lentic WQC, are met when conducting the RP analysis and when developing WQBELs for selenium (see 40 CFR 122.44(d)(1)). Additionally, the EPA regulations at 40 CFR 131.10(b) provide that "[i]n designating uses of a waterbody and the appropriate criteria for those uses, the state shall take into consideration the water quality standards of downstream waters and ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." If an NPDES permitting authority bases RP on the fish tissue elements of the selenium criterion using fish from locations near to the discharge, it is then important to document that discharges of selenium do not cause, have the reasonable potential to cause, or contributes to an in-stream excursion of the fish tissue criterion elements in the downstream lentic environment. For additional information on protection of downstream waters, see *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b) and section 4.1 of the *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* (USEPA 2021).

Q2-7: Does EPA have recommendations that address whether a receiving waterbody should be considered lentic or lotic for purposes of RP analysis and NPDES permit WQBEL determinations?

A2-7: In some cases, it might be unclear whether a receiving or downstream waterbody is lentic or lotic with regard to an appropriate selenium water column criterion element (e.g., run-of-the-river reservoirs). Generally, the classification of fresh waters into lotic or lentic categories is made by the state or authorized Tribe, based on site-specific information. States and authorized Tribes should ensure they use transparent, scientifically defensible approaches to appropriately classify receiving waterbodies. Lotic systems such as rivers and streams are characterized by flowing water. Lentic systems, such as lakes and ponds, are characterized by largely standing water. Sections 2.2.2 and 3.2.4, in the EPA's *Aquatic Life Ambient Water Quality Criterion for*

Selenium—Freshwater 2016 (USEPA 2021) provides further discussion of the bioaccumulation of selenium in aquatic systems and categories of fresh water.

A site-specific study or use of available hydrologic information may be necessary to determine the residence time of the waterbody in order to apply the lentic or lotic water column value in a defensible manner for RP analyses. NPDES permitting authorities should determine whether to use the lentic or lotic water column element for a particular waterbody on a case-by-case basis, factoring in downstream waterbody characteristics (e.g., a lake or other lentic system downstream of a lotic system with a point source discharge). One resource that may be helpful for states and authorized Tribes is a geospatial dataset the U.S. Geological Survey (USGS) developed on lotic and lentic waters, available at https://www.sciencebase.gov/catalog/item/5d420fbde4b01d82ce8da8e9.

3. Calculating Water Quality-based Effluent Limits (WQBELs) in NPDES Permits

Q3-1: How should an NPDES permitting authority derive selenium WQBELs for a noncontinuous or intermittent effluent discharge containing selenium?

A3-1: Where the state or authorized Tribe has an EPA-approved WQS based on the EPA's national CWA section 304(a) recommended selenium criterion, for intermittent discharges that occur within a 30-day period, the NPDES permitting authority can derive WQBELs for noncontinuous or intermittent discharges by using the intermittent water column element of the selenium criterion. Under the EPA's CWA section 304(a) recommended selenium criterion, the intermittent water column element is applicable to any receiving water where the long-term instream background concentration is less than the 30-day average criterion value, and where there are instream spikes of selenium concentrations (above the background value) with a duration of less than 30 days. However, in some cases additional data should be collected to determine the duration of instream spikes (e.g., collection of more frequent instream selenium samples, such as daily samples instead of weekly samples). See section 3.3 as well as Appendix J in the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* (USEPA 2021) for more information on the intermittent exposure water column criterion element.

For intermittent discharges, including those that occur less frequently than once per month, permit writers should, at a minimum, assess the long-term average background concentration of selenium in the portion of the stream not impacted by a discharge, as well as the maximum number of days out of any 30-day period that the instream concentration is expected to exceed the long-term average stream background concentration. With respect to a point source discharge of selenium, these in-stream "pulses" are likely to occur on days when the intermittent discharge is occurring, thus, the number of days a discharge is expected to occur may be used as part of the intermittent criterion element as the fraction of 30 days in which an elevated selenium concentration may occur. For discharges that occur less frequently than once per month, the associated fraction would be less than 1/30 or 0.033. As noted in the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* (USEPA 2021), if the fraction (denoted by the EPA as f_{int}) is assigned a value less than one day per month, the intermittent criterion element value could exceed water concentrations that have been shown to be acutely toxic to sensitive species in two- or four-day toxicity tests (USEPA 2004). Because the concentrations that would be acutely toxic in exposures of less than one day might not be much greater than those observed to be toxic in two- to four-day exposures, the EPA notes that the intermittent fraction of the month should *not* be assigned a value less than 0.033 when applying the intermittent selenium water column element.

The number of days an NPDES discharge is expected to occur can be determined from historical effluent data. If sufficient effluent data are not available, data from other sources such as local precipitation maps or data from discharges at other similar NPDES facilities may be used. In cases where selenium concentration data are not available, such as new facilities, permit writers should consider using data from other sources, such as similar NPDES facilities. With that information, the permit writer can use the equation in Table 1 to estimate the calculated value for the intermittent water column element that would be protective on the days when a discharge would occur and could then calculate a WQBEL for the intermittent discharge. The permit writer would determine RP and establish selenium limits using the applicable NPDES implementation guidance (i.e., state or Tribal implementation procedures) that is used for analysis of other water quality criteria, and the projected WQBEL would be calculated using the intermittent water column criterion element.

Q3-2: Can an NPDES permitting authority develop WQBELs using criterion elements based on the fish tissue criterion elements of the EPA's national CWA section 304(a) recommended selenium criterion (i.e., egg-ovary or whole-body and/or muscle) rather than the water column elements?

A3-2: Where the state or authorized Tribe has an EPA-approved WQS based on the EPA's national CWA section 304(a) recommended selenium criterion, any of the four criterion elements can be used for deriving WQBELs pursuant to 40 CFR 122.44(d)(1). If NPDES implementation guidance (i.e., state or Tribal implementation procedures) for the water column selenium criterion elements is currently available to use in developing and establishing WQBELs in NPDES permits, then they should be used if they are protective of WQS.[12] As described by the EPA's *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b), if a state or authorized Tribe believes that the EPA's national CWA section 304(a) recommended selenium criterion elements are not appropriate for a waterbody capable of supporting fish, it can develop a site-specific value for a water column element. Any site-specific WQC that is used for the NPDES permit program must be either approved by the EPA or derived through an EPA-approved water column translation procedure (e.g., performance-based approach). See CWA section 303(c) and 40 CFR 131.21.

While the water column criterion elements are one approach for calculating WQBELs, the recommended primacy of the fish tissue selenium criterion elements may lead NPDES permitting authorities to develop innovative NPDES implementation guidance (i.e., state or Tribal implementation procedures) for the expression of WQBELs based on fish tissue criterion elements. In this case, for existing discharges where fish tissue data demonstrate steady-state,

[12] Note that many states or authorized Tribes have not established NPDES implementation procedures for use in deriving WQBELs from fish tissue data, so the water column element may be the only available choice for deriving a WQBEL that is consistent with the state or authorized Tribe's established procedures. https://www.epa.gov/sites/default/files/2017-08/documents/federal-water-pollution-control-act-508full.pdf

a WQBEL could be expressed in mg/kg dry weight based on either or both fish tissue selenium criterion elements summarized in Table 1 and in *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) or EPA-approved site-specific fish tissue selenium criterion elements. This expression is appropriate when the state or authorized Tribe has developed the EPA-recommended NPDES implementation procedures for the development of WQBELs that will ensure that the fish tissue criterion elements are met, and permit conditions are incorporated to ensure representative monitoring of fish tissue. The EPA supports the development of such innovative approaches to implement water quality criteria based on the EPA's CWA section 304(a) recommended selenium criterion; however, as with any WQBEL expression, the NPDES limit included in the NPDES permit must be consistent with the requirements of CWA section 301(b)(1)(C) as well as 40 CFR 122.44(d)(1)(i) and 122.45 (see FAQ 1-1).

As noted in Table 1 and in *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021), the recommended frequency for the fish tissue criterion elements of the national CWA section 304(a) recommended selenium criterion is "not to be exceeded." Therefore, to ensure that the fish tissue selenium criterion elements are never exceeded, states and authorized Tribes should consider developing NPDES implementation procedures that *prevent* the likelihood of a fish tissue criterion element excursion in the receiving waterbody. For example, in addition to fish tissue selenium monitoring to address the fish tissue based WQBEL, routine monitoring of effluent selenium concentration, as well as selenium concentrations upstream of the permitted discharge, may help determine whether there has been a substantial increase in selenium inputs that would negate a steady-state condition with respect to fish tissue selenium concentration. NPDES regulations at 40 CFR 122.48(b) require monitoring that yields data representative of the monitored activity.

A second NPDES implementation procedure that an NPDES permitting authority may consider when expressing selenium WQBELs in terms of the fish tissue criterion elements is the incorporation of best management practices (BMPs) in the NPDES permit. BMPs, and specifically a selenium minimization plan, may help reduce the likelihood of increases in effluent selenium concentration that could result in a non-steady-state condition in selenium fish tissue concentration in the receiving waterbody. For example, activities involving management of selenium-bearing materials at a landscape scale may require materials management practices that minimize weathering or other mechanisms that mobilize selenium. For NPDES facilities at which selenium discharges cannot be effectively controlled without materials management, source reduction, and/or other operation-specific practices, 40 CFR 122.44(k)(4) may require the permit writer to incorporate selenium BMPs into the permit in addition to numeric WQBELs.

A third NPDES implementation procedure that NPDES permitting authorities could consider incorporating is a permit reopener clause with respect to the selenium WQBEL. Additionally, if fish tissue data, reported during the permit cycle, or information about new or increasing selenium inputs indicate that the receiving stream fish tissue concentrations are not at steady-state, this could constitute cause for permit modification, pursuant to 40 CFR 122.62(a)(2). In this case, WQBELs expressed as fish tissue concentration (mg/kg) should be replaced by

WQBELs expressed as effluent concentration (µg/L). Both fish tissue and water column WQBELs could be derived at permit issuance with the caveat that the fish tissue WQBELs are applicable unless there is new information that precludes fish tissue from being assumed to be in steady-state (e.g., increasing fish tissue concentrations, new or increasing selenium inputs).

Q3-3: The EPA's national CWA section 304(a) recommended selenium criterion does not include an acute expression. Must permits contain both short- and long-term limit expressions?

A3-3: The EPA's NPDES permit regulations at 40 CFR 122.45(d)(1) require that, for continuous discharges, all NPDES permit limits shall, unless impracticable, be stated as maximum daily limit (MDL) and average monthly limit (AML) for all dischargers other than publicly owned treatment works (POTWs). For POTWs, NPDES permit limits should be stated as average weekly or seven-day average and average monthly or 30-day average limitations for most pollutants. The TSD provides procedures to ensure that the calculated limitations are protective of the designated use (e.g., aquatic life) (USEPA 1991).

As explained in section 4 of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) and the Introduction section of this document, the EPA is not recommending a separate acute criterion because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. To meet the NPDES regulatory requirements established in 40 CFR 122.45(d)(1), the EPA provides information for the following scenarios where the permitting authority derives WQBELs from: (1) the fish tissue criterion elements; (2) the chronic water column criterion element; and (3) the intermittent exposure criterion element.

Where a state or authorized Tribe has adopted fish tissue criterion elements and intends to express WQBELs in terms of fish tissue, it may be appropriate for the NPDES permitting authority to consider whether establishing MDLs and AMLs is "impracticable" as authorized by the NPDES regulation.[13] Where WQBELs are expressed in terms of fish tissue the use of an MDL and an AML may be impractical because fish tissue selenium bioaccumulation requires longer exposure periods (see lotic/lentic exposure periods, FAQs 2-6 and 2-7). For example, fish-tissue WQBELs could be developed based on instantaneous measurements which are compared against the selenium WQC when the fish are known to be at steady-state. If the state or authorized Tribe adopts or establishes (e.g., site-specific) selenium criterion that differ from the EPA's 304(a) criterion, or where a state or authorized Tribe's implementation guidance (i.e., state or Tribal implementation procedures) differ from the EPA's TSD, the permitting authority could provide the "impracticability" justification in the permit record. The justification would need to explain why the NPDES permit limit expressions required in 40 CFR 122.45(d)(1) were

[13] The fish tissue criterion may be more appropriately implemented directly as an instantaneous maximum rather than an average value.

impracticable and demonstrate that the WQBELs included in the permit derive from and ensure attainment with the applicable WQS.

Where a state or authorized Tribe has adopted water column criterion elements and intends to express WQBELs in terms of selenium water column concentrations for continuous discharges, the EPA developed a monthly average exposure water column selenium criterion element that is based on a 30-day average exposure concentration. Where the NPDES permitting authority derives WQBELs from the chronic water column criterion element, the EPA's TSD provides a detailed statistical methodology to calculate both maximum daily and average monthly WQBELs from a single criterion value as required by 40 CFR 122.45(d)(1) (USEPA 1991). Since the EPA's national CWA section 304(a) recommended selenium water column criterion elements are based on a 30-day average, a modification to the formula for calculating the chronic long-term average (LTAc) should be used as described in 64 FR 71976.[14] In some cases, the state or authorized Tribe may retain or establish an acute water column criterion that would also need to be considered when developing permit limits (i.e., compare the long-term average [LTA] for acute to the LTA for the chronic to determine the more limiting LTA). The EPA's TSD permit limit development approach derives a single long-term average to calculate the MDL and AML that is deemed to be protective of any applicable criterion by selecting the lowest LTA.[15]

The EPA also derived an intermittent water column selenium criterion element to address intermittent discharges so that the 30-day average water column selenium criterion element (and therefore the fish tissue criterion elements) are met. For intermittent discharges, a maximum daily limitation will most likely be required to ensure that daily discharges are controlled in a manner to ensure that the calculated intermittent criterion[16] is not exceeded and an average monthly limitation would be required to ensure that the underlying 30-day average is met as well.

[14] Water Quality Criteria; Notice of Availability; 1999 Update of Ambient Water Quality Criteria for Ammonia https://www.govinfo.gov/content/pkg/FR-1999-12-22/pdf/99-33152.pdf

[15] The EPA TSD Tables 5-1 (TSD page 102) indicates the formula for calculating the LTA using a n=4. For selenium, the multiplier for n=30 will need to be calculated and used in the derivation of an LTA. The EPA TSD Table 5-2 (TSD page 103) has the 95th and 99th percentile multipliers for n=30, thus these can be used for derivation of an AML (USEPA 1991). https://www3.epa.gov/npdes/pubs/owm0264.pdf

[16] See section 3.3 of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Q3-4: Should the selenium WQBEL be expressed as total recoverable selenium? If so, how should an NPDES permitting authority translate the dissolved selenium water column criterion element under the EPA's national CWA section 304(a) recommended selenium criterion to a total recoverable selenium concentration for an NPDES permit limit?

A3-4: Yes, WQBELs for selenium should be expressed as total recoverable selenium. While the EPA's national CWA section 304(a) recommended selenium water column criterion elements are expressed as dissolved total selenium, the particulate, as well as dissolved selenium, can have deleterious effects on aquatic life. Total recoverable selenium is further discussed in the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium—Freshwater 2016* (USEPA 2021). In the absence of site-specific data, the EPA recommends that it may be appropriate to use a total recoverable-to-dissolved selenium ratio of 1.00 to determine RP and calculate WQBELs for selenium. A ratio of 1.00 means that the concentration of total recoverable selenium is equal to the concentration of dissolved selenium, which may be a valid assumption for some surface waters, especially certain lotic waters, based on geochemistry.

The geochemical conditions governing solubility of selenium will affect the ratio of total-to-dissolved selenium in a waterbody. Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) oxyanions are typically the dominant species under the pH and redox conditions of most surficial aquatic environments (Presser and Luoma 2010). Selenate is highly mobile due to the solubility of its salts, whereas selenite is more likely to be immobilized by adsorption onto particulates, particularly iron oxyhydroxides (Presser and Luoma 2010). An oxygenated water with neutral to slightly alkaline pH favors selenate (Presser and Luoma 2010). Under such conditions, selenate will be the dominant form and the ratio of dissolved-to-total for selenium in the water column would be 1.00 and this is the reason the EPA recommends the use of a ratio of 1.00.

The EPA is aware of alternative total to dissolved selenium translators that have been developed by states for specific waterbodies. Water quality data collected by a state or authorized Tribe, USGS, the EPA, and others should be consulted when considering such translators for a given NPDES permit. Additionally, the EPA has provided previous guidance on procedures for translating a criterion based on the concentrations of dissolved constituent(s) to total recoverable permit limits; the EPA's guidance also includes recommendations for sampling and analysis (USEPA 1996).

4. NPDES Monitoring (Basis for RP and WQBELs, Compliance Monitoring)

Q4-1: What EPA analytical methods are recommended for analyzing water and fish tissue samples for selenium under the EPA's NPDES permits program?

A4-1: The EPA provides analytical methods approved for measuring selenium in wastewater effluent at 40 CFR 136.3 (see Appendix L in USEPA 2021). Use of any other analytical methods for permit compliance monitoring must first be approved through the EPA's alternate test procedures approval process (see 40 CFR 136.4 and 136.5). For selenium fish tissue sampling, the EPA has developed *Technical Support for Fish Tissue Monitoring for Implementing EPA's 2016 Selenium Criterion*, which describes analytical methods that may be used for measuring selenium in fish tissue samples (USEPA 2024c).

5. Compliance Schedules in NPDES Permits

Q5-1: Could a compliance schedule be considered for implementation of WQS based on EPA's national CWA section 304(a) recommended selenium criterion?

A5-1: The NPDES regulations limit the availability of a compliance schedule for new NPDES dischargers. See 40 CFR 122.29(d)(4) and 122.47(a)(2). Consistent with 40 CFR 122.47(a)(2), a permit for a new NPDES discharger could only include a compliance schedule to meet a WQBEL based on the selenium criterion if the criterion was issued after the discharger had begun construction but less than three years before the discharge of concern had begun. A compliance schedule, defined in CWA section 502(17) and 40 CFR 122.2, may be considered in cases where the applicable WQS for the state or for the authorized Tribe (i.e., a Tribe with treatment-as-a-state for the WQS program, or the state or Tribe's implementing regulations authorize the use of compliance schedules, and the discharger needs additional time to meet NPDES permit limits).[17] The EPA's WQS regulations at 40 CFR 131.15 state that if a state or authorized Tribe intends to allow the use of schedules of compliance for WQBELs in NPDES permits, it must adopt a permit compliance schedule authorizing provision. This authorizing provision would be a WQS subject to EPA review under CWA section 303(c). When the EPA has approved a state or authorized Tribe's authorization of the use of compliance schedules, in accordance with the permitting regulations at 40 CFR 122.47, a discharger may be provided with a compliance schedule in its NPDES permit if the permit writer determines that it is appropriate under the circumstances. The NPDES regulations 40 CFR 122.47(a) require that the permit's compliance schedule must require compliance with the WQBEL "as soon as possible." This timeframe would be based on the actions the discharger will need to take to meet its WQBEL. As further detailed in the May 10, 2007 Memorandum, *Compliance Schedules for Water Quality-Based Effluent Limitations in NPDES Permits*, decisions to provide compliance schedules, and the requirements they include are made on a case-by-case basis, considering the facility and site-specific conditions and the requirements in 40 CFR 122.47.[18] Further information is provided in section 3.4 of the EPA's *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards* (USEPA 2024b).

[17] See EAB decision in *Star-Kist Caribe., Inc*. NPDES No. 88-5, 4 E.A.D. 33 (1992).

[18] https://www3.epa.gov/npdes/pubs/memo_complianceschedules_may07.pdf

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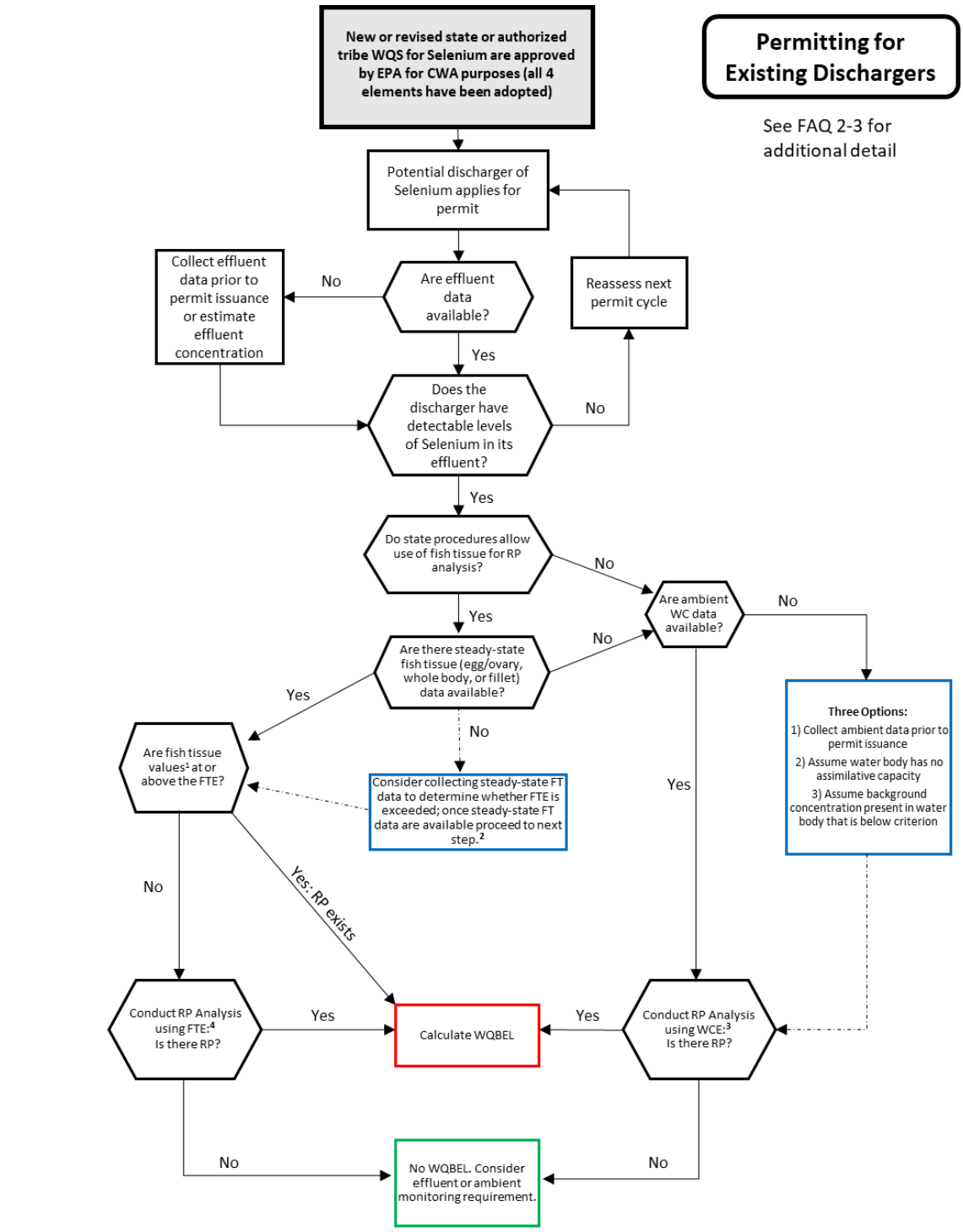
USEPA. 2024b. *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards.* EPA-820-R-24-001. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

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Attachment 1:

The EPA's Recommended Flowcharts for NPDES Implementation of the 2016 National CWA Section 304(a) Recommended Selenium Criterion



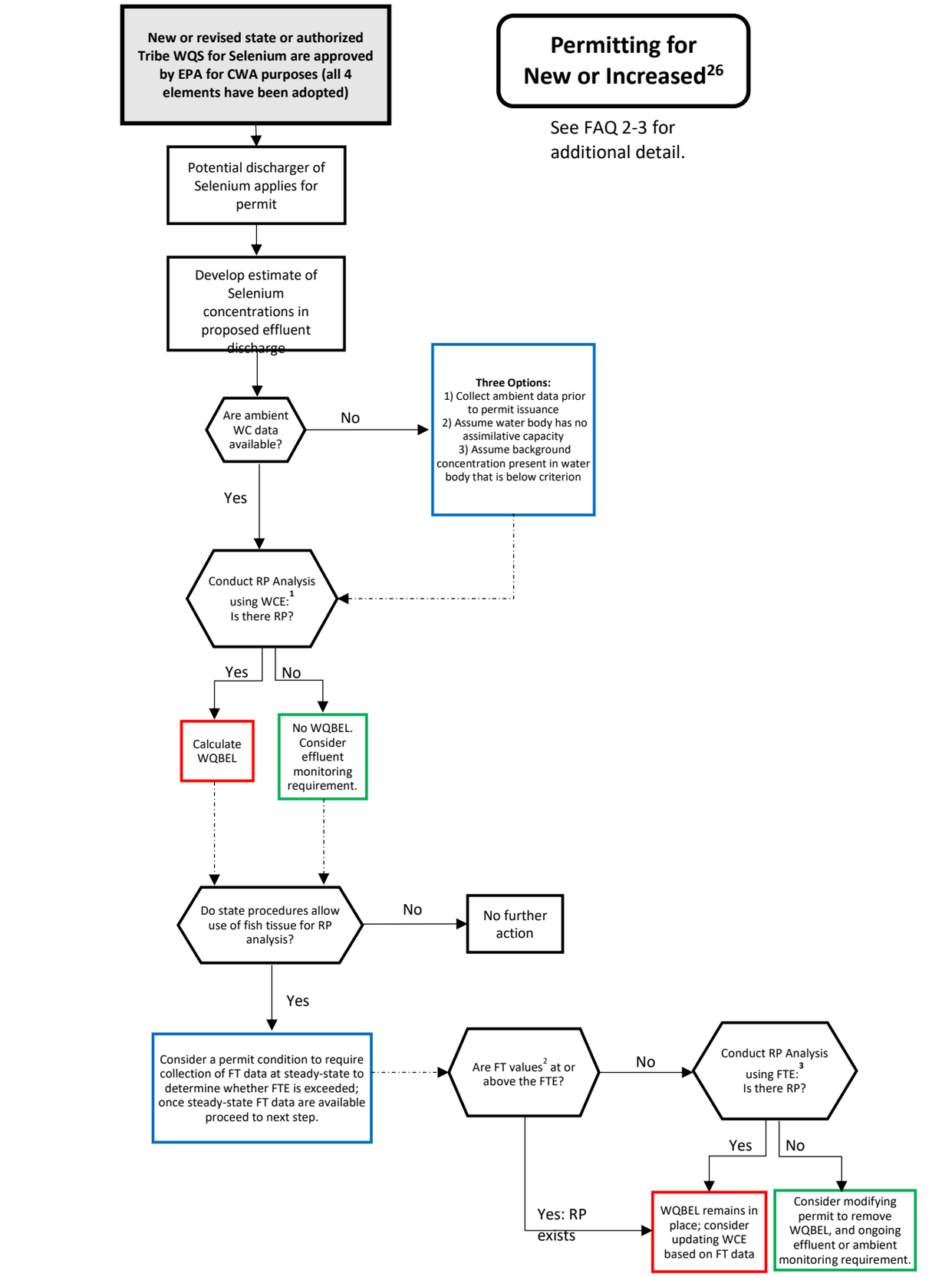
[1] – Fish tissue values may include egg/ovary, whole body, or fillet.
[2] – RP analysis should not be unreasonably delayed pending collection of fish tissue data.
[3] – RP analysis may consider dilution consistent with state WQS and procedures.
[4] – RP analysis using fish tissue must consider the effects of effluent variability and other environmental factors to determine the "potential" for possible excursions of the FTE.

- WCE: Water Column Criterion Element*
- WC: Water Column (Ambient) Selenium Concentration
- RP: Reasonable Potential
- WQBEL: Water Quality Based Effluent Limit
- FT: Fish Tissue
- FTE: Fish Tissue Criterion Element

RP has been determined
Transition box
No RP
Optional pathway

Question

* "Water Column Criterion Element" refers to either:
1. the national default water column criterion element;
2. a state- or site-specific water column value approved by EPA;
3. a site-specific water column value developed from performance-based approach approved by EPA.



[26] This flowchart is appliable for new dischargers or dischargers who have increased their selenium discharges. In these instances, fish tissue selenium concentrations should not be assumed to be in steady-state (see FAQ 1-6 for information on steady-state).

Appendix M

United States Environmental Protection Agency

Office of Water
EPA 820-R-24-003
April 2024

Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion

Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion

While this document cites statutes and regulations that contain requirements applicable to Clean Water Act (CWA) implementation programs it does not impose legally binding requirements on the EPA, states, authorized Tribes, other regulatory authorities, or the regulated community, and may not apply to a particular situation based upon the circumstances. The EPA, state, Tribal and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those provided in this technical support document as appropriate and consistent with statutory and regulatory requirements. The EPA may update this document as new information becomes available. In addition to this document, the EPA has related documents that provide considerations and recommendations on implementing criteria based on the national CWA section 304(a) recommended selenium criterion for freshwater, which are available at the EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

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List of Acronyms and Abbreviations

CF	Conversion Factor
CW	Cold water
CWA	Clean Water Act
EC_{10}	10% effect concentration
µM	Micron
MDL	Method detection limit
MPCA	Minnesota Pollution Control Agency
NAMC-SWG	North American Metals Council-Selenium Work Group
NCCA	National Coastal Condition Assessment
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
PBA	Performance-based approach
QA/QC	Quality assurance/quality control
QL	Quantitation limit
SETAC	Society of Environmental Toxicology and Chemistry
SSD	Species sensitivity distribution
TMDL	Total maximum daily load
TSM	Technical support materials
TTF	Trophic transfer factor
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
WQC	Water quality criteria
WQS	Water quality standards
WW	Warm water
ww	Wet weight

Definitions[1]

Anadromous fish
Fish with a life cycle that is divided between fresh and saltwater, including fish migrating to spawn in freshwater. Migrations should be cyclical, predictable, and cover more than 100 km (FishBase 2016).

Asynchronous spawning
Eggs are released in batches over a period of time that can last days or even months (Murua and Saborido-Rey 2003).

Fecundity
The physiological maximum potential reproductive output of an individual (usually female) over its lifetime (Bradshaw and McMahon 2008).

Gravid
Having the body distended with ripe eggs (FishBase 2016).

Indeterminate fecundity
Potential annual fecundity is not fixed before the onset of spawning and eggs can develop at any time during the spawning season (FishBase 2016).

Iteroparous
Producing offspring in successive batches, for example, annual or seasonal batches, as is the case for most fishes (FishBase 2016).

Method detection limit
The minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results (USEPA 2016).

Quantitation limit
The smallest detectable concentration of analyte greater than the detection limit where the required accuracy (precision and bias) is achieved for the intended purpose (USEPA 2017).

Oocyte
Female sex cell which develops into an ovum. Oogonia become oocytes when meiosis begins, and specialized cells surround each oocyte to form a follicle. The oocyte undergoes maturation in preparation for spawning as an egg (modified from FishBase 2016).

[1]This glossary is meant to provide plain language definitions for key terms used in this document. Individuals should consult the Clean Water Act and the EPA's implementing regulations to identify whether there are legal definitions of these terms.

Potamodromous
Fish species that spend their whole life in freshwater, but generally migrate for spawning purposes, typically back to a natal upstream tributary from a mainstream river or between connected lake and river systems. Migrations should be cyclical, predictable, and cover more than 100 km (FishBase 2016).

Semelparous
Producing all offspring at one time, such as most salmon. Usually these fish die after reproduction (FishBase 2016).

Site
In the context of site-specific criteria, a "site" may be a state, region, watershed, waterbody, or segment of a waterbody. A "site" for fish sampling is a specific waterbody segment.

Synchronous spawning
Eggs are released in a single episode during each breeding season (Murua and Saborido-Rey 2003).

Vitellogenesis
The process by which the yolk is formed and accumulated in the ovum. This is also the period when nutrients stored in the liver are transferred to the developing oocytes in the ovary or ovaries (FishBase 2016).

1.0 Introduction

1.1 The EPA's National CWA Section 304(a) Recommended Chronic Aquatic Life Selenium Criterion in Freshwater

In 2016, the U.S. Environmental Protection Agency updated its national Clean Water Act (CWA) section 304(a) recommended chronic aquatic life criterion for selenium in freshwater systems to reflect the latest scientific information and, in 2021, issued an erratum, *2021 Revision to: Final Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*.[2] The EPA's national CWA section 304(a) recommended selenium criterion still reflects the latest science and the EPA is not aware of any updated scientific information that would change this recommendation. The latest scientific information indicates that selenium toxicity to aquatic life is driven by dietary exposures and that the reproductive life stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium. The recommended criterion has four criterion elements: (1) a fish egg-ovary criterion element; (2) a fish whole-body and/or muscle criterion element; (3) a water column criterion element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent criterion element (to account for potential chronic effects from short-term exposures to high concentrations in lentic and lotic aquatic systems) (see Table 1). Under the EPA's 2016 national CWA section 304(a) recommended selenium criterion, the fish tissue criterion elements have primacy over water column elements, except where there are no fish, where fish tissue data are not adequate, or for waterbodies with new or increased discharges where selenium concentrations in fish tissue might not have stabilized and reached steady-state. The EPA also recommends that the egg-ovary tissue criterion element has primacy over whole-body and muscle tissue criterion elements.

Toxicity data indicate that the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse reproductive effects in aquatic organisms. Toxicity to developing embryos and larvae is directly linked to egg selenium concentration.[3] The EPA derived the whole-body, muscle tissue, and water column elements from the egg-ovary element so that states and authorized Tribes could more readily implement their water quality criteria (WQC) based on the EPA's national CWA section 304(a) recommended selenium criterion. The assessment of the available data on chronic selenium exposure for fish, invertebrates, and amphibians indicates that a criterion element derived from fish is expected to be protective of the aquatic community in a waterbody, since other taxa

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[2] In 2021, the EPA identified that the following text was missing from the second sentence in footnote 4 in the selenium criterion table: "When selenium inputs are increasing" and issued an erratum. The EPA corrected footnote 4 to state: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data."

[3] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

appear to be less sensitive to selenium than fish. The EPA did not develop an acute criterion for selenium when it updated the chronic criterion. Although selenium may cause acute toxicity at high concentrations, the most deleterious effects on aquatic organisms are due to selenium's bioaccumulative properties.

In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from chronic exposure to chemicals via the diet (through the food web pathway). Such studies also do not account for the accumulation kinetics of many bioaccumulative compounds, such as selenium, and may underestimate effects from long-term accumulation in some types of aquatic systems. Therefore, since acute studies do not address the primary exposure pathway for organisms to selenium and because chronic toxicity occurs at lower concentrations, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion. As described in the EPA's *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016 (*hereafter referred to as *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*), the EPA also included an intermittent exposure criterion element to provide protection from the most significant chronic effects of selenium toxicity, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high concentration exposure events.[4]

The EPA recommends, as stated in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* that states and authorized Tribes[5] adopt into their water quality standards (WQS) a selenium criterion that includes all four criterion elements.[6] For more information see the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, which can be found at https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[4] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[5] Throughout this document and in the CWA, the term "states" means the fifty states, the District of Columbia, the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer a CWA WQS program.

[6] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg-ovary[2]**	**Fish Whole-body or Muscle[3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dry weight	8.5 mg/kg dry weight whole-body or 11.3 mg/kg dry weight muscle (skinless, boneless fillet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where $WQC_{30\text{-}day}$ is the water column monthly element for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

1.2 Selenium Technical Support Materials

The EPA has prepared a four-volume set of documents to provide recommendations to states, authorized Tribes, and other agencies for implementing their WQC based on the national CWA section 304(a) recommended selenium criterion for aquatic life.[7] These four documents constitute the Technical Support Materials for the EPA's *Aquatic Life Ambient Water Quality*

[7] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Criterion for Selenium–Freshwater 2016.[8] Each document of the set focuses on a specific aspect of implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. Together, these four EPA documents provide information to assist states and authorized Tribes with adopting WQC based on the EPA's national CWA section 304(a) recommended selenium criterion and implementing them in various CWA programs.

1) *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*: Provides recommendations for the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools.
2) *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*: Provides an overview of how to establish or enhance existing fish tissue monitoring programs to facilitate implementation of fish tissue criterion elements based on the national CWA section 304(a) recommended selenium criterion.
3) *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*: Provides information to help National Pollutant Discharge Elimination System (NPDES) permit writers understand what permitting guidance (i.e., state or Tribal implementation procedures) may be appropriate to implement state and authorized Tribal WQS based on the EPA's national CWA section 304(a) recommended selenium criterion. This set of FAQs also provides recommendations on how to establish water quality-based effluent limits (WQBELs) in NPDES permits.
4) *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*: Provides information on how to complete assessments, list impaired waters, and develop TMDLs to implement the EPA-approved[9] WQS that are based on the EPA's national CWA section 304(a) recommended selenium criterion.

1.3 Document Overview

This document, *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*, is intended to provide recommendations for sampling fish tissue for the implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. The recommendations provide information for states and authorized Tribes to consider so they can collect fish tissue samples that represent the selenium exposure and vulnerable species at a particular site. The recommendations are written assuming all four

[8] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[9] Approved by the EPA includes both WQS adopted by states, territories or authorized Tribes and approved by the EPA and WQS promulgated by the EPA.

criterion elements of the national CWA section 304(a) recommended selenium criterion were adopted by a state or authorized Tribe along with the recommended hierarchical structure of the criterion. These recommendations may also be helpful to states and authorized Tribes that have not yet adopted WQC based on the national CWA section 304(a) recommended selenium criterion but are considering doing so and are interested in evaluating the concentration of selenium within their fish populations.

This document examines technical considerations for developing a robust sampling program to characterize selenium concentrations in fish tissue for a variety of CWA implementation programs (e.g., CWA section 303(d) listing and TMDLs). Some aspects of this document can also be used to support the development of site-specific water column criterion elements. Site-specific water column criterion elements can be developed by performing a site-specific water column translation from the fish tissue elements of the criterion. See Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) for site-specific approaches for translating between fish tissue and water column concentrations.

Site-specific conditions may at times impact field collection decisions, particularly conditions such as fish abundance, species availability, and site accessibility. The EPA recommends that states and authorized Tribes use the following document as a tool for developing field sampling plans with the knowledge that there may need to be deviations from these plans due to actual conditions at a site. To maintain this flexibility, the EPA recommends that states and authorized Tribes incorporate these recommendations into guidance and use them for planning purposes, rather than adopting them into a rule or other legally binding mechanism. The one exception is if a state or authorized Tribe develops a performance-based approach (PBA) for translating fish tissue criterion elements into site-specific water column criterion elements. A PBA is a WQS that is a transparent process, such as a criterion derivation methodology, rather than a specific outcome, such as a concentration of a pollutant. The approval of a PBA would also serve for CWA purposes as the approval of each outcome generated from following that process or method. States and authorized Tribes can adopt PBAs as part of their selenium WQS that must be submitted to the EPA for review and approval or disapproval, in accordance with the CWA section 303(c) and the EPA's implementing regulations at 40 Code of Federal Regulations (CFR) Part 131. Information from this technical support material can be useful in developing parts of the methodology included in a PBA. Since a PBA is used to generate values used for CWA purposes, that methodology must be legally binding. For more information on the PBA, see section 2.2.1 of *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards* (USEPA 2024c).

This document is intended to assist states and authorized Tribes in planning for and enhancing their monitoring programs to collect fish tissue samples that are representative of the selenium exposure and species that are present at a site; it is not intended to limit the fish tissue data that states and authorized Tribes could evaluate for waterbody assessments with the recommended criterion. In other words, these recommendations should not be used to exclude the consideration of data for assessment decisions, but rather could be used in planning the collection of that data. The EPA's regulations require that states and authorized Tribes evaluate

all existing and readily available water quality-related data for assessment decisions (40 CFR 130.7(b)(5)), which for WQS based on this criterion would include selenium data and information for any fish species. For additional information about assessment decisions for this criterion, please refer to the *Frequently Asked Questions: Implementing the 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs* (USEPA 2024a).

This document does not specifically address the site-specific modification of the fish tissue criterion elements, which can be developed using fish assemblage data and the recalculation procedure (USEPA 2013). States and authorized Tribes interested in developing site-specific fish tissue criterion elements should engage their EPA regional office early in the process to evaluate this option.

2.0 Monitoring Strategy

The EPA's national CWA section 304(a) recommended selenium criterion was designed to protect "populations of fish, amphibians, aquatic invertebrates, and plants" (USEPA 2021). As studies have shown that selenium is accumulated in organisms primarily through dietary exposure and that fish are the taxa most sensitive to selenium within the aquatic community, fish tissue criterion elements were developed as part of this criterion (USEPA 2021). When developing monitoring strategies for CWA implementation of criteria based on the national CWA section 304(a) recommended selenium aquatic life criterion, the EPA recommends that states and authorized Tribes review their existing fish tissue monitoring programs and determine how best to incorporate the collection of fish tissue samples for such implementation into their existing monitoring programs. For example, existing fish tissue monitoring programs may already be collecting data to assess the risk of fish consumption to human health. These existing monitoring programs may be designed with different objectives than monitoring activities designed to assess the attainment of an aquatic life criterion. These differences should be considered and reconciled when using an existing fish tissue monitoring program for assessing the fish tissue criterion elements of the selenium aquatic life criterion.

The following sections discuss study design and sampling considerations regarding fish tissue types, sample types, target species, and spatial and temporal variability. These topics should be considered to ensure the collection of a fish tissue sample that is representative of the selenium exposure and the species present at a site. These topics should be considered when collecting data to assess fish tissue concentrations against the fish tissue criterion elements of the states or authorized Tribes' selenium aquatic life criterion or collecting data to translate a fish tissue criterion element into a site-specific water column criterion element.

Additionally, the relationship between fish sampling locations and timing, species' habits and natural history, and the selenium source(s) should be understood and accounted for during the design of sampling plans for criterion implementation. Fish tissue sampling methods should be designed to characterize the variability of selenium in the target population, including areas of high selenium bioaccumulation.

Since the national CWA section 304(a) recommended selenium criterion establishes population-level protection, the EPA generally recommends implementing these fish tissue criterion elements with a measure of central tendency (e.g., mean, median) when the selenium concentration in fish tissue is determined to be at steady-state, as this will represent the level of exposure that the population as a whole is experiencing. When characterizing the sample population's exposure, the monitoring data set and information about the underlying distribution of the sample population should be used to determine which measure of central tendency is most appropriate. In addition, the variability around that measure of central tendency (e.g., standard deviation, standard error, confidence interval) should be quantified and considered when making implementation decisions. The EPA recommends using a measure of central tendency only for characterizing a data set that was collected during a single sampling event (identified location over a specified period of time (generally less than a week)). For implementation purposes, a measure of central tendency should not be calculated between different sampling events; instead, different sampling events should all be considered as discrete data sets.

However, there are some situations where it may be more appropriate to implement the criterion using a metric other than a measure of central tendency, such as using the upper bound of the confidence interval or using the maximum measured value of all data sets. For example, it could be appropriate to use the maximum value from a single sampling event to determine reasonable potential for an NPDES permitted discharge (USEPA 1991). For more information, see *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*.

The EPA encourages states and authorized Tribes to have early discussions with their EPA regional office to ensure that studies capture the appropriate data. Detailed examples of field collection procedures and sampling design considerations can be found in *Sampling Protocols for Collecting Surface Water, Bed Sediment, Bivalves, and Fish for Priority Pollutant Analysis* (USEPA 1982); *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis* (the EPA's *Fish Advisory Guidance Volume 1*) (USEPA 2000); *Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue* (USEPA 2002a); the EPA's National Aquatic Resource Survey Field Operations Manuals for rivers and streams and Great Lakes and coastal waters (USEPA 2019a, 2019b, 2015); and *Protocols Manual for Water Quality Sampling in Canada* (CCME 2011). Appendix A of this document presents egg and ovary collection and sample preparation methods.

2.1 Tissue Type

The EPA recommends sampling egg-ovary tissue, when possible. From a toxicological standpoint, the most representative measure of exposure to a toxic substance is its concentration at the site of toxic action. In fish (the aquatic species most sensitive to selenium), the most ecologically relevant sites of toxic action are the mature reproductive tissues of adults (ovaries) or early life stages (vitellogenic eggs/larvae). This was a major point of consensus at

the 2009 Society of Environmental Toxicology and Chemistry (SETAC) Pellston workshop on selenium risk assessment (Chapman et al. 2009). Therefore, the national CWA section 304(a) recommended selenium criterion is based on reproductive effects in fish, as represented by selenium concentrations in egg-ovary tissue.

While egg-ovary tissue of adult female fish is the most direct reflection of reproductive toxicity in fish, samples of muscle or whole-body tissue from adult fish serve as robust alternatives and may be collected for implementation purposes when egg-ovary tissue is impractical to sample.

Collection of fish samples for egg-ovary analysis poses special challenges as only gravid female fish can be sampled. Due to this constraint, the decision of what tissue type to collect should be made based on the following considerations:

- *Temporal*: Most fish species that are synchronous spawners spawn in the spring; whereas fish tissue collection for fish consumption advisories typically occurs in the late summer or early fall, when contaminant loads in the edible portion of the fish are highest. Spring sampling may also be challenging in states or on authorized Tribal lands where rivers and streams have high flows due to storm run-off and spring snow melt. Timing the collection of mature eggs from asynchronous spawners can also be challenging, as these species can have eggs in various stages of development at once.

- *Spatial*: Some fish species migrate to upstream areas to spawn, and these areas may be harder to access than higher order downstream segments that are inhabited during non-spawning seasons.

- *Size*: It is difficult to collect and analyze egg-ovary tissue samples from small fish species (e.g., certain species in the family Cyprinidae or Cyprinodontidae) due to the logistics of the collection and the small amount of tissue available for analysis (number of eggs or biomass).

Due to these considerations, states and authorized Tribes have considerable discretion when selecting the fish tissue type to collect in their sampling protocols. The flexibility provided by being able to collect data from multiple fish tissue types allows for leveraging existing monitoring capacity. A number of the species that are good target species for selenium sampling could also be commonly collected as muscle (fillet) samples in state and authorized Tribal fish tissue monitoring programs for other contaminants (e.g., trout, salmon, bass, sunfish) (see section 2.3 for more information on target species). The whole-body tissue criterion element also simplifies the collection and processing of small fish species that may be the dominant trophic level in lower order stream networks.

When developing a new–or modifying an existing–fish tissue monitoring strategy, states and authorized Tribes may want to consider funding and staff resources, opportunities to work with other fish tissue monitoring programs, existing information on the spawning habits and size of target species, and potential population level effects associated with using lethal sampling techniques when deciding what fish tissue type to sample. To keep the public, stakeholders,

and the EPA well informed, it is good practice for monitoring programs to describe in their sampling protocols why they are sampling a particular tissue type. Similar considerations might also be evaluated when selecting a tissue to sample for the development of a site-specific water column criterion element. If possible, the EPA recommends that egg-ovary tissue be sampled to conduct a site-specific water column translation (see section 2.1.1). Sampling considerations associated with different types of fish tissue are presented in Table 2.

Table 2. Sampling Considerations Associated with Different Types of Fish Tissue.

Issue	Egg-ovary	Whole-body	Muscle/Fillet	Comments
Criterion Hierarchy Considerations	Has primacy in the criterion; supersedes other fish tissue criterion elements and the water column criterion elements	Supersedes water column criterion elements; equal consideration to muscle tissue	Supersedes water column criterion elements; equal consideration to whole-body tissue	While selenium concentrations in all three tissue types are significantly correlated to reproductive toxicity effects seen in fish, egg-ovary concentrations have the strongest relationship.
Ease of collection	Difficult	Easy[a]	Easy – except on small fish[a]	Egg-ovary samples are only collected from gravid females; there are seasonal and logistical considerations, and species-specific sampling windows. See Appendices A and B.
Consistency with existing state & authorized Tribal methods	Not typically collected	Sometimes collected	Primary tissue collected	Whole-body samples might be collected in special cases, such as for certain human populations that consume whole fish or for ecological risk assessments.
Sample availability	Limited–only from gravid females	Always	Always	For waterbodies with small sized species at top trophic levels, whole-body may be the only option due to issues collecting a sufficient mass of muscle tissue.

Issue	Egg-ovary	Whole-body	Muscle/Fillet	Comments
Ability to make composite sample	Yes	Yes	Yes	Compositing can be used to reduce the overall cost of an analytical program, primarily by reducing the number of samples that must be analyzed to represent an average concentration. Compositing can also ensure that enough mass is available for chemical analyses. However, by compositing samples, information on the range of selenium concentrations in individual organisms is lost.
Ability to test individual sample	Yes, on larger species; may be difficult on small species	Yes, on larger species; may be difficult on small species	Yes, on larger species; may be difficult on small species	Individual samples are valuable when sampling from waters known or suspected to be impacted by selenium discharges (see section 3.2.2); however, the need to analyze multiple individual samples versus a few composite samples can make them more resource intensive to prepare and expensive to analyze.

[a] Availability of fish may make collections challenging. If sampling will result in detrimental impacts on the fish populations of a waterbody, the EPA recommends collecting water samples instead.

2.1.1 Egg-ovary Tissue Sample

Selenium concentrations in all three tissue types are significantly correlated to reproductive toxicity effects seen in fish; however, egg-ovary concentrations have the strongest relationship. The EPA recommends sampling fish egg-ovary tissue for assessment of the selenium aquatic life criterion or for development of a site-specific water column criterion element, when possible. Egg-ovary tissue refers to mature eggs, pre-spawn ovary tissue that contains mature eggs, or both. As an oocyte grows into a mature egg, it passes through several stages of development (i.e., oogenesis, primary oocyte growth, cortical alveolus stage, vitellogenesis, maturation, and ovulation) (Tyler and Sumpter 1996). During this egg development process, the oocyte size increases dramatically as the yolk is developed. For example, the diameter of an undeveloped oocyte of the Rainbow Trout is around 20 µm and the fully developed egg is about 4 mm (Nagahama 1983). Selenium is transferred from an adult female fish to her eggs during vitellogenesis. Eggs should not be collected until after this transfer has occurred as this will

more accurately represent larval exposure to selenium. Appendix A of this document presents egg and ovary collection and sample preparation methods.

Egg-ovary tissue from pre-spawn, reproductively mature (also called "gravid" or "vitellogenic") females is the preferable tissue to collect because it will give the most accurate representation of potential selenium hazard to reproduction. Egg-ovary tissue data provide point measurements that reflect integrative dietary accumulation, transfer, and deposition of selenium over time and space in female fish at a given site. Research has shown that selenium concentrations in egg-ovary tissue is strongly correlated with selenium in the maternal diet (Janz et al. 2010).

When using egg-ovary tissue for the implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, states and authorized Tribes should carefully consider the difficulty in timing egg-ovary sampling with spawning periods. Ovary tissue sampled from a female that is not gravid will not be representative of the selenium concentrations of this tissue for a gravid individual. A female is typically gravid for a small window of time for most synchronous species; the timing of the spawning season will depend on the species, geography, and a number of environmental cues (e.g., temperature, flow, photoperiod). In northern latitudes or higher elevations, spring spawning may occur slightly later and fall spawning may occur slightly earlier than in southern latitudes or lower elevations. For more information on expected spawning seasons, see Appendix B. The EPA recommends that states and authorized Tribes consult with local fish biologists, who may work at other state or authorized Tribal agencies (e.g., Departments of Natural Resources, Departments of Fish and Game), when designing sampling plans.

Reproductively mature females of most fish species, except indeterminate spawning species and viviparous species (i.e., live bearing), will produce eggs that can be sampled for selenium. However, it may be impractical to sample eggs from small-bodied fish. In this instance, the whole-body should be collected (with the eggs if the fish is female) and compared to the whole-body criterion element. Ovary tissue of synchronous spawners (e.g., species in the genus *Oncorhynchus*) typically contain oocytes that are all in the same stage of development. Fish species that spawn multiple times per season (asynchronous, e.g., some species in the family Cyprinidae) have variable cycles of oogenesis and commonly have ovary tissue that contains oocytes and eggs at all stages of development (Nagahama 1983). In these asynchronous spawners, egg maturation may occur well before, immediately prior to, or during the spawning season. For example, Green Sunfish can spawn multiple times per season (Osmundson and Skorupa 2011, Chapman et al. 2010). Thus, special care should be taken when sampling asynchronous species for egg-ovary tissue, as the pre-spawn window can be hard to predict.

Given these considerations, the EPA has the following recommendations when sampling female asynchronous spawners: (1) if the fish is too small to easily sample the egg-ovary tissue, the whole-body should be sampled (including the eggs) and the selenium concentration should be compared to the whole-body criterion element; (2) if fish are sampled during the reproductive season and they are large enough to easily sample the egg-ovary tissue, this tissue should be

sampled (the 75% rule does not apply to egg-ovary composite samples (see section 2.2.1 below)); and (3) muscle tissue should not be sampled during the reproductive season as selenium may be depleted from this tissue during this time.

The egg-ovary tissue criterion element has primacy over all other criterion elements of the national CWA section 304(A) recommended selenium criterion. The EPA recognizes that many states and authorized Tribes do not currently collect egg-ovary tissue as part of their regular monitoring programs and may not have the resources to augment their existing monitoring programs to include egg-ovary tissue collection. While egg-ovary remains the preferable tissue type, whole-body or muscle tissue samples can be used as an alternative.

2.1.2 Whole-body and Muscle Tissue Samples

Whole-body and muscle tissue samples may be collected as an alternative to egg-ovary tissue. States or authorized Tribes might choose to use whole-body or muscle tissue samples because egg-ovary tissue is not available year-round and because of the difficulty with appropriately timing the collection of egg-ovary tissue. States or authorized Tribes might also choose to use whole-body samples because a small-bodied species might be the most appropriate species to sample in a particular situation, such as in a low order streams where only small-bodied species are available for sampling (Beatty and Russo 2014). In addition, states and authorized Tribes may sample whole-body or muscle tissue because existing monitoring programs can more readily incorporate such selenium analysis into their existing fish tissue monitoring strategies.

Selenium concentrations in these tissues will provide representative information on selenium bioaccumulation and ecological exposure at almost any time of the year (except pre- and post-spawn windows for females). However, there will likely be some variation across seasons due to dietary composition, temperature, depuration of selenium from tissue during vitellogenesis prior to spawning, and other factors. The only time of year that should be avoided for collecting whole-body or muscle tissue samples from female fish is directly pre- and post-spawn because they could have reduced selenium concentrations in their tissues due to the recent transfer of selenium to eggs (unless eggs are included in a pre-spawn whole-body sample) (USEPA 2021).

Summer and fall may be prime periods for whole-body and muscle tissue collection from spring spawners due to the engorgement of populations to replenish fat and energy reserves post-spawn and for over-wintering. If sampling fall spawners, spring and summer should be targeted for sampling. Winter tissue collection is discouraged, except in subtropical regions where metabolic changes due to lower water temperatures do not occur. Whole-body and muscle tissue data provide point measurements that reflect integrative dietary accumulation and deposition of selenium in fish tissues over time and space in fish population(s) at a given site.

The EPA is aware that some states and authorized Tribes use muscle plugs in their monitoring programs as a non-lethal alternative to collecting muscle fillets. States or authorized Tribes that utilize plugs should be aware of certain considerations. Contaminant concentrations can vary considerably depending on where the plug is taken from a fish. Plugs should be collected from a

descaled meaty portion of the dorsal muscle tissue, between the dorsal fin and lateral line (USEPA 2019a). Waddell and May (1995) found that selenium concentrations in plugs from this location were significantly correlated to adjacent muscle tissue. Studies with mercury have also shown that this location results in a sample that has homogeneous concentrations and concentrations that were similar to mean concentrations for a muscle fillet (Cizdziel et al. 2002). Plugs provide very small tissue masses (about a gram of tissue per fish) and may not provide enough biomass for reanalysis or quality assurance/quality control (QA/QC) analysis. This may lead to difficulty confirming the quality of the sample analysis. In addition, relatively small individuals may not recover from a muscle plug biopsy punch. Care should be taken to ensure that there is enough tissue for the analytical method. States and authorized Tribes may want to consider compositing muscle plugs to attain tissue masses that are needed for analysis (see section 2.2.1 for more information on compositing samples).

In addition, states and authorized Tribes may want to establish species-specific conversion factors or regressions at the start of their sampling program that quantify the relationship between the muscle plug concentration and the muscle fillet concentration so that selenium concentrations from plugs can be appropriately compared to the muscle tissue criterion element. If taking this approach, the EPA recommends consulting a statistician about determining a sufficient sample size for establishing the regression. The EPA has also conducted a study to test the applicability of plugs for monitoring fish tissue for selenium and better define the relationship between selenium concentrations in muscle plugs and muscle fillets. The EPA found that there were no statistically significant differences between log concentrations of fillet plugs and log concentrations of homogenized fillets at the community level. However, the authors of this study did caution that when using a selenium plug monitoring alternative, samplers must employ a sufficiently sensitive analytical method and consider total solids in the tissue samples (Stahl et al. 2021).

A specific case where sampling whole-body or muscle tissue is recommended over sampling egg-ovary is for sampling anadromous juvenile Pacific salmonids. This is because the whole-body and muscle tissue of these juvenile fish are more representative of the selenium exposure of a site than the adults of these species. Anadromous fish species start their lives in freshwater, then as juveniles the fish undergo smoltification and migrate to the ocean, where they stay until adulthood before migrating back into freshwater to spawn. These species include but are not limited to Coho, Chum, and Chinook Salmon, and marine adapted Rainbow Trout (steelhead).[10] Adult anadromous females (in the genus *Oncorhynchus* – except steelhead and Brown Trout) stop eating prior to re-entering freshwater environments as part of the physiological modifications required for the migratory spawning process, and thus, lack exposure to freshwater selenium sources. They are also semelparous (except steelhead), meaning they die after spawning so there is no post-spawn residual exposure. Since adults of these species are not residents of the waterbody, the selenium concentrations will not be

[10] Before sampling these species, states and authorized Tribes should determine whether or not they are listed as threatened or endangered in the sampling location and whether a "take" permit may be required for sampling or where an alternative species should be sampled.

representative of localized freshwater selenium sources (see section 6.4.1 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*) (USEPA 2021). Given this lifecycle, it is not appropriate to sample adult semelparous anadromous fish for assessment of freshwater waterbodies, as their selenium concentrations will not be representative of selenium exposure from where they are sampled. Instead, the juvenile fish from these species should be sampled, so they will reflect local selenium concentrations. An exception are landlocked variants of Striped Bass that cannot migrate out to sea, or hybrids (e.g., "wipers" which are Striped Bass-White Bass crosses) in the Midwest. Adult fish in these landlocked populations may be representative of localized freshwater selenium concentrations, and thus appropriate for sampling. Although more uncertain, some studies indicate that selenium might affect endpoints such as juvenile growth and survival (Hamilton et al. 1990, DeForest and Adams 2011), therefore the most appropriate tissue to sample for Pacific anadromous salmon smolt is the whole-body.

Researchers are still investigating, on an ongoing basis, the impacts of reproductive strategies on fish selenium concentrations; however, it appears the reproductive strategy employed by a fish species may impact the distribution of selenium throughout a fish's tissues. This relationship, quantified by a conversion factor (CF), can be dynamic between tissue types, particularly between egg-ovary tissue and other fish tissue depending on the stage of the reproductive cycle (Conley et al. 2014). This may be especially true with asynchronous spawners, which can have oocytes in multiple stages of development at once (Conley et al 2014). Asynchronous spawners may transfer selenium more quickly to eggs and ovaries than synchronous spawners, which may make their egg-ovary to whole-body and muscle ratios more variable than synchronous spawners. In addition, the amount of selenium in muscle tissue can be limited compared to other fish tissue such as the liver (Mathews et al 2008) or egg-ovary (Herrmann et al. 2020). As selenium could be limited in muscle tissue, states and authorized Tribes may want to consider sampling whole-body tissue, rather than muscle, particularly for asynchronous spawners to capture the selenium that is distributed to other tissues.

Due to the variability in CFs within and between species, there can be times where data from one tissue type may be higher than its criterion element while data from another tissue type may be below its criterion element for the same waterbody. In these situations, the hierarchy (i.e., egg-ovary has primacy over all other tissue types) should be followed, and an implementation decision should be made based on which tissue type has primacy. This is more likely to be an issue for species that have large CFs between tissue types, such as Mountain Whitefish. If possible, egg-ovary samples should be taken from these species to make the most accurate assessment about potential selenium effects on fish.

2.2 Sample Type

States and authorized Tribes have flexibility in the type of sample that is collected to represent an instantaneous measurement of selenium in a fish population at a given site (see criterion duration footnote 6). Samples can include composites of multiple fish or the collection of

individual fish in the population. The field sampling and analytical considerations for both sample types are described below.

2.2.1 Composite Samples

Composite samples are homogeneous mixtures of one type of tissue (e.g., egg-ovary, whole-body, or muscle tissue) from two or more individual organisms of the same species, collected at a particular place and time, and analyzed as a single sample. Composite samples can be useful for collecting enough tissue from small fish species to perform the appropriate analyses. Composite samples also allow for the analysis of additional target analytes if fish tissue samples are being collected as part of a broader fish tissue monitoring effort. Because chemical analytical costs are usually higher than field costs, using composite samples may be a cost-effective way to represent average selenium tissue concentrations in target species sample populations by reducing the number of individual chemical analytical samples that are needed to characterize concentrations in the sample population (Patil et al. 2011). Composite sampling may also help with the issue of determining how to incorporate a sample with a concentration below the method detection limit (MDL) into an average, as the composite represents a physical averaging of the samples (USEPA 2011). Composite samples have also been shown to provide equivalent estimates of the mean compared to individual samples (Zhou et al. 2018, USEPA 1995). However, with composite samples, extreme contaminant concentration values for individual organisms are attenuated (Patil et al. 2011). Information from each individual sample is lost, which may mean losing information about variability within a site. Also, if a set number of fish are being analyzed, compositing those fish rather than analyzing them individually will result in fewer data points, which can potentially lead to having less power in statistical analyses. However, this will be dependent on the data set and knowledge about the underlying sampling distribution. Hitt and Smith (2015) found that composites of two to four fish did not decrease power relative to individual samples when the sampling distribution was known, because the composites had greater precision for estimating the mean (but power did decrease when an empirical sampling distribution was constructed from prior research instead of being known).

The EPA's *Fish Advisory Guidance Volume 1* recommends collecting three to ten individuals for a composite sample for each target species, as availability allows (USEPA 2000). This is similar to recommendations from the National Water Quality Assessment Program administered by the U.S. Geological Survey (USGS) for collecting at least five fish, but eight, if possible, per composite (Crawford and Luoma 1993). It is also in line with the Base Monitoring Program of the Great Lakes Fish Monitoring and Surveillance Program. This program also generally includes five fish in each of its composite samples, as five represents a reasonable number of fish that also satisfy statistical requirements for that study (USEPA 2012). Section 6.1.2.7.1 ("Guidelines for Determining Sample Sizes") of the EPA's *Fish Advisory Guidance Volume 1* maintains that it is not possible to recommend a single set of sample size requirements for all fish contaminant monitoring studies (USEPA 2000). Rather, the EPA presents a more general approach to sample size determination that is both scientifically defensible and cost-effective. Table 6-1 in the EPA's *Fish Advisory Guidance Volume 1* shows the varying precision achieved by using additional

numbers of individuals per composite and additional replicate composite samples. The data suggest that greater precision in the estimated standard error is gained by increasing the number of replicate composite samples than by increasing the number of fish per composite (USEPA 2000). The EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends collecting at least two composite samples at each site, and encourages a third, in order to properly estimate the site variance. An alternative sampling approach may be to collect a greater number (five or greater) of smaller composites (two to three fish), which would increase sample size and statistical power, but still minimize resource expense compared to individual sampling (USEPA 2018, Hitt and Smith 2015).

As a rule of thumb, if a state or authorized Tribe does not have previous data available for its sampling location, the EPA recommends composites of at least five fish be used for fish tissue monitoring for CWA implementation of the selenium criterion (USEPA 1982). The EPA recognizes that sometimes it might not be possible to collect a five-fish composite or collecting that many fish may impact the fish population at a location. In these cases, the EPA encourages the state or authorized Tribe to get as close to five fish as possible in the composite given resources and the fish population at a site. Given the large number of variables that go into fish collection, the EPA does not recommend including rigid minimum sample size requirements in CWA implementation programs. Rather, implementation decisions (e.g., impairment decisions) should be made based on the available data for a waterbody. The one exception is if a state or authorized Tribe adopts a PBA for translating water column criterion elements into fish tissue criterion elements. The PBA, as a criterion derivation process, includes information (such as minimum sample sizes) to ensure scientifically defensible, transparent, and repeatable results are obtained (see section 2.2.1 of USEPA 2024c).

The spatial variability of a site should also be considered when collecting composite samples. If a site is particularly large with high variability in selenium throughout, the site may need to be divided into subsites and composites collected from within each subsite to appropriately quantify the selenium impacts at that site.

The EPA recommends that fish used in a composite sample meet the following specifications:

- All the same species (USEPA 1982, USEPA 2000, Crawford and Luoma 1993, USEPA 2012);
- Of similar size (Crawford and Luoma 1993), so that the smallest individual in a composite is no less than 75% of the total length (size) of the largest individual (USEPA 2000, USEPA 2012) (the "75% rule"; this "75% rule" does not apply to fish from which egg-ovary samples are collected);
- Collected at the same time (i.e., collected as close to the same time as possible, but all samples should be collected within a week of each other) (USEPA 2000, USEPA 2012); and
- Collected in sufficient numbers to provide a composite homogenate tissue sample of at least 20 grams wet weight for selenium analysis (USEPA 2000).

The EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends including an equal number of fish in each composite sample and collecting two to three composite samples. However, when sampling fish in waters potentially impacted by selenium, the number of composite replicates may be determined on a case-by-case basis. This decision would primarily be based on the amount of variation in selenium concentration expected at the site and the number of individuals of the target species present at the site.

As species have different selenium bioaccumulation potentials and different sensitivities to selenium (USEPA 2021), it is not scientifically defensible to create a composite sample that consists of more than one species. Compositing individuals that are the same genus, but not the same species is not appropriate. Accurate taxonomic identification is essential to prevent the mixing of species in a sample.

The EPA recognizes that, in contrast to some other bioaccumulative contaminants in fish, selenium concentrations are generally conserved across fish size (May et al. 2008). But many states and authorized Tribes may add selenium monitoring to their existing fish tissue monitoring programs that assess the risk of fish consumption to human health from a number of chemicals. Many other chemicals that are assessed through this monitoring are persistent, bioaccumulative chemicals, such as mercury, polychlorinated biphenyls (PCBs), and other substances that biomagnify through the food web and build up over the lifetime of the fish, resulting in greater concentrations of chemicals in larger fish. Therefore, for these fish consumption monitoring efforts, a common guideline is that fish to be composited should be a similar size so that the smallest individual in a composite is no less than 75% of the total length (size) of the largest individual (USEPA 2000). If a state or authorized Tribe is combining their monitoring efforts for selenium and fish consumption advisories, the EPA also recommends following the "75% rule" for the sizes of individual specimens included within a composite when sampling whole-body or muscle tissue (this 75% rule recommendation does not apply to fish collected for egg-ovary samples). In addition, following the 75% rule for whole-body samples included in a composite ensures that all fish samples are making relatively equal contributions to the composite sample.

Individual fish used in a composite sample should ideally be collected at the same time (if possible) so that temporal changes in contaminant concentrations are minimized. A best practice is to collect all fish included in the composite sample within a week of each other so that the composite sample accurately reflects the selenium concentration of fish in that waterbody at that time.

The EPA recommends collecting a tissue sample of at least 20 grams wet weight (ww) for analysis, for both composite samples and individual samples. When creating composite samples from muscle tissue or egg-ovary tissue, an equal mass of homogenized tissue from each fish should be combined to create the composite, and then 20 grams ww should be sampled from the composite for analysis. To make sure that similar masses of tissue are added from each fish when creating composite samples from whole bodies, all fish included in a composite should meet the 75% rule. While the specific amount of tissue needed for analysis will be dependent

on the laboratory and analytical method used for analysis, 20 grams ww is a reasonable estimate of the amount of tissue needed for typical selenium analyses. This mass allows for five grams of tissue for the selenium analysis, five grams of tissue for a matrix spike sample, five grams of tissue for dry weight analysis, and a final five grams of tissue available in case there is a problem with one of the other analyses and a procedure needs to be repeated. In addition, a sample of 20 grams ww allows for a quality control sample to be processed, which can ensure homogeneity of the tissue sample. To allow for all analyses and contingencies, 20 grams is the ideal mass to collect, although these analyses can be run on smaller quantities (6-12 grams ww). The EPA recommends discussing the mass requirements for these analyses with an analytical lab if 20 grams ww cannot be collected.

Monitoring agencies typically collect composite samples for other analytes when sampling for fish consumption advisories. If agencies currently discard or archive the composite homogenates that are in excess of their current analytical needs, the excess tissue could be used, if adequate in mass, for selenium analysis. Agencies could also collect additional tissue mass and add selenium as an analyte to their current sampling protocol. If after these analyses enough remaining tissue mass is available, agencies may want to retain tissue from the individual fish in case future analyses are needed on the individuals.

Most fish tissue samples for selenium analysis are processed as wet tissue, resulting in a selenium concentration based on wet weight. As the national CWA section 304(a) recommended selenium criterion is based on a dry weight selenium concentration, an analysis of the moisture content of the tissue needs to be performed and the wet weight concentration needs to be converted to a dry weight concentration. See Appendix C of this document for more information on how to perform a wet weight to dry weight conversion.

The EPA recognizes that if a state or authorized Tribe collects muscle plugs, they will likely be collecting sample masses of less than 20 grams ww per fish. States and authorized Tribes should ensure that the mass of tissue they are collecting and the analytical methods that they are using will allow for accurate quantification of selenium. Stahl et al. (2021) used a modification to EPA Method 200.8 that utilized an inductively coupled plasma instrument with a triple quadrupole mass spectrometer detection system (Agilent Model 8800 ICP-QQQ) to get adequate sensitivity for measuring selenium in samples with small tissue masses. Paired with the digestion method of EPA SW-846 Method 3050B (USEPA 1996), the EPA was able to effectively measure selenium in samples in the one- to two-gram ww range. This analysis will also need to include a percent moisture analysis. Compositing muscle plugs (one per fish from multiple fish) may be one way to achieve sufficient mass for analysis.

The EPA also recommends collecting an equal number of fish in each composite, as this simplifies the statistical methods needed to analyze the results from this analysis. With equal numbers of fish, the arithmetic average of the replicate composite measurements is an unbiased estimator of the population mean. When unequal numbers are used, the arithmetic average is no longer unbiased. Instead, a weighted average of the composite measurements is calculated, where the weight for each composite reflects the number of fish in each composite

sample. At times, fish are lost or damaged prior to compositing. When several fish are damaged or lost, the allocation of the remaining fish to composites may be reconfigured to allow equal numbers of fish in composites. During this reconfiguration process, a sampler may be faced with the choice of either making composites of an equal number of fish or to follow the 75% rule. The EPA recommends adhering with the 75% rule over having an equal number of fish in each composite if both parameters cannot be met. If an equal number of fish cannot be included in each composite, care should be taken to adjust the statistical procedures to account for the unequal allocations (USEPA 2000).

While sampling, the EPA recommends documenting and reporting additional information about the fish samples to assist with interpretation of the data. Useful information to document includes the species, length, age (if it can be estimated), weight, and sex of the fish samples. Documenting information about the sampling site and day can also be useful for data interpretation. Samplers should note the location of collection by the Global Positioning System (GPS) if possible, sampling date, and weather. Samplers may also want to note flow rate and other characteristics about the site that may affect the concentration of selenium at the sampling sites.

2.2.2 Individual Samples

An individual sample is a discrete sample from a single fish, and can be an egg-ovary, whole-body, or muscle (fillet) tissue sample. Use of composite samples for selenium fish tissue monitoring is acceptable, but there are some instances where collecting individual fish may be desirable.

Analysis of individual fish samples may be of interest when collecting data for a site-specific study or for statistically evaluating patterns in selenium concentrations over time or space. Analysis of individual fish may also be important when evaluating selenium concentrations in critical species, where understanding potential toxicity at the individual level is important. A critical species is a resident species that (1) is commercially or recreationally important at the site; (2) is listed as threatened or endangered under section 4 of the Endangered Species Act; or (3) is a species for which there is firm evidence that its loss would yield an unacceptable impact on the site's commercially or recreationally important species, endangered species, abundances of a variety of other species, or structure or function. Analysis of individual samples also allows for the evaluation of spatial and temporal differences among individuals of a species or across the population of a species residing in a specific waterbody.

For waterbodies or segments that are known to be impacted by selenium, individual samples describe the range of variability within a population, including characterizing extreme values. Individual samples may provide better information about selenium source-exposure relationships in large waterbodies. Individual samples may also allow for the identification of fish that are migrant or transient in a population, since those fish may have higher or lower concentrations of selenium than other fish in the area. If studies are being conducted to

monitor trends, the EPA recommends sampling fish of the same species throughout the entire course of the study so that data from multiple sampling events are comparable.

If using individual samples to calculate a central tendency of selenium fish tissue concentrations, all fish should be the same species and collected from the same waterbody (or site for large waterbodies) within the same sampling period (ideally within the same week) (USEPA 1982). The samples should be of the same tissue type. Individual fish samples that are collected for the implementation of fish muscle or whole-body criterion element do not need to be constrained to the 75% rule, but samplers should be cognizant that fish may change their diets as they mature and mature fish may be migratory or more motile than small fish. Therefore, selenium concentrations may shift as fish grow due to ontogenetic shifts in fish diet (Stewart et al 2010). Thus, assessors should be cautious about calculating a central tendency from a data set with both juvenile and adult fish data.

The EPA recommends targeting at least five fish (USEPA 1982) (per waterbody or per site for larger waterbodies) for individual analysis. Greater or fewer fish samples may be needed based on the variation of selenium at a particular site. Those entities desiring greater statistical power for their analyses should collect additional fish samples. If collecting at least five individuals of one species is not possible, fewer specimens may be collected, but the statistical power of analyses will be affected (Hitt and Smith 2015). As with composite samples, the EPA recommends collecting 20 grams ww as a minimum tissue mass per individual fish for analysis and QA/QC.

Similar to the collection of composite samples, when sampling individual fish the EPA recommends documenting and reporting additional information about the fish samples to assist with interpretation of the data. Useful information to document includes the species, length, age (if it can be estimated), weight, and sex of the fish samples. Documenting information about the sampling site and day can also be useful for data interpretation. Samplers should note the location of collection by GPS if possible, and sampling date and weather. Samplers may want to also note flow rate and other characteristics about the site that may affect the concentration of selenium at the sampling sites.

2.3 Target Species

The two main factors to consider when selecting a target fish species to sample for the implementation of criteria based on the national CWA section 304(a) recommended selenium criterion are (1) a species' toxicological sensitivity to selenium; and (2) a species' bioaccumulation potential for selenium. In addition, it is important to consider a species' home range and how that will impact its ability to represent selenium exposure at a site and the species' potential for exposure to selenium within the site. The EPA recommends that states and authorized Tribes create a priority list of target species based on these factors for sampling teams. This list should identify the primary target species and alternative species if the primary species is not present, not present in sufficient numbers for sampling, or if sampling could impact the primary target species population at that site. When developing a priority list, states

and authorized Tribes should also ensure that sampling does not impact any protected species (e.g., threatened, endangered) or fisheries management goals. If data cannot be acquired for a desired target species, data from any fish species can be used to make assessment decisions, as the criterion was developed for use with any fish species.

For the purposes of implementing criteria based on the national CWA section 304(a) recommended selenium criterion, a species' toxicological sensitivity to selenium, is defined as a species or a surrogate species' 10% effect concentration (EC_{10}). An EC_{10} is the concentration of a chemical that is estimated to result in a 10% effect in a measured chronic endpoint (e.g., growth, reproduction, or survival). For the national CWA section 304(a) recommended selenium criterion, a species' EC_{10} is the concentration of selenium within egg-ovary tissue that results in a 10% effect on a reproductive endpoint for that species. Based on the best available and acceptable reproductive-effect studies, as well as extensive analyses, the EPA developed a species sensitivity distribution (SSD) to support the derivation of the national CWA section 304(a) recommended selenium criterion based on a species' EC_{10} value (see Table 3.2 in USEPA 2021). The SSD for the national CWA section 304(a) selenium criterion indicates that the four most sensitive genera for fish reproductive effects (in decreasing order) are *Acipenser*, *Lepomis*, *Salmo*, and *Oncorhynchus*.

The bioaccumulation potential of a species is largely determined by its dietary composition and the exposure of its prey to selenium. Consumption of benthic invertebrates tends to drive greater selenium bioaccumulation than consumption of plankton (Schneider et al. 2015, Simmons and Wallschläger 2005, Stewart et al. 2010, Ponton and Hare 2015). Among benthic organisms, the consumption of mollusks tends to drive greater selenium bioaccumulation than consumption of other benthic invertebrates (Luoma and Presser 2009, Stewart et al. 2004). Mollusks, such as mussels and clams, can accumulate selenium to a much greater extent than planktonic crustaceans and insects due to higher ingestion rates of both suspended particulate-bound selenium (algae) and dissolved selenium from the water column through filter feeding. Mollusks can also have a lower selenium elimination rate (Johns et al. 1988, Reinfelder et al. 1997, Stewart et al. 2010). However, this tendency for greater selenium accumulation in bivalves may be more prominent in marine or estuarine habitats than in freshwater habitats (Stewart et al. 2010). The greater bioaccumulation of selenium in benthic organisms suggests that bottom feeding fish may have higher selenium levels, at least for the portion of their lifecycle that ties their energy needs to food webs with benthic organisms. Other studies (Saiki et al. 1993, Saiki and Lowe 1987) have shown that detritivores may also be exposed to high levels of dietary selenium, as high concentrations of selenium were measured in detritus.

The bioaccumulation potentials of organisms at higher trophic levels (such as piscivores) are dependent on its food chain's cumulative exposure to and bioaccumulation of selenium. Trophic transfer factors (TTFs) provide a numeric representation of bioaccumulation between a consumer and its diet. A composite TTF ($TTF^{composite}$), which is the product of TTFs at each trophic level of a consumer's food chain, represents the overall TTF for a higher trophic level organism. In addition, composite TTFs account for the proportion of different food sources in a consumer's diet. Evaluations of composite TTFs may be helpful in determining which species to

target for sampling. Local influences, such as prey availability, selenium distribution, and selenium speciation, can also impact a species ultimate bioaccumulation potential at a site. The EPA recommends using site specific information to help inform the selection of target species, if available. More information about TTFs can be found in Section 3.0 of Appendix B in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). In addition, an explanation of how composite TTFs are calculated is included in Appendix E of this document.

The sensitivity of a species indicates at what selenium concentration a species will experience reproductive effects, while bioaccumulation potential influences how much selenium a species will accumulate. The intersection of these two will determine to what extent a species is impacted by selenium at a site (i.e., the species has accumulated enough selenium to exceed its EC_{10}). As both a species' sensitivity to selenium and bioaccumulation of selenium influences whether it will be impacted by selenium, states and authorized Tribes should consider both when selecting a target species and designing fish tissue sampling plans. The EPA recommends the following prioritization scheme for selecting a target species. Other considerations, however, such as home range and habitat preferences, may also factor into a state or authorized Tribe's prioritization scheme.

1) Sample the species that has the greatest bioaccumulation potential from within the four most sensitive genera (sensitive according to the SSD from USEPA 2021).
2) If no species are present from the four most sensitive genera, sample the species with the greatest bioaccumulation potential.
3) If no species are present from the four most sensitive genera and if all species have similar bioaccumulation potential, sample the species from within the most sensitive genera present at the site.

States and authorized Tribes should begin with targeting the fish species that has the greatest bioaccumulation potential from within the four most sensitive genera. As described above, the SSD for the national CWA section 304(a) recommended selenium criterion determined that the four most sensitive genera for fish reproductive effects (in decreasing order) are *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus.* When selecting a fish species to sample from within these genera, states and authorized Tribes should consider the diet and exposure of all the species at the site that are within those four most sensitive genera and select the species that has the greatest potential to bioaccumulate selenium. For example, if a site has multiple species of *Lepomis* present (e.g., Bluegill and Redear Sunfish), the state or authorized Tribe should sample the species that has the greatest bioaccumulation potential (Redear Sunfish, depending on prey available).

When determining the bioaccumulation potential of a fish species at a site it is important to consider both the diet of that species and the prey available at the site. Many species are opportunistic feeders, so their diet will be driven by what prey are available. A species may consume mollusks if available, but they should not be considered a part of the species' diet if no mollusks are present at the sampling site. In the San Francisco Bay estuary, White Sturgeon

are monitored not only because they are sensitive to the toxic effects of selenium (low EC_{10}), but also because their primary prey at that site (clams) bioaccumulate selenium very efficiently. As a result, White Sturgeon receive large doses of selenium and may be more likely to bioaccumulate selenium to levels of concern than another species. Table 3 provides a summary of these genera (highlighting information for the specific species tested), their relative sensitivity, their general habitat type (warm water (WW) or cold water (CW)), and their estimated relative bioaccumulation potential based on consideration of typical diet and trophic level. Fish that consume primarily benthic organisms will tend to exhibit greater selenium bioaccumulation than fish that feed higher in the water column at the same trophic level (Schneider et al. 2015, Simmons and Wallschläger 2005). Table 3 also provides a representative list of species that are within the same genus as the tested sensitive species and that could be considered as surrogates for tissue collection. A more comprehensive list of these surrogate species, along with details about relevant characteristics and occurrence is presented in Appendix D.

If no species from the four most sensitive genera are present at the site, then the state or authorized Tribe should target the fish species with the greatest bioaccumulation potential. As stated above, bioaccumulation potential will be predominantly determined by the diet of the species. Composite trophic transfer factors can provide a numeric representation of bioaccumulation between a consumer and its diet and help inform the decision about which species to sample.

If all species that are present at a site have similar diets and bioaccumulation potential and are not from the four most sensitive genera, then the state or authorized Tribe should target the most sensitive species. This will be the species with the lowest EC_{10}. If the EC_{10} of a particular species is unavailable, sensitivity can be estimated from the EC_{10} of a closely related taxon.

In addition to sensitivity and bioaccumulation potential, there are a number of factors that should be considered when identifying appropriate fish species for collection. The following summarizes some key points for consideration:

1) White Sturgeon (and available surrogates) are in the most sensitive genera (based on available data) and are distributed in large river systems in the U.S. These species are typically sampled by specialized monitoring programs (e.g., U.S. Fish and Wildlife Service (USFWS) and National Oceanic and Atmospheric Administration-National Marine Fisheries Service (NOAA-NMFS)). If a state or authorized Tribe would like to sample White Sturgeon, coordination with these existing programs may provide for expanded sampling opportunities or the use of existing selenium fish tissue data. Collecting and processing this species may be challenging and states and authorized Tribes should determine whether they have the appropriate resources to sample this genus. Several species and specific populations of species within the *Acipenser* genus are federally listed species under the Endangered Species Act and may not be appropriate to sample. USFWS, NOAA-NMFS, and appropriate state agencies should be consulted before sampling any federal or state listed threatened or endangered species.

2) Bluegill, and the related sunfish species in the genus *Lepomis* are widely distributed in warm water habitats, while trout species (particularly Rainbow and Brown Trout) are widely distributed in cold water habitats. These species are frequently targeted by monitoring programs in states and on authorized Tribal lands with warm water and cold water habitats, respectively, offering a possible opportunity for leveraging these existing sampling programs for the collection of tissue for selenium analysis.
3) Smaller warm and cold water systems (e.g., wadeable streams), which are not typically targeted by state and authorized Tribal fish tissue contaminant monitoring programs, are often dominated by cyprinid (minnow) species and may represent a source of fish tissue for selenium sampling in waterbodies where other species may not be present. Some of these species are shown in Table 3 and a broader range of these species are shown in Appendix D. Given the large number of minnow genera and species, and the diversity of their trophic strategies and habitats, the sensitivity and bioaccumulation potential of individual members in this diverse group should be considered when evaluating a candidate species for consideration in tissue sampling. However, state and authorized Tribal biomonitoring programs typically sample these waters for index of biological integrity metrics; therefore, their expertise and sampling program could be leveraged to target species and obtain representative samples.
4) Although generalizations can be made about the potential for bioaccumulation within fish species, when developing a sampling plan, the potential for bioaccumulation should be considered for the specific area being evaluated. Potential for bioaccumulation within any given species can vary significantly with location-specific factors, including prey type and availability, and the nature of selenium distribution in the environment.
5) There are a number of species for which toxicity data are not available, but for which dietary information could allow states and authorized Tribes to characterize the potential for selenium bioaccumulation. Some of these species are collected as target species in state and authorized Tribal monitoring programs and could be considered to characterize selenium tissue concentrations in the absence of sensitive species if available data indicate these species bioaccumulate selenium.

Along with sensitivity and bioaccumulation, it is also important to consider how a species' habitat preferences, feeding regimes, and/or home ranges will affect their selenium exposure. Species with smaller home ranges (typically smaller-bodied fishes or juveniles) may be at risk of greater selenium exposure if the elevated selenium is localized to their home range or their prey's home range. Species with larger home ranges may not represent local selenium exposure. States and authorized Tribes should target species with limited motility and home ranges that closely match the site being evaluated, so that the fish reflect exposure to selenium at that particular site. Local fish biologists may be able to help states and authorized Tribes identify species' habitat preferences, feeding regimes, motility, and home ranges. If possible, migratory species and highly motile species should be avoided. Highly motile fish species, such as potamodromous and anadromous species, could travel back and forth between areas with low and elevated selenium concentrations, resulting in variable selenium tissue concentrations (Beatty and Russo 2014). It is possible that typical selenium exposure concentrations of migratory adults would be lower than concentrations at rearing grounds; therefore, these fish

Table 3. Potential Target Species for Collection Based on Selenium Sensitivity and Bioaccumulation Potential.

Sensitivity Ranking (Based on Egg-Ovary EC_{10})	*Genus*, Common Name	Habitat (WW/CW)	Bioaccumulation			Additional Representative Surrogate Species[b]
			Expected Relative Bioaccumulation Potential	Adult Diet	Trophic Level (TL)	
1	*Acipenser,* White Sturgeon[a]	WW	Medium-High	Invertivore Piscivore Molluscivore	TL3/TL4	Shortnose Sturgeon[a], Lake Sturgeon[a]
2	*Lepomis*, Bluegill	WW	Medium	Invertivore Piscivore	TL3	Pumpkinseed[a], Redear Sunfish[a], Green Sunfish[a], Redbreast Sunfish[a], Longear Sunfish, Warmouth, Orangespotted Sunfish, Redspotted Sunfish, Bantam Sunfish, Northern Longear Sunfish, Spotted Sunfish
3	*Salmo,* Brown Trout[a]	CW	Medium-High	Invertivore Piscivore Molluscivore	TL3/TL4	None
4	*Oncorhynchus*, Rainbow Trout	CW	Medium-High	Invertivore Piscivore	TL3/TL4	None
	Oncorhynchus, Cutthroat trout	CW	Medium	Invertivore	TL3	None
5	*Micropterus* Largemouth Bass	WW	Medium – High	Invertivore Piscivore	TL4	Smallmouth Bass, Spotted Bass, Redeye Bass
Not Ranked	*Pimephales* Fathead Minnow[c]	WW	Low-Medium	Herbivore Invertivore	TL2/TL3	WW: Satinfin Shiner, Red Shiner, Golden Shiner[a], Bull Chub[a], Creek Chub, Roundtail Chub, Thicklip Chub[a], Striped Shiner, Central Stoneroller, Blacknose Dace, Speckled Dace, Cutlips Minnow[a], River Darter[a], Arkansas Darter[a]
						CW: Redside Shiner, Peamouth Chub, Mottled Sculpin

WW = warm water, CW = cold water, TL = trophic level

[a] Fish species that consume mollusks (e.g., clams, mussels, snails) as part of their diet and are anticipated to have relatively high bioaccumulation potential. For Brown Trout, molluscivory is incidental and will likely only be significant on a site-specific basis where mollusks are abundant.

[b] Species are surrogates for sensitivity based on taxonomic relationships.

[c] Fathead minnow is a surrogate for small-bodied fish species inhabiting wadeable streams.

may not reflect selenium concentrations that other fish species would experience at their time of spawning. Given this issue, resident species should be the first choice when selecting a target species. Recently stocked fish should also be avoided, regardless of species, since their residence time before sampling may be too short to provide a representative sample. The EPA recommends speaking with local fish and game authorities to understand stocking frequency in waters where fish are being sampled for selenium analysis. If stocked fish must be sampled, the EPA recommends sampling fish regularly after their addition to the waterbody to see when their selenium concentrations stabilize. Before this stabilization point, it would not be appropriate to use stocked fish for implementation purposes.

If migratory or highly motile species must be sampled, the EPA recommends that sampling plans account for the life history of these species so that the correct locations for sampling within a watershed are selected. Before sampling these species, knowledge about the migratory extent of these species and the time spent in each location should be known so that the influence of selenium at each habitat location can be accounted for. Depending on the species, it may be possible to target sampling during a non-migratory phase for the species. At times, additional studies may be needed to determine where selenium exposure occurs for these species. The EPA recommends consulting local fish biologists for information about the migratory patterns of local fish populations and considering this information in making a target species selection and determining when sampling will occur. If Pacific anadromous species are selected as target species to be used for sampling, the EPA recommends that states and authorized Tribes sample the whole-body of juveniles and compare the concentration to the whole-body criterion element. This recommendation is due to the lack of selenium exposure to adult salmonids from freshwater prior to reproduction (see section 6.4.1.1 in USEPA 2021).

One or more species that is/are sensitive to selenium (e.g., Bluegill, Rainbow Trout, Brown Trout) are commonly present in waterbodies. However, if selenium-sensitive species or species that potentially bioaccumulate high levels of selenium are not available in sufficient numbers to sample, then another species that is available in sufficient numbers may be used for fish tissue monitoring, including species known to be tolerant to selenium. These selenium tissue samples can still be compared to the appropriate tissue criterion elements (see Table 1), which are designed to be protective of the entire aquatic community and can still be used to make an assessment decision. In addition, if fish tissue data from other fish species than the ones recommended in this section were collected for an alternate purpose, those data can also be used for an assessment decision.

As there are many considerations involved in the selection of a target species, the EPA recommends that sampling teams develop a sampling priority plan before going into the field. This plan should start by identifying the species and factors that will increase selenium exposure at a site. From there, an initial target species should be selected and any decisions about sampling locations can be made. The plan can then identify a prioritized list of species that the state or authorized Tribe will sample if the primary target species is not present in sufficient numbers or if sampling could impact the primary target species population at that site. The EPA recommends that the plan also include what type of sampling gear is required for

the collection of each species. In addition, the plan should specify if collection permits are required for fish tissue sampling, and if so, include instructions for acquiring them before the sampling event. Having a clear plan will ensure that the fish collected will provide the most representative data of selenium conditions at the site.

States and authorized Tribes may want to limit the number of target species that are sampled in their state or authorized Tribal waters. The use of a limited number of target species allows for the comparison of fish contaminant data across sites over a broad geographic area. It is difficult to compare contaminant monitoring results within a state or authorized Tribe or among states or authorized Tribes unless the data are from the same species because of differences in habitat, food preferences, and rate of contaminant uptake among various fish species. Limiting the number of species collected across a state or authorized Tribal waters could allow for the better comparison of contaminant data from across a state or region. Given this, the EPA recommends that states and authorized Tribes evaluate the range of sensitive species with high bioaccumulation potential across their state or Tribal waters and determine which ones may be able to be sampled at multiple locations across the state or Tribal lands.

If a state or authorized Tribe is sampling fish tissue for the development of a site-specific water column criterion element, the state or authorized Tribe only needs to sample one species but may want to consider expanding the sampling to include multiple species to better understand the system at the site. The species that is sampled should have both high bioaccumulation potential and high sensitivity to selenium (as described earlier in this section). Sampling additional species that have high bioaccumulation potential, as well as species with high sensitivity to selenium, could provide a more complete picture of selenium dynamics at the site.

Lastly, care should be taken to avoid sampling federal-, state-, and Tribal-protected, threatened, or endangered species when selecting a target species. For example, although *Acipenser*, *Salmo*, and *Oncorhynchus* are three of the four most sensitive genera, many species within these genera are listed as threatened or endangered under the federal Endangered Species Act and thus, are not suitable for sampling. Before sampling from these genera and other genera with federally listed and other protected species, the EPA recommends speaking to local fish biologists and other relevant government entities (e.g., USFWS, NOAA-NMFS, state and authorized Tribal wildlife agencies). When conducting monitoring to ensure these species are protected, states and authorized Tribes should target surrogate species that have similar taxonomy (preferably at the genus level), diets, and trophic levels. Species with similar taxonomy, diets, and trophic levels should have similar selenium sensitivity and bioaccumulation as the threatened or endangered species. If a taxonomically similar surrogate is not present, then states and authorized Tribes should target a species with a similar diet and trophic level.

2.4 Sampling Locations

Several factors should be considered when selecting where fish should be sampled (to be analyzed either individually or as a composite) to accurately characterize the concentration of

selenium at a site of interest. The spatial extent of the site should be defined and the factors that may affect selenium variability throughout the site should be identified so that they can be considered in the design of the sampling plan. The selection of a site and how its boundaries are defined will be influenced by the objective of the monitoring and by past monitoring activities (see section 3.0 *Leveraging Existing Fish Tissue Monitoring Programs and Sample Design*). The factors informing the site definition and the subsequent selection of sampling locations will vary, and may include:

- Monitoring objectives (e.g., assessment, site-specific selenium studies);
- Waterbody type and site hydrology (lotic vs. lentic);
- Waterbody size;
- Selenium sources and their location;
- Aquatic habitat variability; and
- Physical barriers to fish movement.

These factors (and potentially others) will influence the definition of the site boundaries, decisions about where fish are collected from within the site, and decisions about how many fish need to be collected at the site. Sections 2.4.1 – 2.2.4 discuss a non-exhaustive set of factors that the EPA recommends states and authorized Tribes consider when selecting sites and sample locations; however, particular situations may warrant the consideration of other important factors.

2.4.1 Waterbody Type

Selenium concentrations and bioaccumulation patterns are different in lotic (flowing waters, such as rivers and streams) versus lentic (very slow moving or still waters, such as lakes and reservoirs) environments. Water residence time is typically shorter in lotic systems than in lentic systems, and subsequently, aquatic organisms living in lentic systems tend to bioaccumulate proportionately more selenium than organisms living in lotic systems (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). In addition, lentic waterbodies tend to have greater reducing conditions (conditions that lead to reduction reactions and reduced ionic species of selenium, such as selenite), which create an environment where selenium accumulates in sediment more readily and may also lead to higher bioavailability in the water column (Luoma and Rainbow 2008, Simmons and Wallschlägel 2005). Benthic organisms in lentic systems can then be exposed to higher concentrations of selenium in the sediment, leading to increased bioaccumulation potential in other organisms feeding on the benthic organisms (Simmons and Wallschläger 2005, Orr et al. 2006). For example, Hillwalker et al. (2006), found that the body burden concentrations of selenium in insects within similar taxa were up to seven times greater in lentic systems than lotic systems within the same watershed.

When sampling fish, consideration should be given to the different flow characteristics of the site that is being sampled along with the locations where fish are feeding and obtaining their

selenium body burdens. Some areas of a lotic site may have lentic characteristics and vice versa. For example, some rivers may have slow moving pools or backwaters that have characteristics similar to lentic environments. Human-made lakes and reservoirs may have some features that are intermediate between typical lotic and lentic systems. For example, reservoirs tend to be longer and narrower than natural lakes, and generally have a shorter water retention time than a natural lake of comparable volume (Thornton et al. 1990). When sampling sites, attempts should be made to sample all habitat types to appropriately characterize the range and distribution of selenium concentrations at a site.

2.4.2 Waterbody Size

Generally, the variability of selenium within resident fish populations would be expected to be low when the spatial and temporal variability of selenium concentrations across all compartments of the ecosystem are low (e.g., water column, sediment). As the area of a site increases, the spatial and temporal variability is expected to also increase, thus increasing the number of samples needed to characterize the selenium concentrations in the resident fish populations. If the waterbody is sufficiently large that sub-populations are expected to be present (potentially applicable to large reservoirs), it could be advantageous for the sub-populations to be represented separately in the data set.

2.4.3 Site-specific Studies for Water Column Translations

If a site-specific water column criterion element is being developed, a study should be designed that captures data that appropriately reflect the whole site (i.e., captures spatial, temporal, and habitat variability). To support the development of a site-specific water column criterion, data beyond what are necessary for other CWA implementation purposes (e.g., listing) are typically necessary (e.g., additional sampling locations, sampling times, species of fish, and/or selenium measured in multiple matrices) to ensure that the designated use is appropriately protected by the site-specific water column translation. The extent of the sampling and type of data collected will depend on the size and complexity of the site. It will also depend on whether there are any discharges of selenium into the site. A “site” may be a state, region, watershed, waterbody, segment of waterbody, category of water (e.g., ephemeral stream), etc. Regardless of how the site is defined, the site-specific water column translation should be derived to protect aquatic life for the entire site, including both areas upstream and downstream of a discharge if one is present at the site, in accordance with CWA section 303(c) and the EPA’s implementing regulations at 40 CFR Part 131. To ensure protection for aquatic life throughout the entire site, fish should be sampled from locations where selenium is expected to bioaccumulate the most (areas of the site with more lentic properties and areas where selenium may be elevated due to source contributions). In addition to sampling from the area of greatest exposure, states and authorized Tribes may sample fish from various locations at the site to understand the dynamics of selenium within that site. With that knowledge, the site-specific water column translation can generally be designed to be protective of the most vulnerable fish community. If selenium concentrations are significantly different between

different locations within a site, the state or authorized Tribe may consider whether the site should be broken into two sites. In addition, a site-specific water column criterion element would need to be protective of downstream waters (40 CFR 131.10(b)).

Additional information related to sampling fish tissue to support a site-specific water column translation can be found in Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) and in *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards* (USEPA 2024c). One example of a method for conducting a site-specific water column translation can be found in the *Draft Translation of Selenium Tissue Criterion Elements to Site-Specific Water Column Criterion Elements for California Version 1, August 8, 2018* (USEPA 2018). As there are a large number of factors to consider when designing a site-specific study, the EPA strongly recommends engaging with the EPA regional offices early in the development of a site-specific water column translation to discuss study design and data needs.

2.4.4 Point Sources

When selecting sampling locations, samplers should consider where and how selenium is entering a waterbody and determine whether exposure is relatively equal throughout the waterbody or if some sections of the waterbody have greater exposure. The sampling objectives will provide direction on how known sources (e.g., discharge, tributary with elevated selenium, irrigation return canal, groundwater discharge) and any associated mixing zones (if allowed under state or authorized Tribal water quality standards) should be taken into consideration when collecting fish tissue samples. Two potential objectives and the associated sampling recommendations are explored here.

When the objective is to collect data to support waterbody assessment decisions, the goal is to measure the central tendency of the selenium concentration in the target population throughout the sampling reach. Therefore, when a point source is located within the defined sampling reach, states and authorized Tribes should not avoid sampling fish from areas of incomplete mixing resulting from a discharge or tributary. Given the mobility of many fish taxa, it is reasonable to expect that fish freely move in and out of areas of incomplete mixing when the conditions do not elicit an avoidance response (e.g., due to chemical or temperature gradients). In some discharge situations, fish can be attracted to the effluent and spend a significant portion of their time in the area of incomplete mixing. Also, depending on their life history, some fish species have a limited mobility range and may spend more time in the area of incomplete mixing if it overlaps with their territory, breeding grounds, or feeding grounds. Ultimately, states and authorized Tribes should consider and document if there are any reasons to avoid tissue collection from a location adjacent to a known point source prior to sampling (e.g., conditions are not representative of the rest of the segment). States and authorized Tribes may also want to consider whether further segmentation of an assessment unit into upstream and downstream segments is warranted due to the presence of a discharge.

When the sampling objective is to characterize the contribution of selenium that a known point source(s) is making to the waterbody, samples collected upstream and downstream of the point source(s) should be assessed independently (i.e., not composited or averaged). The downstream sampling reach should be large enough to include samples collected within and downstream of areas of incomplete mixing to characterize the range of bioaccumulation potential in the tissue samples as the water column concentrations decrease. One way to do this would be to collect fish and water samples at regular intervals from the discharge to observe how the selenium concentrations in both types of samples decrease downstream of the discharge. It is important to understand the hydrology in the system as this will influence the range and direction of transport of selenium from the discharge source(s) to other portions of the waterbody/site. It is also important to understand the ecology of that target species to understand their home range and potential for exposure. For more information on considerations related to selenium sources and the locations of those sources, see section 2.1 in *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021).

3.0 Leveraging Existing Fish Tissue Monitoring Programs and Sample Designs

3.1 Augmenting Existing Fish Tissue Monitoring Programs

Many states and authorized Tribes have existing fish tissue monitoring programs that can be leveraged to collect fish tissue data to assess against the fish tissue criterion elements of criteria based on the EPA's 2016 national CWA section 304(a) recommended selenium criterion. In 2010, 45 states monitored chemical contaminants in fish tissue to assess risks to human health. Twenty-eight states identified selenium as a contaminant in their human health monitoring programs (USEPA 2011). These states can potentially modify their current fish tissue monitoring programs to not only assess human health risks, but also assess attainment of the aquatic life selenium criterion.

The design of an agency's existing fish tissue monitoring program will likely drive its approach to selenium monitoring. Agencies should evaluate how current sampling and analytical protocols can be modified to meet both the objectives of fish tissue monitoring for risk to human health and aquatic life protection. In the following sections, the EPA provides case studies with examples of programs that might have the capacity and framework to augment their existing monitoring strategies to include fish tissue monitoring for the selenium aquatic life criterion.

3.1.1 Consistency with Existing Programs

The EPA recommends states and authorized Tribes evaluate current fish tissue monitoring programs to determine how they can be augmented to implement criteria based on the national CWA section 304(a) recommended selenium criterion. To the extent possible within a

state or authorized Tribal program, the EPA recommends that fish tissue monitoring for the selenium aquatic life criterion should be consistent with the state or authorized Tribe's current fish tissue monitoring practices regarding spatial and temporal considerations, species collected, and sample type collected. In this way, logistical modifications to a state or authorized Tribe's fish tissue monitoring program can be minimized. Muscle tissue is the most common type of sample collected and analyzed by monitoring programs. Less frequently, states and authorized Tribes collect and analyze whole-body samples. To maximize efficiency, a portion of these samples can be submitted for selenium analysis. States and authorized Tribes can realize cost efficiencies by choosing to use whole-bodies or muscle tissue (fillets) that are already being collected for an existing monitoring program.

When using existing sampling programs that are designed for human health protection to assess selenium levels for protection of aquatic life, states and authorized Tribes should recognize the potential limitations of these data. Those data may not represent the area's most likely to be contaminated by selenium, the most relevant time periods for sampling, and/or the most appropriate species. State and authorized Tribal programs may need to alter their typical monitoring protocols due to spatial, temporal, or species/sample type considerations specific to selenium. To address these additional sampling needs, states and authorized Tribes may be able to leverage expertise and logistical assistance from other agencies with existing fish tissue monitoring programs. Since the EPA's national CWA section 304(a) recommended selenium criterion applies to ecological risk and not human health, monitoring agencies could evaluate their target species list and determine if they include appropriate species for assessing selenium risk to aquatic life. See the discussion in section 2.3 about selecting target species and see *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater* (USEPA 2021) for more information about species' sensitivity and bioaccumulation potential.

A survey conducted in 2010 reported that 40 state agencies conduct fish sampling at regular intervals, and several conduct statewide, rotating basin sampling programs over a multi-year period (USEPA 2011). Many states and authorized Tribes may be able to use their current fish tissue sampling programs to monitor for the selenium criterion as well. Agencies could monitor state- or basin-wide, and track progress in individual basins relative to other areas. Regular yearly sampling could be conducted, with intensified sampling in targeted basins as needed (see Table 4 for several documents that provide guidance for sampling and survey designs). Several states use a probabilistic survey design to select sampling sites. This type of sampling design can produce estimates that represent the condition of the whole watershed, and an estimate of random spatial variability (USEPA 2000). Probability-based sampling provides the basis for estimating the resource (i.e., fish population(s)) extent and condition, for characterizing trends in resource extent or condition, and for representing spatial patterns, all with known certainty (USEPA 2009). Additional or more targeted sampling approaches may be needed in areas where elevated selenium is associated with a known point of discharge. The case study below presents the Kansas Department of Health and the Environment's (KDHE) fish

tissue monitoring program, which uses several designs for selecting sites. Based on the information available, it is likely that a state or authorized Tribe with a similar program could take advantage of their current sampling strategy to perform screening level selenium analysis throughout their state or Tribal area. Where selenium is already a primary parameter of interest, the state or authorized Tribe may have data to support more intensive studies in certain waterbodies.

CASE STUDY: The Kansas Department of Health and the Environment

The Kansas Department of Health and the Environment (KDHE) currently collects fish samples annually from 30-50 fixed and rotating stations. The KDHE selects sites based on targeted, census, and probability-based sampling designs. Specific sub-program objectives determine the numbers, species, and sizes of fish collected from a particular waterbody, and the tissues and parameters of interest (https://www.kdhe.ks.gov/1268/Fish-Tissue-Contaminant-Monitoring-Progr).

Highlights:

- Whole fish, muscle, muscle plugs, or other specific tissues were collected for different programs;
- Selenium was a primary parameter of interest; and
- Specific tissues (such as egg-ovary) were analyzed for specific chemicals of concern known to accumulate in certain organs.

This program is an example of an existing fish sampling program that could be enhanced to collect data for the implementation of a state or authorized Tribal criterion based on the national CWA section 304(a) recommended selenium criterion.

https://www.kdhe.ks.gov/DocumentCenter/View/11902/Fish-Tissue-Contaminant-Monitoring-Programs-Quality-Assurance-Management-Plan-PDF

3.1.2 Temporal Considerations

Various temporal considerations will influence fish tissue monitoring strategies for selenium. These can include considerations related to the ecology of the fish (e.g., species' spawning season) or to abiotic environmental factors (e.g., weather conditions and river flows). Temporal considerations will influence decisions regarding which tissue type is sampled: egg-ovary, whole-body, or muscle tissue. For example, most fish species that are synchronous spawners spawn in the spring, making spring the prime season to sample egg-ovary tissues, yet sampling for health advisories is typically done in the fall when concentrations of contaminants are highest in muscle tissue (fillets). Agencies will need to consider their resources and determine

which fish tissue type they would like to sample and at what time of year. If agencies plan to sample egg-ovary tissue, they should plan to sample it right before spawning. If an agency plans to conserve resources and sample for both fish consumption advisories and the selenium criterion at the same time, whole-body or muscle tissue should be sampled outside of species-specific pre- and post-spawning windows. In this case, muscle (or whole-body) tissue can be composited and evaluated for the selenium aquatic life criterion in addition to contaminants of interest for fish consumption advisories. If the agency has information indicating that there may be seasonal differences in whole-body or muscle tissue concentrations, then agencies should plan to sample during the season when the highest selenium concentrations are expected. Agencies may want to sample spring spawners in late summer or fall to avoid the potential for underestimating selenium body burdens. Selenium body burdens can be decreased directly post-spawn due to the selenium depuration from the body via the maternal transfer of selenium to eggs and the subsequent release of eggs to the environment.

For egg-ovary tissue sampling, the EPA recommends that agencies with fish tissue monitoring responsibilities consult with local fisheries biologists to determine the appropriate time for sampling specific species in their region to capture the specimens in their pre-spawning phase. These regional experts will be familiar with the local species and are able to use their best professional judgment to determine which species are appropriate for egg-ovary sampling and the appropriate sampling period based on spawning season.

3.1.3 Spatial Considerations

Monitoring agencies generally target high-use fishing areas, areas of special concern, and areas of suspected contamination (such as waterbodies where fish advisories have been issued), when selecting sites for sampling fish tissue (USEPA 2011). As current fish tissue monitoring programs are typically designed to specifically address the risk to human health from fish consumption, these programs predominantly sample locations where fishing is common. This may lead to mostly sampling lakes and higher order streams. States and authorized Tribes using this sampling design should consider if these existing programs will adequately capture waterbodies impacted by point and non-point sources of selenium and potential areas of selenium contamination. If not, agencies may want to modify sampling designs to target such areas for sampling.

Some states and authorized Tribes may incorporate fish tissue sampling for selenium into a statistical survey designed to understand the distribution of tissue concentrations across the state or Tribal lands. The underlying geology of a region may produce elevated selenium concentrations in certain areas and make nearby waterbodies prone to selenium bioaccumulation, particularly if anthropogenic activities increase the release of selenium into

the system. This should be kept in mind when selecting sites, and when analyzing data from these areas (Beatty and Russo 2014).[11]

Additional sampling locations may need to be added to a current fish tissue monitoring program that are outside of areas that are typically targeted due to fishing use when sampling for the assessment of the selenium aquatic life criterion. For example, mine runoff may elevate selenium concentrations in headwater streams, which may not be normally targeted for fish tissue monitoring. Agencies should also consider a species' home range in relation to the location of a known selenium source (e.g., the migratory patterns of a certain species versus the location of a power plant on a reservoir). It is also important to consider the relationship of an upstream source to downstream habitats, particularly when downstream habitats have characteristics that will lead to greater selenium bioaccumulation (e.g., lentic systems). Monitoring plans may need to be adjusted to reflect the species of fish available in a waterbody (e.g., small streams), temporal issues (e.g., spring flood/safety, low flow), and the types of appropriate sampling gear.

The monitoring strategy in the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) discusses two tiers of studies used to identify locations where fish consumption advisories may be needed. Information from these studies may be utilized to develop selenium specific monitoring programs for the assessment of the aquatic life criterion. Tier 1 studies are screening studies that evaluate a large number of sites for chemical contamination with few samples per site. These would be most useful for waterbodies or regions where there are no known or expected selenium problems. Screening studies can help states and authorized Tribes identify those sites where selenium concentrations are elevated relative to other waterbodies. Information from screening studies can be used to prioritize waterbodies for future fish tissue monitoring, thus enabling resources to be used more efficiently. For example, waterbodies with fish having low selenium concentrations may be monitored less frequently in the future, while waterbodies with fish having selenium concentrations at or near the tissue criterion elements may be prioritized for more frequent or more intensive monitoring. In addition, data collected during these screening studies can be used to inform assessment determinations for the waters where the samples were collected.

Tier 2 studies are intensive studies of areas identified as potential problems in screening studies. The purpose of a Tier 2 study is to determine the magnitude of chemical contamination in sensitive fish species, and to assess the geographic extent of the contamination. If a Tier 2 study is being conducted for selenium, fish species from a sensitive genus with high bioaccumulation potential should be sampled either in addition to or in place of sensitive species. Agencies will typically use Tier 2 studies to determine the overall magnitude and variability of a specific contaminant that was found at elevated levels during a Tier 1 study. In many areas, selenium sources have been well characterized; in these areas a screening study may not provide any additional information that would change the course of the investigation.

[11] See the USGS's map of selenium concentrations in soils and stream segments at https://mrdata.usgs.gov/geochem/doc/averages/se/usa.html.

At these sites, it may be most useful to move directly to an intensive study designed to capture the magnitude and geographical extent of the selenium contamination in fish tissue. These studies may be helpful as a basis for developing a site-specific water column criterion element, if relevant.

3.2 Existing Resources and Information

3.2.1 Available Expertise

Within each state or authorized Tribe, the agency that develops the WQS and the agency that typically conducts fish sampling may not be the same. When designing sampling plans to assess the selenium aquatic life criterion, agencies with experience in the development and execution of fish sampling programs can be consulted to aid in designing an effective fish tissue monitoring plan. State and authorized Tribal agencies should also determine whether there is any overlap in current sampling efforts. Various state and authorized Tribal (e.g., Department of Natural Resources) and federal agencies (e.g., EPA, USFWS, NOAA-NMFS, USGS) have expertise in fish sampling, biology, and ecology, and may be able to assist with designing a sampling plan.

All states and many authorized Tribes and interstate commissions have established biological assessment programs, and most have fisheries biologists and managers. This could provide a state or authorized Tribe with the capacity to establish or modify existing fish tissue monitoring programs to facilitate implementation of the fish tissue-based criterion elements of criteria based on the national CWA section 304(a) recommended selenium criterion. In addition to individual state and authorized Tribal agencies and local expertise, federal (e.g., USFWS) and state and authorized Tribal resource agency collaborations could be used, as necessary, to help fill in data gaps and provide supporting data. By using all available resources for information and expertise, monitoring agencies should be able to:

- Identify potential sites/locations, waterbodies, and watersheds for selenium sampling beyond the coverage of current monitoring programs;
- Design an appropriate monitoring strategy (including selection of fish tissue type and sample type (i.e., individual or composite samples));
- Select target species;
- Identify pre-spawning periods; and
- Procure analytical support.

The case study below presents Minnesota's Fish Contaminant Monitoring Program, which is implemented through a collaborative partnership of four state agencies to maximize available expertise. Based on the available information, a state or authorized Tribe with a similar collaborative program could take advantage of their joint resources to devise the most efficient approach for adding selenium to their current fish tissue monitoring strategy. They could also use their extensive database to determine where to conduct more intensive studies.

CASE STUDY: Minnesota's Fish Contaminant Monitoring Program

Minnesota's Fish Contaminant Monitoring Program is implemented through a partnership of Minnesota Departments of Natural Resources, Health, and Agriculture and the Minnesota Pollution Control Agency (MPCA). The data are used to issue fish consumption advisories, identify impaired waters, research mercury cycling, and document long term trends for PCBs and mercury.

Highlights (MPCA 2008, P. McCann, personal communication, May 7, 2018):

- Approximately 130 lakes and river sites are sampled annually;
- The Fish Contaminant Monitoring Program database contains over 52,000 data records; and
- As of 2016, the program has sampled 1,410 lakes of the estimated 5,500 fishing lakes in the state.

This program is a robust example of how interagency cooperation can maximize available expertise, resources, and cost effectiveness.

https://www.health.state.mn.us/communities/environment/fish/techinfo/index.html

3.2.2 Existing Guidance

The EPA and other stakeholders have produced numerous documents on bioassessment techniques. Specific sections of these documents contain information that may be helpful in developing guidelines for sampling fish for selenium fish tissue analysis, particularly for species like cyprinids, which are not typically targeted by state and authorized Tribal monitoring programs. For example, *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish - Second Edition Chapter 3* (Barbour et al. 1999) provides guidance and information on the elements of biomonitoring, including seasonality and methods for fish collections.

If sampling for both the selenium criterion and for fish consumption advisories, the EPA's *Fish Advisory Guidance Volume 1* discusses study design considerations and the major protocols that must be specified for fish collection, such as site selection, analyte screening values, sampling times, sampling type, and QA/QC.

Also, the EPA's *Fish Advisory Guidance Volume 1* provides useful information on the collection of whole-body and muscle tissue samples. Specifically, section 7.2.2 of the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) includes detailed directions for preparing muscle and whole-

body samples. The limitation to this guidance is that it was developed specifically for assessing human health risks associated with consumption of fish and shellfish. As a result, there are aspects of implementing the aquatic life selenium fish tissue-based criterion that are not specifically addressed by the EPA's *Fish Advisory Guidance Volume 1* (e.g., fish egg-ovary sampling, ecological risks). A selection of recommended documents for additional guidance is presented in Table 4.

Table 4. Recommended Documents for Additional Guidance.

Title	Author	Link
Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis[1]	USEPA 2000	https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf
Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish - Second Edition	Barbour et al. 1999	https://www.epa.gov/sites/production/files/2019-02/documents/rapid-bioassessment-streams-rivers-1999.pdf
Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue[1]	USEPA 2002a	http://www.epa.gov/sites/production/files/2015-07/documents/fish-study-fieldplan.pdf
The National Study of Chemical Residues in Lake Fish Tissue (Final Report)[1]	USEPA 2009	https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005P2Z.PDF?Dockey=P1005P2Z.PDF
Concepts and Approaches for the Bioassessment of Non-Wadeable Streams and Rivers	Flotemersch et al. 2006	https://nepis.epa.gov/Exe/ZyPDF.cgi/600006KV.PDF?Dockey=600006KV.PDF
Guidance on Choosing a Sampling Design for Environmental Data Collection	USEPA 2002b	http://www.epa.gov/sites/production/files/2015-06/documents/g5s-final.pdf
Spatially Balanced Survey Designs for Natural Resources. Design and Analysis of Long-Term Ecological Monitoring Studies	Olsen et al. 2012	https://www.cambridge.org/core/books/design-and-analysis-of-longterm-ecological-monitoring-studies/spatially-balanced-survey-designs-for-natural-resources/F06C6F53022E46D694D1233782D5F274
Spatially Balanced Sampling of Natural Resources	Stevens and Olsen 2004	https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.files/fileID/13339
Application of Global Grids in Environmental Sampling	Olsen et al. 1998	https://archive.epa.gov/nheerl/arm/web/html/abolsen98.html
National Rivers and Streams Assessment 2018/19: Field Operations Manual – Non-Wadeable[1]	USEPA 2019a	https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_nonwadeable_version_1.2.pdf
National Rivers and Streams Assessment 2018/19: Field Operations Manual – Wadeable[1]	USEPA 2019b	https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_wadeable_version_1.2_0.pdf

Title	Author	Link
National Coastal Condition Assessment 2015 Field Operations Manual[1]	USEPA 2015	https://www.epa.gov/sites/production/files/2016-03/documents/national_coastal_condition_assessment_2015_field_operation_manual_version_1.0_1.pdf
Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants	Schmitt et al. 1999	https://www.cerc.usgs.gov/pubs/center/pdfDocs/91116.pdf

[1] Fishing sampling in these references are designed specifically for assessing risk to human health through fish consumption.

3.2.3 Using Existing Data to Enhance Selenium Monitoring

The EPA recommends states and authorized Tribes consider and use all available data, as appropriate, to inform and enhance selenium monitoring. According to the EPA's *2010 Fish Advisory Survey Report*, 28 states identify selenium as a contaminant in their fish monitoring program (USEPA 2011). Several states have conducted extensive statewide assessments and could have existing selenium data. Other organizations may also have selenium data available. For example, the Ohio River Valley Water Sanitation Commission collects fish tissue samples for selenium analysis as part of their Fish Consumption Advisory Program and has data available online (http://www.orsanco.org/fish-tissue). National scale data sources for selenium in fish tissue samples include the EPA's *2008-2009 National Rivers and Streams Assessment* (available at https://www.epa.gov/national-aquatic-resource-surveys/national-rivers-and-streams-assessment-2008-2009-results) and the National Listing of Fish Advisories Fish Tissue Search database (available at https://fishadvisoryonline.epa.gov/FishTissue.aspx). The EPA also has concentration data available from 100 paired mercury and selenium fish fillet samples collected in 2007 (available at http://www.epa.gov/sites/production/files/2015-07/mercury-finaldata2012.xlsx). Sample sites for this 2007 study were randomly selected from U.S. locations where mercury advisories for fish consumption were in place at the time of sampling. Available data can be used to conserve limited resources by providing baseline information which can inform future collections by indicating which areas may and may not need additional monitoring.

4.0 Sample Analysis

4.1 Analytical Chemistry

Fish tissue sampling to support implementation of criteria based on the national CWA section 304(a) recommended selenium criterion should address many of the same analytical concerns as those associated with other fish tissue monitoring programs. Various researchers have shown that analytical results on the same population of fish can differ between studies and even within studies. These uncertainties, inherent in any sampling program, can be minimized

through a rigorous study design, clear data quality objectives, meticulous QA/QC protocols, and careful execution of the monitoring program in the field. Standardized sampling methods should be followed in the field to ensure the appropriate samples (that have been handled, preserved, and shipped according to protocol) are analyzed in the laboratory (Beatty and Russo 2014). Consistent analytical methods and procedures should be used across implementation programs that are utilizing fish tissue data. Analytical methods should be selected that are sufficiently sensitive to address study objectives (e.g., analytical methods with detection limits below the selenium fish tissue criterion elements after allowing for conversion to dry weight concentrations) and minimize the number of values that are below the MDL. Results should be reported to the appropriate significant figures for the precision of the analytical method.

Laboratories should be selected based on relevant laboratory accreditations, strong QA/QC protocols, and experience with using analytical methods for selenium and the fish tissue matrix. Samples should be prepared in accordance with the tissue type. Section 7.2.2 of the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) includes detailed directions for preparing muscle and whole-body samples. Appendix A of this document includes directions for preparing egg and ovary samples.

As of the publication date of this technical support material (TSM), the EPA does not have approved 40 CFR Part 136 analytical methods for measuring selenium in fish tissue. However, states and authorized Tribes are not required to use EPA-approved analytical methods for the monitoring and assessment of criteria attainment or criteria development. Additionally, in the case of pollutants or pollutant parameters for which there are not approved analytical methods under 40 CFR Part 136 or methods are not otherwise required under 40 CFR Chapter I, subchapter N or O, monitoring for activities related to NPDES permit applications, permit limits, or permit compliance reports shall be conducted according to a test procedure specified in the NPDES permit for such pollutants or pollutant parameters.[12] In the assessment of criteria attainment and establishment of lists of waters not attaining criteria, however, states and authorized Tribes are required to assemble and evaluate all existing and readily-available water quality-related data and information (40 CFR 130.7(b)(5)). If a state or authorized Tribe has additional laws concerning data acceptability or laboratory accreditation programs, then the fish tissue analytical methods implemented by the state or authorized Tribe should follow these laws.

Before selecting an analytical method and a laboratory to conduct selenium analyses, states and authorized Tribes should discuss with laboratories their MDLs for detecting selenium in fish tissue using a particular analytical method. States and authorized Tribes should confirm whether those MDLs are for wet weight or dry weight and ensure that they are sensitive enough for the assessment of the selenium criterion or for site-specific study purposes. Table 5 presents several analytical procedures for measuring selenium in solids and biota with MDLs that are sufficiently sensitive for comparison to the tissue criterion elements. Exact MDLs and

[12] The standard conditions of a NPDES permit (40 CFR 122.41(j)(4) and 122.44(i)) require, when available, permittees use test procedures specified in 40 CFR Part 136.

quantitation limits (QL) for these analytical methods are not provided, as those values are laboratory and project specific; however, all the analytical methods listed below should be able to detect a selenium concentration of at least 1.5 mg/kg dry weight (dw). States and authorized Tribes should decide which value they want the laboratory to use for reporting, whether they would like it to be equal to the MDL, QL, or some alternative value that they have confidence in using for regulatory decisions. See section 4.2 of this document for discussion about evaluating data that are below the MDL or falls in between the MDL and the QL. Furthermore, some of the analytical methods and procedures identified in Table 5 do not include specific QC requirements and acceptance limits. Therefore, states and authorized Tribes should work closely with the laboratory to establish appropriate requirements so that data meet the selenium monitoring objectives.

Table 5. List of Test Procedures for Total Selenium in Solids and Biota.

Method	Digestion / Preparation in reference method?	Links to Analytical Methods
EPA Method 6020B[a] – Inductively Coupled Plasma - Mass Spectrometry (ICP - MS)	No – Recommended: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/esam/epa-method-6020b-sw-846-inductively-coupled-plasma-mass-spectrometry https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf
EPA Method 7742[a] – Selenium (Atomic Absorption, Borohydride Reduction)	No- Recommended: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/sites/production/files/2015-12/documents/7742.pdf (See links for digestion methods above)
USGS I-9020-05 – Determination of Elements in Natural-water, Biota, Sediment, and Soil Samples using Collision /Reaction Cell ICP – MS	No – References 3052 (total) Recommended: 3052 (total), or 3050B (total recoverable)	https://pubs.usgs.gov/tm/2006/tm5b1/PDF/TM5-B1.pdf (See links for digestion methods above)

Method	Digestion / Preparation in reference method?	Links to Analytical Methods
NOAA 140.1 - Graphite Furnace-Atomic Absorption for the Analysis of Trace Metals in Marine Animal Tissues	Yes – Teflon Bomb	https://www.nemi.gov/methods/method_summary/7185/
EPA Method 200.8, Rev 5.4[1,2] – Determinations of Trace Elements in Waters by ICP- MS (USEPA 1994)	Yes - Section 11.3 May also use: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf

[1] These EPA methods are not included in 40 CFR Part 136 for fish tissue analysis. The EPA does not currently have any 40 CFR Part 136 methods for analyzing parameters in fish tissue.

[2] Tissue samples must be digested before using this method.

Fish tissue samples should be homogenized and digested prior to analysis using strong acid and either a closed-vessel microwave digestion or an open-vessel heated digestion procedure. If samples are to be dried before homogenization and digestion, freeze drying is a good drying technique to use to minimize selenium losses from the sample. The possibility of volatilization of selenium from the sample is more likely when oven drying so, if possible, freeze drying should be used for selenium samples (Iyengar et al 1978). However, undried tissues may be homogenized and digested, and a dry weight conversion can be determined using a separate aliquot of the homogenized tissue. The suitability of a given technique should be discussed with the individual laboratory given its capabilities and preference. The laboratory and the agency submitting the samples should mutually decide on a technique that meets the purposes of the monitoring. Care should be taken to use a process that minimizes the loss of volatile selenium. Reference materials (from a source like the National Institutes of Standards and Technology, the National Research Council of Canada, or other traceable source), analytical duplicates, and matrix spike samples are recommended to determine the applicability of the selected digestion and analysis procedures.

The North American Metals Council-Selenium Work Group (NAMC-SWG) has published comprehensive discussions of analytical concerns relevant to selenium: Ohlendorf et al. (2008) and Ohlendorf et al. (2011). An additional NAMC-SWG document, Ralston et al. (2008), presents guidance on analytical methods for selenium. Inductively coupled plasma mass spectrometry is the typical method used for analyzing selenium in tissue and other matrices; however, this method is sensitive to interferences. When using this method, these potential interferences should be addressed. Alternative methods for analyzing selenium are discussed in D'Ulivo (1997), Ohlendorf et al. (2008), and Ralston et al. (2008). States and authorized Tribes should choose an analytical method that is sufficiently sensitive to implement its WQS for selenium or meet the site-specific study objectives.

States and authorized Tribes can also consider adapting methods for analyzing selenium in water to measure selenium in fish tissue, as long as the fish tissue samples are appropriately digested. In particular, EPA Method 200.8, Rev 5.4, *Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry* (USEPA 1994) can easily be adapted to tissue analyses by the addition of an appropriate digestion procedure. Additional information regarding analytical methods for water samples can be found in Appendix L of the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). Complete descriptions of analytical methods appropriate for analyzing selenium in different media can be found in the National Environmental Methods Index at http://www.nemi.gov.

4.2 Data Analysis

Generally, for implementation purposes, states and authorized Tribes can calculate a measure of central tendency (e.g., mean, median) for their selenium fish tissue data sets. The monitoring data set and information about the underlying distribution of the sample population should be used to determine which measure of central tendency is most appropriate. In addition, the variability around that measure of central tendency (e.g., standard deviation, standard error, confidence interval) should be quantified and considered when making implementation decisions. The EPA recommends using a measure of central tendency only for characterizing a data set that was collected during a single sampling event (identified location over a specified period of time (generally less than a week)). Different sampling events should all be considered as discrete data sets.

At times, states and authorized Tribes may conduct resource management studies that include the collection of fish tissue data from several locations within a region of interest or for multiple time periods (e.g., seasons or years) from a single location, or a combination of both. Data from intensive studies such as these may be used to perform spatial or temporal analyses to provide information on selenium variability in a target species population. The EPA's *Fish Advisory Guidance Volume 1* provides recommended statistical approaches for comparing contaminants measured at different locations or over time (See Appendix N of USEPA 2000). The EPA recommends that states and authorized Tribes consult a statistician to determine the specific statistical tests needed for a particular study question and data set. Consulting a statistician at the time of the study design may be useful for assuring that the appropriate data are collected to answer the desired question.

When performing these data analyses, states and authorized Tribes should consider how they will address potential data quality concerns, such as the use of analytical results that are below the MDL and/or analytical results that are in between the MDL and the QL. These results can be largely avoided with proper quality assurance project planning. The collection of sufficient tissue mass and use of a sufficiently sensitive analytical method will provide results with a minimal number of values below the MDL and between the MDL and QL. However, if a state or authorized Tribe is using a data set that includes values below the MDL or in between the MDL and the QL, it should decide how it will evaluate these values.

There are various options to deal with these measurements. The EPA notes that identifying and developing approaches to statistically analyze data sets containing non-quantified chemical concentration values (i.e., "censored data") is an active area of research and no one method can be recommended in all circumstances (for more information see: Helsel 2005, Pleil 2016, and Singh and Nocerino 2002). The EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends using one-half of the MDL for values below the MDL in calculating mean values (section 9.1.2). The guidance also recommends that measurements that fall between the MDL and the QL be assigned a value of the MDL plus one-half the difference between the MDL and the QL. The EPA notes, however, that these conventions provide a biased estimate of the average concentration (Gilbert 1987) and, where the computed average is close to the criterion, might suggest an impairment when one does not exist or, conversely, suggest no impairment when one does exist. As an alternative to this option, some states, authorized Tribes, and laboratories may choose to apply what is called a "J" flag to any results reported at or above the MDL, but below the QL. The "J" flag would indicate that the chemical is present, but the reported value is an estimate of the true concentration since it was detected below the QL. Some states and authorized Tribes may choose to use these "J" flagged values for data analysis. The EPA used this option for the National Study of Chemical Residues in Lake Fish Tissue, including all the "J" flagged data in analyses of the fish tissue data (USEPA 2009).

States or authorized Tribes can also calculate the average of a data set that includes values below the MDL using other statistical methods (e.g., robust regression on order statistics, maximum likelihood estimation, Kaplan-Meier) (Helsel 2012, Millard 2013). George et al. (2021) have published a review of several methods for data reporting and analyzed the potential bias each can introduce into the calculation of the mean.

One approach that a state or authorized Tribe could take to ascertain the effect of what value is used to quantify samples below the MDL is to conduct a sensitivity analysis. In a sensitivity analysis, the state or authorized Tribe would compute the mean concentration by first using the value of the MDL to quantify samples below the MDL and then using a zero value for samples below the MDL. For example, if the MDL is 1.5 mg/kg dw, first the mean would be calculated with all values below the MDL being assigned the value of 1.5 mg/kg dw. Then the mean would be recalculated with the value of 0.0 mg/kg dw being assigned to all values below the MDL. If both calculated means are above or below the criterion, the choice of how to quantify samples below the MDL does not affect the decision. However, if one calculated mean is below the criterion and the other is above, the choice of how to quantify samples below the MDL does affect the decision, and a state or authorized Tribe may want to use a more sophisticated approach such as the ones presented in Helsel (2012) or Millard (2013).

All data handling options have tradeoffs. A state or authorized Tribe should understand the tradeoffs of which option it uses, especially if the choice makes a difference as to whether a waterbody is considered impaired. Furthermore, a state or authorized Tribe should be clear about which approach it used in its assessment methodology. The selected approach must be consistent with the state or authorized Tribe's EPA-approved WQS and should generally adhere to any published assessment method associated with them. For further discussion on handling

values below the MDL, see USEPA 2000 (section 9.1) and USEPA 2010 (section 4.3.1). In general, states and authorized Tribes should not have issues with measurements of selenium in fish tissue being below the MDL when a sufficient mass of tissue is collected as the method sensitivities are low enough and all fish should have selenium concentrations higher than those MDLs. Similarly, with sufficient sample mass and appropriate analytical methods, it is unlikely that many states and authorized Tribes will have selenium measurements between the MDL and QL for fish tissue.

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Appendix A:
Egg and Ovary Sample Preparation

Scope

This guidance is for egg and ovary collection from freshwater fish. The egg extraction method is excerpted and adapted from a more comprehensive guidance, *Standard operating procedure for evaluating selenium-induced deformities in early life stages of freshwater fish* (Janz and Muscatello 2008), that includes gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish. The ovary dissection method was compiled from peer-reviewed literature.

1) Field collection and handling of adult fish

> "Spawning adults can be collected in the field using a wide variety of techniques, including fish traps (e.g., hoop or trap nets), electrofishing or angling in areas close to spawning areas. Gillnets are also effective in capturing fish during spawning migrations, but it is essential to monitor these nets constantly to remove fish immediately after capture. If possible, the use of passive capture methods (e.g., hoop or trap nets) is recommended since this is the least stressful capture technique of those listed above. Trap nets are usually set up in creeks, streams or narrows in lakes, although successful fish capture can also occur when these nets are set perpendicular to shore in lentic habitats. Trap or hoop nets can be purchased from fisheries suppliers, or even constructed in creeks and streams using chicken wire, baling wire and reinforcing bar." (Janz and Muscatello 2008)

Fish should be held in livewells until adult female fish are selected for egg collection.

2) Egg collection procedures

Fish should be carefully observed for signs of physical damage, mortality, or other sources of stress. Since any handling of the fish will remove the protective body layer of slime, fish should be handled as little as possible using dip nets and soft material gloves. Adult fish for egg collection should be randomly selected from livewells.

> "Eggs should not be in contact with water; thus, it is imperative to dry the area surrounding the urogenital opening with paper towels. All the material used for egg collection should be carefully cleaned and dried. Precautions to avoid fecal, blood or urine contamination should be taken. [Eggs] must be kept covered to avoid direct sun exposure. [Egg collection] should proceed after recording weight and length [of the gravid female]. Gentle pressure from behind the pectoral fins towards the anus is applied to express the eggs. This process needs to

be repeated several times. Check that eggs are released 'clean' (e.g., without feces) before starting collection to avoid contamination of the entire egg batch. Eggs are individually collected into pre-cleaned stainless steel bowls and kept covered in a cool place until use. Collected eggs should be closely inspected and eggs with adhered feces, urine or blood discarded by using a clean plastic pipette." (Janz and Muscatello 2008)

Eggs are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate. An individual or composite homogenate tissue sample of 20 grams ww should be collected for analysis of selenium.

3) Ovary dissection procedures

Fish designated for ovary collection should be humanely euthanized, and necropsy procedures should commence immediately following euthanasia (Wolf et al. 2004). The fish should be placed in right lateral recumbency on a piece of aluminum foil. The left body wall should be removed by using fine dissecting instruments (Wolf et al. 2004). To identify female specimens for ovary collection, sex is determined by macroscopic inspection when the body cavity is opened. The ovaries are paired organs suspended from the dorsal wall, with color ranging from clear to white to yellow-orange. A yellow-orange color is indicative of a ripening or ripe adult specimen. Further, increased blood flow during the reproductive season causes the ovaries to become highly vascularized and appear reddish. In cross-section, the ovaries are round to elliptical and contain a central cavity (lumen). In young fish, the texture of the ovaries varies from smooth to slightly granular. The ovarian texture in a ripe fish will be highly granular (Fisheries Information Network 2006). If inspection of the ovaries reveals that the specimen is immature or developing, it is not recommended that the eggs/ovarian tissue be used for tissue monitoring for selenium.

After confirmation that the specimen is a ripe female, the ovaries should be excised by severing the oviducts and mesenteric attachments. All gonads are dissected in a caudal to cranial direction (Wolf et al. 2004). Ovaries are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate (Orr et al. 2012). An individual or composite homogenate sample of 20 grams ww of tissue should be collected for analysis of selenium.

4) Storing fish eggs and ovaries

"Eggs and ovaries should be kept frozen until analysis. After collection, samples should be kept in a container with ice or freezer packs until transfer to a freezer (–20°C) for storage" (Janz and Muscatello 2008). It is recommended to transfer the samples collected from each individual female into sealed resealable plastic storage bags to "prevent water (from ice melting) entering the sample" (Janz and Muscatello 2008). Recommendations for the storage, preservation and holding time for egg and ovary samples are equivalent to other tissue samples. Samples should be frozen at –20°C in plastic, borosilicate glass, quartz, or PTFE bottles. The recommended

maximum holding time is six months but can be up to two years for most trace metals, including selenium (USEPA 2000).

5) Laboratory preparation of egg and tissue samples for metal analysis

> "Egg and tissue samples should be thawed, and wet weight recorded for each individual sample. To prevent cross contamination between samples, a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is required to record the moisture content for each individual sample in order to express analytical data on a dry weight basis. Trace element (e.g., selenium) analysis is routinely performed using hydride generation atomic absorption spectrophotometry (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry-weight basis." (Janz and Muscatello 2008)

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Appendix B:
Spawning Seasons for Example Fish Assemblages from Select U.S. Watersheds

This appendix contains spawning season calendars for fish assemblages from selected watersheds in six different areas of the United States. The calendars are intended to provide examples of spawning periods for fish species commonly collected in those areas. The EPA recommends that monitoring agencies use all available locally relevant resources to determine the appropriate time to collect fish for the purpose of implementing the selenium criterion, including contacting their local natural resources or fish and game agency.

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Table B-1. Spawning Seasons for Example Fish Assemblages in the Merrimack River, MA and NH Watershed.

Family	Scientific Name	Common Name	Spawning Season
Atherinopsidae	*Menidia menidia*	Atlantic Silverside	April through August
Catostomidae	*Catostomus commersonii*	White Sucker	March through July
Centrarchidae	*Ambloplites rupestris*	Rock Bass	April through July
Centrarchidae	*Enneacanthus obesus*	Banded Sunfish	April through July
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	April through July
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	June through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through June
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	April through July
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	March through August
Cyprinidae	*Carassius auratus*	Goldfish	March through August
Cyprinidae	*Cyprinus carpio*	Common Carp	April through August
Cyprinidae	*Luxilus cornutus*	Common Shiner	May through July
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	May through July
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	May through June
Cyprinidae	*Notropis bifrenatus*	Bridle Shiner	May through August
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	May through September
Cyprinidae	*Rhinichthys atratulus*	Blacknose Dace	April through July
Cyprinidae	*Rhinichthys cataractae*	Longnose Dace	April through June
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	March through June
Cyprinidae	*Semotilus corporalis*	Fallfish	April through May
Esocidae	*Esox lucius*	Northern Pike	March through May
Esocidae	*Esox niger*	Chain Pickerel	March through May
Fundulidae	*Fundulus diaphanus*	Banded Killifish	April through August
Fundulidae	*Fundulus heteroclitus*	Mummichog	June through July
Gadidae	*Lota lota*	Burbot	January through April
Gasterosteidae	*Apeltes quadracus*	Fourspine Stickleback	April through May
Gasterosteidae	*Gasterosteus aculeatus*	Threespine Stickleback	March through June
Gasterosteidae	*Pungitius pungitius*	Ninespine Stickleback	April through August
Ictaluridae	*Ameiurus catus*	White Catfish	May through July
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through June
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	April through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through September
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through July
Ictaluridae	*Noturus insignis*	Margined Madtom	June through July
Moronidae	*Morone americana*	White Perch	May through June
Percidae	*Etheostoma fusiforme*	Swamp Darter	April through May
Percidae	*Etheostoma olmstedi*	Tessellated Darter	March through May
Percidae	*Perca flavescens*	Yellow Perch	May through July

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Sander vitreus*	Walleye	April through May
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	April through June
Salmonidae	*Salmo trutta*	Brown Trout	October through February
Salmonidae	*Salvelinus fontinalis*	Brook Trout	September through November

(Scarola 1973, Page and Burr 1991)

Table B-2. Spawning Seasons for Example Fish Assemblages in the Delaware River, DE Watershed.

Family	Scientific Name	Common Name	Spawning Season
Aphredoderidae	*Aphredoderus sayanus*	Pirate Perch	April through May
Atherinopsidae	*Membras martinica*	Rough Silverside	May through August
Atherinopsidae	*Menidia peninsulae*	Tidewater Silverside	May through August
Atherinopsidae	*Menidia menidia*	Atlantic Silverside	April through August
Catostomidae	*Catostomus commersonii*	White Sucker	March through May
Catostomidae	*Erimyzon oblongus*	Creek Chubsucker	March through May
Centrarchidae	*Acantharchus pomotis*	Mud Sunfish	May through June
Centrarchidae	*Enneacanthus chaetodon*	Blackbanded Sunfish	May through July
Centrarchidae	*Enneacanthus gloriosus*	Bluespotted Sunfish	May through September
Centrarchidae	*Enneacanthus obesus*	Banded Sunfish	June through September
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	May through June
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	May through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis annularis*	White Crappie	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through June
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through June
Cyprinidae	*Carassius auratus*	Goldfish	June through July
Cyprinidae	*Cyprinus carpio*	Common Carp	May through July
Cyprinidae	*Hybognathus nuchalis*	Mississippi Silvery Minnow	April through May
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Cyprinella analostana*	Satinfin Shiner	March through July
Cyprinidae	*Notropis bifrenatus*	Bridle Shiner	March through August
Cyprinidae	*Notropis chalybaeus*	Ironcolor Shiner	April through May
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	April through July
Cyprinidae	*Rhinichthys atratulus*	Blacknose Dace	May through June
Esocidae	*Esox americanus americanus*	Redfin Pickerel	February through March
Fundulidae	*Fundulus diaphanus*	Banded Killifish	April through August
Fundulidae	*Fundulus heteroclitus*	Mummichog	April through September
Fundulidae	*Fundulus majalis*	Striped Killifish	April through September
Fundulidae	*Lucania parva*	Rainwater Killifish	May through July
Ictaluridae	*Ameiurus catus*	White Catfish	April through July
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	May through July
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	May through July
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through July
Moronidae	*Morone americana*	White Perch	April through June
Percidae	*Etheostoma fusiforme*	Swamp Darter	April through May
Percidae	*Etheostoma olmstedi*	Tessellated Darter	March through May

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Perca flavescens*	Yellow Perch	March through April
Poeciliidae	*Gambusia affinis*	Mosquitofish	May through August
Umbridae	*Umbra pygmaea*	Eastern Mudminnow	April through June

(Wang and Kernehan 1979, Page and Burr 1991)

Table B-3. Spawning Seasons for Example Fish Assemblages in the Cahaba River, AL Watershed.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Atherinopsidae	*Labidesthes sicculus*	Brook Silverside	June through August
Catostomidae	*Carpiodes cyprinus*	Quillback	March through September
Catostomidae	*Carpiodes velifer*	Highfin Carpsucker	May through July
Catostomidae	*Erimyzon oblongus*	Creek Chubsucker	March through May
Catostomidae	*Erimyzon sucetta*	Lake Chubsucker	March through April
Catostomidae	*Erimyzon tenuis*	Sharpfin Chubsucker	March through April
Catostomidae	*Hypentelium etowanum*	Alabama Hog Sucker	April through June
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	March through April
Catostomidae	*Minytrema melanops*	Spotted Sucker	April through May
Catostomidae	*Moxostoma carinatum*	River Redhorse	April
Catostomidae	*Moxostoma duquesnii*	Black Redhorse	April through May
Catostomidae	*Moxostoma erythrurum*	Golden Redhorse	April through June
Catostomidae	*Moxostoma poecilurum*	Blacktail Redhorse	April
Centrarchidae	*Ambloplites ariommus*	Shadow Bass	May through October
Centrarchidae	*Centrarchus macropterus*	Flier	February through May
Centrarchidae	*Lepomis macrochirus*	Bluegill	March through May
Centrarchidae	*Lepomis marginatus*	Dollar Sunfish	May through August
Centrarchidae	*Lepomis megalotis*	Longear Sunfish	May through August
Centrarchidae	*Lepomis microlophus*	Redear Sunfish	March through May; September through November
Centrarchidae	*Lepomis miniatus*	Redspotted Sunfish	March through September
Centrarchidae	*Micropterus coosae*	Redeye Bass	May through July
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	March through May
Centrarchidae	*Micropterus punctulatus*	Spotted Bass	April through May
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis annularis*	White Crappie	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	February through May
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through May
Clupeidae	*Dorosoma petenense*	Threadfin Shad	April through August
Cottidae	*Cottus carolinae*	Banded Sculpin	January through March
Cyprinidae	*Campostoma oligolepis*	Largescale Stoneroller	April through May
Cyprinidae	*Cyprinella callistia*	Alabama Shiner	March through May
Cyprinidae	*Cyprinella trichroistia*	Tricolor Shiner	June through July
Cyprinidae	*Cyprinella venusta*	Blacktail Shiner	March through October
Cyprinidae	*Hybognathus nuchalis*	Mississippi Silvery Minnow	March through April
Cyprinidae	*Hybopsis winchelli*	Clear Chub	February through April
Cyprinidae	*Luxilus chrysocephalus*	Striped Shiner	April through August

Family	Scientific Name	Common Name	Spawning Season
Cyprinidae	*Lythrurus bellus*	Pretty Shiner	April through June
Cyprinidae	*Macrhybopsis storeriana*	Silver Chub	May through August
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Notropis ammophilus*	Orangefin Shiner	April through October
Cyprinidae	*Notropis asperifrons*	Burrhead Shiner	April through June
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	May through July
Cyprinidae	*Notropis baileyi*	Rough Shiner	May through October
Cyprinidae	*Notropis buccatus*	Silverjaw Minnow	March through June
Cyprinidae	*Notropis candidus*	Silverside Shiner	June through September
Cyprinidae	*Notropis chrosomus*	Rainbow Shiner	May through June
Cyprinidae	*Notropis edwardraneyi*	Fluvial Shiner	May through June
Cyprinidae	*Notropis stilbius*	Silverstripe Shiner	March through August
Cyprinidae	*Notropis texanus*	Weed Shiner	February through October
Cyprinidae	*Notropis uranoscopus*	Skygazer Shiner	May through July
Cyprinidae	*Notropis volucellus*	Mimic Shiner	April through August
Cyprinidae	*Opsopoeodus emiliae*	Pugnose Minnow	April through September
Cyprinidae	*Phenacobius catostomus*	Riffle Minnow	April through May
Cyprinidae	*Pimephales notatus*	Bluntnose Minnow	April through August
Cyprinidae	*Pimephales vigilax*	Bullhead Minnow	May through August
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	April through May
Cyprinidae	*Semotilus thoreauianus*	Dixie Chub	April through May
Elassomatidae	*Elassoma zonatum*	Banded Pygmy Sunfish	March through April
Esocidae	*Esox americanus*	Redfin Pickerel	April through May
Esocidae	*Esox niger*	Chain Pickerel	April through October
Fundulidae	*Fundulus olivaceus*	Blackspotted Topminnow	March through September
Hiodontidae	*Hiodon tergisus*	Mooneye	April through May
Ictaluridae	*Ameiurus melas*	Black Bullhead	May through August
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	April through June
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	April through August
Ictaluridae	*Ictalurus furcatus*	Blue Catfish	April through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through July
Ictaluridae	*Noturus funebris*	Black Madtom	May through June
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through September
Ictaluridae	*Pylodictis olivaris*	Flathead Catfish	June through July
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	May through July
Lepisosteidae	*Lepisosteus osseus*	Longnose Gar	April through August
Moronidae	*Morone chrysops*	White Bass	February through March
Percidae	*Ammocrypta beanii*	Naked Sand Darter	March through October
Percidae	*Etheostoma meridianum*	Southern Sand Darter	April through June
Percidae	*Etheostoma chlorosomum*	Bluntnose Darter	April
Percidae	*Etheostoma jordani*	Greenbreast Darter	April through May

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Etheostoma nigrum*	Johnny Darter	March through May
Percidae	*Etheostoma parvipinne*	Goldstripe Darter	March through April
Percidae	*Etheostoma ramseyi*	Alabama Darter	March through May
Percidae	*Etheostoma rupestre*	Rock Darter	March through April
Percidae	*Etheostoma stigmaeum*	Speckled Darter	March through May
Percidae	*Etheostoma swaini*	Gulf Darter	March through April
Percidae	*Percina kathae*	Mobile Logperch	April through June
Percidae	*Percina maculata*	Blackside Darter	March through June
Percidae	*Percina nigrofasciata*	Blackbanded Darter	May through June
Percidae	*Percina vigil*	Saddleback Darter	February through April
Percidae	*Sander vitreus*	Walleye	March through April
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	May through June

(Boschung and Mayden 2004)

Table B-4. Spawning Seasons for Example Fish Assemblages in the Chicago River, IL Watershed.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Catostomidae	*Catostomus commersonii*	White Sucker	April through May
Centrarchidae	*Ambloplites rupestris*	Rock Bass	May through July
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	June through August
Centrarchidae	*Lepomis humilis*	Orangespotted Sunfish	May through July
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	May through July
Centrarchidae	*Lepomis gulosus*	Warmouth	May through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through June
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through July
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	May through July
Cyprinidae	*Campostoma anomalum*	Central Stoneroller	April through July
Cyprinidae	*Carassius auratus*	Goldfish	May through June
Cyprinidae	*Cyprinella spiloptera*	Spotfin Shiner	May through August
Cyprinidae	*Cyprinus carpio*	Common Carp	May through August
Cyprinidae	*Hybopsis dorsalis*	Bigmouth Shiner	May through June
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	May through August
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	April through August
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	June through July
Cyprinidae	*Notropis stramineus*	Sand Shiner	May through July
Cyprinidae	*Pimephales notatus*	Bluntnose Minnow	May through August
Cyprinidae	*Pimephales promelas*	Fathead Minnow	May through August
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	April through June
Cyprinodontidae	*Fundulus notatus*	Blackstripe Topminnow	May through August
Esocidae	*Esox americanus*	Grass Pickerel	May through June; November
Esocidae	*Esox lucius*	Northern Pike	March through May
Gobiidae	*Neogobius melanostomus*	Round Goby	April through May
Ictaluridae	*Ameiurus melas*	Black Bullhead	May through June
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through August
Moronidae	*Morone americana*	White Perch	May through June
Moronidae	*Morone chrysops*	White Bass	April through June
Moronidae	*Morone mississippiensis*	Yellow Bass	April through May
Percidae	*Etheostoma nigrum*	Johnny Darter	April through June
Percidae	*Sander vitreus*	Walleye	April through May
Percidae	*Perca flavescens*	Yellow Perch	May through July
Umbridae	*Umbra limi*	Central Mudminnow	April through May

(Auer 1982, Page and Burr 1991)

Table B-5. Spawning Seasons for Example Fish Assemblages in the Truckee and Carson River, NV Watersheds.

Family	Scientific Name	Common Name	Spawning Season
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through July
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through July
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through July
Ictaluridae	*Ictaluridae*	Catfish species	June through July
Moronidae	*Morone saxatilis*	Striped Bass*	April through June
Moronidae	*Morone chrysops*	White Bass	April through June
Percidae	*Sander vitreus*	Walleye	January through April
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	March through May
Salmonidae	*Salmo trutta*	Brown Trout	January through March
Salmonidae	*Prosopium williamsoni*	Mountain Whitefish	October through December

This population of striped bass is landlocked and cannot migrate out to sea.

(Nevada Division of Environmental Protection 2006)

Table B-6. Spawning Seasons for Example Fish Assemblages in the Rio Grande and Colorado River, TX Watersheds.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Anguillidae	*Anguilla rostrata*	American Eel	February through June
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	March through September
Catostomidae	*Ictiobus cyprinellus*	Bigmouth Buffalo	April through May
Catostomidae	*Ictiobus niger*	Black Buffalo	April through May
Centrarchidae	*Lepomis macrochirus*	Bluegill	April through September
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	April through August
Centrarchidae	*Lepomis megalotis*	Longear Sunfish	May through June
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	April through October
Centrarchidae	*Lepomis microlophus*	Redear Sunfish	May through July
Centrarchidae	*Lepomis gulosus*	Warmouth	March through October
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	February through May
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through May
Centrarchidae	*Micropterus punctulatus*	Spotted Bass	April through June
Centrarchidae	*Micropterus treculii*	Guadalupe Bass	March through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	March through May
Centrarchidae	*Pomoxis annularis*	White Crappie	March through May
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande Cichlid	March through August
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through June
Clupeidae	*Dorosoma petenense*	Threadfin Shad	April through September
Cyprinidae	*Ctenopharyngodon idella*	Grass Carp	April through July
Cyprinidae	*Cyprinus carpio*	Common Carp	March through June
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	April through September
Cyprinidae	*Cyprinella venusta*	Blacktail Shiner	April through September
Cyprinidae	*Notropis amabilis*	Texas Shiner	February through September
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Pimephales promelas*	Fathead Minnow	May through September
Esocidae	*Esox niger*	Chain Pickerel	December through February
Ictaluridae	*Ictalurus furcatus*	Blue Catfish	April through May
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through June
Ictaluridae	*Pylodictis olivaris*	Flathead Catfish	June through July
Ictaluridae	*Ameiurus melas*	Black Bullhead	April through June
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through July
Lepisosteidae	*Atractosteus spatula*	Alligator Gar	April through May
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	April through June

Family	Scientific Name	Common Name	Spawning Season
Lepisosteidae	*Lepisosteus osseus*	Longnose Gar	April through July
Lepisosteidae	*Lepisosteus platostomus*	Shortnose Gar	May through July
Moronidae	*Morone chrysops*	White Bass	March through May
Moronidae	*Morone mississippiensis*	Yellow Bass	April through June
Moronidae	*Morone saxatilis*	Striped Bass*	February through April
Percidae	*Sander vitreus*	Walleye	February through April
Polyodontidae	*Polyodon spathula*	Paddlefish	February through June
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	November through February
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	April through June
Sciaenidae	*Sciaenops ocellatus*	Red Drum	August through October

*This population of striped bass is landlocked and cannot migrate out to sea.

(Hendrickson and Cohen 2015, Texas Parks and Wildlife Department 2016)

Appendix C:
Conversion of Wet to Dry Tissue Weight

Conversion of Wet to Dry Tissue Weight

Selenium data in fish tissues can be reported in either dry weight (dw) or wet weight (ww) concentrations. It is essential that exposure assessors be aware of this difference so that they may ensure consistency between units when comparing data. If the contaminant concentration is measured in wet weight of fish, then the concentration must be converted to dry weight units in order to be compared to the selenium criterion, which is expressed in dry weight (USEPA 2021). Wet weight may be converted to dry weight, and vice versa, using the following equations:

ww = dw x [1 - (percent moisture/100)] (USEPA 2011)

dw = ww / [1 - (percent moisture/100)] (USEPA 2011)

Measurements reported as wet weight can be converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data are unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) may be used. Table C-1 lists percent moisture of some species by tissue type (USEPA 2021). Percent moisture can vary within species; therefore, the data in Table C-1 should generally be used when dealing with historical data. When using field collected data, measuring percent moisture within the field collected sample will provide the most accurate measurement of percent moisture, thus giving more accurate conversions between dry weight and wet weight data.

Table C-1. Percent Moisture by Species and Tissue Type.

Scientific Name	Common Name	Average % Moisture	% Moisture by Tissue			Reference
			Whole -body	Muscle	Egg-ovary	
Cyprinus carpio	Common Carp	75.64[a]		75.81[b]		USEPA 2014[a] Chatakondi et al. 1995[b]
Rhinichthys cataractae	Longnose Dace	73.25				USEPA 2014
Rhinichthys atratulus	Blacknose Dace	73.75				USEPA 2014
Semotilus atromaculatus	Creek Chub	76.71				USEPA 2014
Pimephales promelas	Fathead Minnow	76.64			75.3	USEPA 2014
Pimephales notatus	Bluntnose Minnow	74.8				USEPA 2014

Scientific Name	Common Name	Average % Moisture	% Moisture by Tissue			Reference
			Whole -body	Muscle	Egg-ovary	
Nocomis micropogon	River Chub	75.2				USEPA 2014
Ictalurus punctatus	Channel Catfish			81.22[a] 78.43[b]		Pinkney 2003[a] May et al. 2009[b]
Ictalurus melas	Black Bullhead	76.82				USEPA 2014
Pylodictis olivaris	Flathead Catfish			75.97		May et al. 2009
Catostomus commersonii	White Sucker	77.37				USEPA 2014
Coregonus clupeaformis	Lake Whitefish			80		Rieberger 1992
Oncorhynchus kisutch	Coho Salmon			80		Rieberger 1992
Oncorhynchus mykiss	Rainbow Trout			77.54	61.2	USEPA 2021
Sander canadensis	Sauger	77				USEPA 2014
Perca flavescens	Yellow Perch	73.98				USEPA 2014
Micropterus salmoides	Largemouth Bass	75.74[a]		79.06[b] 78.53[c]		USEPA 2014[a] Pinkney 2003[b] May et al. 2009[c]
Micropterus dolomieu	Smallmouth Bass	74.22				USEPA 2014
Pomoxis annularis	White Crappie			80.57		May et al. 2009
Pomoxis nigromaculatus	Black Crappie			79.75		May et al. 2009
Lepomis macrochirus	Bluegill		74.8	80.09	76	USEPA 2021
Ambloplites rupestris	Rock Bass	74.95				USEPA 2014
Esox lucius	Northern Pike			78		Rieberger 1992
Pylodictis olivaris	Flathead Catfish				58.97	May et al. 2009
Scaphirhynchus platorynchus	Shovelnose Sturgeon			77.13	47.18	May et al. 2009

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Appendix D: Extended List of Potential Target Species for Monitoring of Selenium in Fish Tissue

This appendix is intended for use with the recommendations in section 2.3 and Table 3. This appendix provides detailed information for the target species identified in Table 3, as well as additional species that may be considered appropriate for monitoring in certain situations (e.g., when species in Table 3 are unavailable for collection).

The tables in this appendix are generally organized by taxon (with the exception of a group of molluscivores) and includes nomenclature, distribution within US states, basic habitat information (warm water [WW] or cool or cold water [CW]), presence in waterbodies (e.g., lotic, lentic), adult diet, and adult trophic level. Information is presented by the following groupings:

1) Sturgeon in the family Acipenseridae
2) Sunfish and other genera in the family Centrarchidae
3) Trout and other genera in the family Salmonidae
4) Freshwater molluscivores & related genera (*Catostomidae*)
5) Minnows in the family Cyprinidae
6) Darters in the family Percidae
7) Sculpin in the family Cottidae

Information sources for these species include NatureServe (https://explorer.natureserve.org/Search), and the USGS NAS - Nonindigenous Aquatic Species Database (https://nas.er.usgs.gov/). Users of this Appendix should consult with both as they examine available information to make decisions about target species in state and authorized Tribal waters as they develop sampling plans.

1) Sturgeon

The family Acipenseridae comprise 27 species in four genera, with two genera (*Acipenser* and *Scaphyrhynchus*) occurring in the U.S. Sturgeon in the genus *Acipenser* (e.g., White Sturgeon) include three freshwater species. Sturgeons are long-lived, late maturing bottom feeding fishes inhabiting large river systems and estuaries. Independent monitoring for these species is generally discouraged since most populations are under pressure from habitat loss and other stressors and coordination with federal agencies (USFWS or NOAA-NMFS) is therefore recommended prior to developing sampling plans that may include these species.

Table D-1. Species in the Family Acipenseridae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Genus *Acipenser*			
White Sturgeon (*Acipenser transmontanus*)	US: AK, AZ, CA, ID, MT, OR, WA	WW Lotic Estuarine	Invertivore Piscivore Molluscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=300 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100679/Acipenser_transmontanus			
Shortnose Sturgeon* (*Acipenser brevirostrum*)	US: CT, DC, DE, FL, GA, MA, MD, ME, NC, NH, NJ, NY, PA, RI, SC, VA	WW Lotic Estuarine	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105033/Acipenser_brevirostrum			
Lake Sturgeon* (*Acipenser fluvescens*)	US: AL, AR, GA, IA, IL, IN, KS, KY, MI, MN, MO, NC, ND, NE, NY, OH, PA, SD, TN, VT, WI, WV	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=299 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104232/Acipenser_fulvescens			
Other Sturgeon:			
Shovelnose Sturgeon	US: AL, AR, IA, IL, IN, KS, KY, LA, MN, MO, MS, MT, ND, NE, NM,	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
(*Scaphirhynchus platorynchus*)	OH, OK, PA, SD, TN, TX, WI, WV, WY	Estuarine	
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103361/Scaphirhynchus_platorynchus			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

2) Sunfish (genus Lepomis), and other genera in the family Centrarchidae

The Bluegill is a species of freshwater fish and a member of the sunfish family Centrarchidae of the order Perciformes. It is native to North America and lives in streams, rivers, lakes, and ponds. The centrarchid family comprises 38 species of fish and includes many recreational and sportfish familiar to North Americans, including the Rock Bass, Largemouth Bass, Pumpkinseed, and crappies. This family typically inhabits medium to large warm water river systems and all sizes of lentic waterbodies. All species in the family are native to only North America.

Table D-2. Species in the Family Centrarchidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Genus *Lepomis*			
Bluegill (*Lepomis macrochirus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=385 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101764/Lepomis_macrochirus			
Pumpkinseed* (*Lepomis gibbosus*)	US: AL, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NV, NY, OH, OR, PA, RI, SC, TN, TX, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=382 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105048/Lepomis_gibbosus			
Redear Sunfish* (*Lepomis microlophus*)	US: AL, AR, AZ, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MI, MO, MS, NC, NE, NM, NV, OH, OK, OR, PA, SC, TN, TX, VA, VT, WV	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=390 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100707/Lepomis_microlophus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Green Sunfish (*Lepomis cyanellus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NJ, NM, NN, NV, NY, OH, OK, OR, PA, SC, SD, TN, TX, UT, VA, WA, WI, WV, WY	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=380 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103917/Lepomis_cyanellus https://pubs.usgs.gov/pp/1803/pdf/pp1803.pdf			
Redbreast Sunfish* (*Lepomis auritus*)	US: AL, AR, CT, DC, DE, FL, GA, KY, LA, MA, MD, ME, MS, NC, NH, NJ, NY, OK, PA, RI, SC, TN, TX, VA, VT, WV	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=379 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101339/Lepomis_auritus			
Longear Sunfish (*Lepomis megalotis*)	US: AL, AR, FL, GA, IA, IL, IN, KS, KY, LA, MD, MN, MO, MS, NC, NJ, NM, OH, OK, PA, TN, TX, VA, WI, WV	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=388 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.885331/Lepomis_megalotis			
Warmouth (*Lepomis gulosus*)	US: AL, AR, AZ, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, NJ, NM, NV, NY, OH, OK, OR, PA, SC, TN, TX, VA, WA, WI, WV	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=376 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102803/Lepomis_gulosus			
Orangespotted Sunfish (*Lepomis humilis*)	US: AL, AR, CO, FL, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=383 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103679/Lepomis_humilis			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Redspotted Sunfish (*Lepomis miniatus*)	US: AL, AR, IL, IN, KY, LA, MO, MS, OK, TN, TX	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=391 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105029/Lepomis_miniatus			
Bantam Sunfish (*Lepomis symmetricus*)	US: AR, IL, IN, KY, LA, MO, MS, OK, TN, TX	WW Lentic Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103718/Lepomis_symmetricus			
Northern Longear Sunfish (*Lepomis peltastes*)	US: IL, IN, MI, MN, NY, OH, PA	WW Lentic Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.883965/Lepomis_peltastes			
Spotted Sunfish (*Lepomis punctatus*)	US: FL, GA, NC, SC, TN	WW Lentic Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100708/Lepomis_punctatus			
Other Centrarchids			
Largemouth Bass (*Micropterus salmoides*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=401 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101622/Micropterus_salmoides			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Smallmouth Bass (*Micropterus dolomieu*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=396 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104786/Micropterus_dolomieu			
Spotted Bass (*Micropterus punctulatus*)	US: AL, AR, AZ, CA, FL, GA, IA, IL, IN, KS, KY, LA, MO, MS, NC, NE, NM, NV, OH, OK, PA, TN, TX, VA, WV	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=397 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.872252/Micropterus_punctulatus			
White Crappie (*Pomoxis annularis*)	US: AL, AR, AZ, CA, CO, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=408 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106200/Pomoxis_annularis			
Black Crappie (*Pomoxis nigromaculatus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=409 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103134/Pomoxis_nigromaculatus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Rock Bass (*Ambloplites rupestris*)	US: AL, AR, AZ, CT, DC, DE, GA, IA, IL, IN, KS, KY, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TN, TX, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=373 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105635/Ambloplites_rupestris			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

3) Salmonids, including Brown, Rainbow, and Cutthroat Trout and whitefish

Trout is the common name for species of freshwater fish belonging to the genera *Oncorhynchus*, *Salmo*, and *Salvelinus* in the family Salmonidae. Trout are considered cold water fish and are usually found in clear streams, rivers and lakes with temperatures not exceeding 60ºF (16ºC).

Table D-3. Species in the Family Salmonidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Family Salmonidae			
Brown Trout (*Salmo trutta*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SD, TN, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=931 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103603/Salmo_trutta			
Rainbow Trout (*Oncorhynchus mykiss*)	US: AK, AL, AR, AZ, CA, CO, CT, DE, GA, HI, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=910 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105164/Oncorhynchus_mykiss			
Cutthroat Trout (*Oncorhynchus clarkii*)	US: AK, AR, AZ, CA, CO, ID, MD, MT, ND, NM, NN, NV, OR, UT, WA, WY	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=890 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103888/Oncorhynchus_clarkii			
Dolly Varden (*Salvelinus malma*)	US: AK, NV, NM, WA, WY	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=941 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104555/Salvelinus_malma			
Brook Trout (*Salvelinus fontinalis*)	US: AK, AR, AZ, CA, CO, CT, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OR, PA, RI, SC, SD, TN, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=939 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103972/Salvelinus_fontinalis			
Mountain Whitefish (*Prosopium williamsoni*)	US: CA, CO, ID, MT, NV, OR, UT, WA, WY	CW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=924 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104696/Prosopium_williamsoni			
Round Whitefish (*Prosopium cylindraceum*)	US: AK, CT, IL, ME, MI, MN, NH, NY, VT, WI	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=921 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102380/Prosopium_cylindraceum			
Lake Whitefish* (*Coregonus clupeiformes*)	US: AK, ID, IL, IN, ME, MI, MN, MT, ND, NH, NV, NY, OH, PA, SD, VT, WA, WI	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=887 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105498/Coregonus_clupeaformis			

*Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

4) Freshwater Molluscivores and Related Genera

Molluscivorous fish feed either preferentially or opportunistically on a variety of mollusks (e.g., clams, mussels, and snails) in freshwater systems. Although taxonomically diverse, physiologically these fish are adapted to feed on mollusks due to the presence of teeth or plates on the lower (and in some species upper) pharyngeal jaws, as well as mouth gape and jaw muscle structure that accommodates feeding on mollusks (Eastman 1977). A study by the Army Corps of Engineers (Kirk, 2001) documents at least 17 species* of North American fish that consume invasive zebra mussels and quagga mussels in the genus *Dreissena*. Several of these species are sunfish in the genus *Lepomis*; they are presented in the table addressing sunfish. Molluscivores may have elevated exposure to selenium, as mollusks bioaccumulate more selenium than other classes of aquatic invertebrates. These taxa typically inhabit larger warm water lentic and lotic systems.

Table D-4. Molluscivores and Related Genera That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Freshwater Molluscivores			
Freshwater Drum* (*Aplodinotus grunniens*)	US: AL, AR, CO, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, MT, NC, ND, NE, NM, NY, OH, OK, PA, SD, TN, TX, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore Molluscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=946 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100338/Aplodinotus_grunniens			
White Perch* (*Morone americana*)	US: CO, CT, DC, DE, GA, IN, MA, MD, ME, MI, NC, NE, NH, NJ, NY, PA, RI, VA, VT, WI	WW Lentic Lotic Estuarine	Invertivore Molluscivore Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=777 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100436/Morone_americana			
White bass* (*Morone chrysops*)	US: AL, AR, AZ, CA, CO, DC, FL, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NM, NV, NY, OH, OK, PA, SC, SD, TN, TX, UT, VA, WI, WV	WW Lentic Lotic	Piscivore Invertivore Molluscivore TL4

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=779 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100951/Morone_chrysops			
Round Goby* (*Neogobius melanostomus*)	US: IL, IN, MI, MN, NY, OH, PA, WI	WW Lentic Lotic	Piscivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=713 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100501/Neogobius_melanostomus			
Brown Bullhead* (*Ameiurus nebulosus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, VA, VT, WA, WI, WV	WW Lentic Lotic	Omnivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=734 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103081/Ameiurus_nebulosus			
Yellow Perch* (*Perca flavescens*)	US: AL, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore Molluscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=820 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102985/Perca_flavescens			
Common carp* (*Cyprinus carpio*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Omnivore Molluscivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=4 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105636/Cyprinus_carpio			
Catostomidae			
Smallmouth Buffalo (*Ictiobus bubalus*)	US: AL, AR, AZ, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, MT, NC, ND, NE, NM, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=361 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105191/Ictiobus_bubalus			
Black Buffalo (*Ictiobus niger*)	US: AL, AR, AZ, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NC, ND, NE, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=363 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101227/Ictiobus_niger			
White sucker* (*Catostomus commersoni*)	US: AL, AR, CO, CT, DC, DE, GA, IA, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NM, NN, NY, OH, OK, PA, RI, SC, SD, TN, UT, VA, VT, WI, WV, WY	WW Lentic Lotic	Herbivore Molluscivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=346 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.833297/Catostomus_commersonii			
Largescale Sucker (*Catostomus macrocheilus*)	US: ID, MT, NV, OR, WA	CW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1098871/Catostomus_macrocheilus			
Greater Redhorse* (*Moxostoma valencienni*)	US: IL, IN, KY, MI, MN, ND, NY, OH, VT, WI	WW Lentic Lotic	Invertivore Molluscivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101488/Moxostoma_valenciennesi			
Shorthead Redhorse (*Moxostoma macrolepidutum*)	US: DC, DE, IA, IL, IN, KS, MD, MI, MN, MO, MS, MT, NC, ND, NE, NY, OH, OK, PA, SC, SD, TX, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=366 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.791411/Moxostoma_macrolepidotum			
River Redhorse (*Moxostoma carinatum*)	US: AL, AR, FL, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NC, NY, OH, OK, PA, TN, VA, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106031/Moxostoma_carinatum			
Golden redhorse (*Moxostoma erythrurum*)	US: AL, AR, DC, GA, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, ND, NY, OH, OK, PA, SD, TN, TX, VA, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=365 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100778/Moxostoma_erythrurum			
Silver Redhorse (*Moxostoma anisurum*)	US: AL, AR, GA, IA, IL, IN, KY, MI, MN, MO, MS, ND, NY, OH, PA, TN, VA, VT, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2912 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100712/Moxostoma_anisurum			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

5) Minnows (Cyprinidae)

The family Cyprinidae (carps and minnows) is naturally distributed throughout most of the world and is the largest family of freshwater fishes with about 2,010 species in 210 genera. About 300 species in 50 genera are native to North America (Canada, Mexico, United States; Nelson, 2006). Cyprinids exhibit considerable variation in morphology, diet, and habitat use, and are often the only fish taxa (along with darters and sculpins) occurring in small order streams. Although cyprinids are not typically considered monitoring targets for contaminant analysis in their tissues, they are routinely collected as part of state biomonitoring programs that use the fish index of biotic integrity to assess stream health in wadeable streams.

The EPA recommends that fish tissue monitoring programs collaborate with state or authorized Tribal biomonitoring programs to leverage expertise, experience, and resources to collect cyprinids and related species in watersheds located in geographic areas of elevated selenium where anthropogenic activities may introduce selenium to surface waters if other more sensitive species are not present.

Table D-5. Species in the Family Cyprinidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Fathead Minnow (*Pimephales promelas*)	US: AL, AR, AZ, CA, CO, CT, DE, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NM, NN, NV, NY, OK, OR, PA, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=621 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102599/Pimephales_promelas			
Bluntnose Minnow (*Pimephales notatus*)	US: AL, AR, CT, DC, GA, IA, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, NC, ND, NE, NJ, NY, OH, OK, PA, SD, TN, VA, VT, WI, WV	WW Lotic Lentic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=620 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103436/Pimephales_notatus			
Bullhead Minnow (*Pimephales vigilax*)	US: AL, AR, CO, GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, NE, NM, OH, OK, PA, SD, TN, TX, VA, WI,	WW Lentic Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=623 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106123/Pimephales_vigilax			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Cutlip Minnow (*Exoglossum maxillingua*)	US: CT, DC, DE, MD, NC, NJ, NY, PA, VA, VT, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=530 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102719/Exoglossum_maxillingua			
Suckermouth Minnow (*Phenacobius mirabilis*)	US: AL, AR, CO, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NE, NM, OH, OK, SD, TN, TX, VA, WI, WV, WY	WW Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=617 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104716/Phenacobius_mirabilis			
Blackstripe Topminnow (*Fundulus notatus*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MI, MO, MS, OH, OK, TN, TX, WI	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=690 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100269/Fundulus_notatus			
Starhead Topminnow (*Fundulus dispar*)	US: AL, AR, FL, IA, IL, IN, KY, LA, MI, MO, MS, OK, TN, WI	WW Lentic Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105342/Fundulus_dispar			
Western Blacknose Dace (*Rhinichthys obtusus*)	US: AL, GA, IA, IL, IN, KS, KY, MI, MN, MO, MS, NC, ND, NE, NY, OH, PA, SC, SD, TN, VA, WI, WV	CW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.790464/Rhinichthys_obtusus			
Eastern Blacknose Dace (*Rhinichthys atratalus*)	US: CT, DC, DE, GA, MA, MD, ME, NC, NH, NJ, NY, PA, RI, VA, VT, WV	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=637 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.828296/Rhinichthys_atratulus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Longnose Dace (*Rhinichthys cataractae*)	US: CO, CT, DC, DE, GA, IA, ID, IL, IN, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=638 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101847/Rhinichthys_cataractae			
Finescale Dace (*Chrosomus neogaeus*)	US: ME, MI, MN, ND, NE, NH, NY, SD, VT, WI, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2556 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102927/Chrosomus_neogaeus			
Speckled Dace (*Rhinichthys osculus*)	US: AZ, CA, CO, ID, NM, NN, NV, OR, UT, WA, WY	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=640 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100335/Rhinichthys_osculus			
Satinfin Shiner (*Cyprinella analostana*)	US: DC, DE, MD, NC, NJ, NY, PA,	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=516 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106108/Cyprinella_analostana			
Red Shiner (*Cyprinella lutrensis*)	US: AL, AR, AZ, CO, GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, NC, ND, NE, NM, NN, NV, OK, SD, TN, TX,	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=518 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105504/Cyprinella_lutrensis			
Bigmouth Shiner (*Notropis dorsalis*)	US: CO, IA, IL, IN, KS, MI, MN, MO, ND, NE, NY, OH, PA, SD, TN, WI, WV, WY	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=593 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104308/Notropis_dorsalis			
Chub Shiner (*Notropis potteri*)	US: AR, LA, OK, TX	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=606 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105179/Notropis_potteri			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Sand Shiner (*Notropis stramineus*)	US: AR, AZ, CO, IA, IL, IN, KS, KY, MI, MN, MO, MT, ND, NE, NM, NN, NY, OH, OK, PA, SD, TN, TX, UT, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=600 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104717/Notropis_stramineus			
Redside Shiner (*Richardsonius balteatus*)	US: AZ, CO, ID, MT, NV, OR, UT, WA, WY	CW Lentic Lotic	Herbivore Invertivore Molluscivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=644 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100279/Richardsonius_balteatus			
Thicklip Chub (*Cyprinella labrosa*)	US: NC, SC, VA	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101215/Cyprinella_labrosa			
Streamline Chub (*Erimystax dissimilis*)	US: AL, IN, KY, NY, OH, PA, TN, VA, WV	WW Lotic	Invertivore Molluscivore TL3
Map: & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106034/Erimystax_dissimilis			
Shoal Chub (*Macrhybopsis hyostoma*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MN, MO, MS, NE, OH, OK, TN, TX, WI, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106278/Macrhybopsis_hyostoma			
Silver Chub (*Macrhybopsis storeriana*)	US: AL, AR, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, NY, OH, OK, PA, SD, TN, TX, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101653/Macrhybopsis_storeriana			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
River Chub (*Nocomis micropogon*)	US: AL, DC, GA, IL, IN, KY, MD, MI, NC, NY, OH, PA, SC, TN, VA, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=577 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101786/Nocomis_micropogon			
Bull Chub* (*Nocomis raneyi*)	US: NC, VA	WW Lotic	Herbivore Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101374/Nocomis_raneyi			
Peamouth (*Mylocheilus caurinus*)	US: ID, MT, OR, WA	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2349 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100544/Mylocheilus_caurinus			
Creek Chub (*Semotilus atromaculatus*)	US: AL, AR, CO, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NY, OH, OK, PA, SC, SD, TN, TX, UT, VA, VT, WI, WV, WY	WW Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=649 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104867/Semotilus_atromaculatus			
Central Stoneroller (*Campostoma anomalum*)	US: AR, CO, CT, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, ND, NE, NM, NY, OH, OK, PA, SC, SD, TN, TX, VA, WI, WV, WY	WW Lotic	Herbivore
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=506 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.844144/Campostoma_anomalum			
Largescale Stoneroller (*Campostoma oligolepis*)	US: AL, AR, GA, IA, IL, IN, KY, MN, MO, MS, ND, OK, VA, WI	WW Lotic	Herbivore

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=507 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102552/Campostoma_oligolepis			
Sacramento Splittail (*Pogonichthys macrolepidotus*)	US: CA	WW Lotic Estuarine	Herbivore Invertivore
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105438/Pogonichthys_macrolepidotus			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

6) Darters (Percidae)

Darters are small, perch-like fish in the family Percidae and are found in freshwater streams in North America. Darters typically occur in riverine systems, inhabiting cold to cool streams and small river systems in North America. Species distributions range from single watersheds in one state to multiple watersheds in several states. Darters are typically benthic omnivores, practicing herbivory as well as preying on invertebrates and for some species, small fish and fish eggs as well.

Table D-6. Species in the Family Percidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Greenside Darter (*Etheostoma blennioides*)	US: AL, AR, DC, GA, IL, IN, KS, KY, MD, MI, MO, MS, NC, NY, OH, OK, PA, TN, VA, WV	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=808 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.790349/Etheostoma_blennioides			
Arkansas Darter (*Etheostoma cragini*)	US: AR, CO, KS, MO, OK	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=810 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103800/Etheostoma_cragini			
Iowa Darter (*Etheostoma exile*)	US: CO, IA, IL, IN, MI, MN, MT, ND, NE, NM, NY, OH, PA, SD, UT, WI, WY	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=812 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100441/Etheostoma_exile			
Fantail Darter (*Etheostoma flabellare*)	US: AL, AR, DC, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, NY, OH, OK, PA, SC, TN, VA, VT, WI, WV	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.832912/Etheostoma_flabellare			
Least Darter (*Etheostoma microperca*)	US: AR, IA, IL, IN, KS, KY, MI, MN, MO, OH, OK, WI	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103616/Etheostoma_microperca			
Johnny Darter (*Etheostoma nigrum*)	US: AL, AR, CO, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, ND, NE, NY, OH, OK, PA, SD, TN, UT, VA, WI, WV, WY	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=814 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100152/Etheostoma_nigrum			
Tessellated Darter (*Etheostoma olmstedi*)	US: CT, DC, DE, FL, GA, MA, MD, NC, NH, NJ, NY, PA, RI, SC, VA, VT, WV	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=816 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106063/Etheostoma_olmstedi			
Cypress Darter (*Etheostoma proeliare*)	US: AL, AR, FL, IL, KY, LA, MO, MS, OK, TN, TX	WW Lotic Lentic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101593/Etheostoma_proeliare			
Redline Darter (*Etheostoma rufilineatum*)	US: AL, GA, KY, MS, NC, TN, VA	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2886 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103471/Etheostoma_rufilineatum			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Orangebelly Darter (*Etheostoma radiosum*)	US: AR, OK, TX	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1156842/Etheostoma_radiosum			
Orangethroat Darter (*Etheostoma spectabile*)	US: AR, CO, IA, IL, IN, KS, KY, MI, MO, NE, OH, OK, TN, TX, WY	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102592/Etheostoma_spectabile			
Speckled Darter (*Etheostoma stigmaeum*)	US: AL, AR, FL, GA, KY, LA, MO, MS,	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1006953/Etheostoma_stigmaeum			
Gulf Darter (*Etheostoma swaini*)	US: AL, FL, GA, KY, LA, MS, TN	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102176/Etheostoma_swaini			
Variegate Darter (*Etheostoma variatum*)	US: IN, KY, NY, OH, PA, VA, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102018/Etheostoma_variatum			
Banded Darter (*Etheostoma zonale*)	US: AL, AR, GA, IA, IL, IN, KS, KY, MD, MI, MN, MO, NC, NY, OH,	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=818 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106576/Etheostoma_zonale			
River Darter (*Percina shumardi*)	US: AL, AR, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO,	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
	MS, ND, OH, OK, PA, TN, TX, WI, WV		
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=826 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101870/Percina_shumardi			
Slenderhead Darter (*Percina phoxocephala*)	US: AL, AR, IA, IL, IN, KS, KY, MN, MO, MS, OH, OK, SD, TN, WI, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104090/Percina_phoxocephala			
Shield Darter (*Percina peltata*)	US: DC, DE, MD, NJ, NY, PA, VA, WV	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2775 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105028/Percina_peltata			
Blackbanded Darter (*Percina nigrofasciata*)	US: AL, FL, GA, LA, MS, NC, SC, TN	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=824 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1255463/Percina_nigrofasciata			
Blackside Darter (*Percina maculata*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, NY, OH, OK, PA, SD, TN, TX, VA, WI, WV	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=823 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106566/Percina_maculata			

7) Sculpins (Scorpionidae)

Sculpins are members of the family Scorpionidae (Scorpionfish), and most species in the Northern Hemisphere are saltwater fishes. Most freshwater sculpins in the U.S. are in the genus *Cottus* and are small benthic predators consuming mainly invertebrates. Sculpins typically prefer cooler, headwater streams, but can be found in larger warmer streams in some states.

Table D-7. Species in the Family Scorpionidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Mottled Sculpin (*Cottus bairdii*)	US: AL, AZ, CO, DE, GA, IA, ID, IL, IN, KY, MD, MI, MN, MO, MS, MT, NC, NM, NN, NV, NY, OH, OR, PA, SC, TN, UT, VA, VT, WA, WI, WV, WY	CW/WW Lotic Lentic	Herbivore Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=502 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819868/Cottus_bairdii			
Paiute Sculpin (*Cottus beldingii*)	US: CA, CO, ID, NV, OR, UT, WA, WY	CW/WW Lotic	Herbivore Molluscivore Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101884/Cottus_beldingii			
Banded Sculpin (*Cottus carolinae*)	US: AL, AR, GA, IL, IN, KS, KY, MO, MS, NC, OK, TN, VA	CW/WW Lotic	Herbivore Invertivore Piscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819914/Cottus_carolinae			
Slimy Sculpin (*Cottus cognatus*)	US: AK, CT, IA, ID, IL, IN, MA, ME, MI, MN, MT, NH, NJ, NY, PA, VA, VT, WA, WI, WV	CW Lotic Lentic	Herbivore Invertivore Piscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101449/Cottus_cognatus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Shorthead Sculpin (*Cottus confuses*)	US: ID, NV, OR, WA	CW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.905574/Cottus_confusus			
Riffle Sculpin (*Cottus gulosus*)	US: CA, OR, WA	CW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103055/Cottus_gulosus			
Spoonhead Sculpin (*Cottus ricei*)	US: IL, MI, MN, MT, NY, OH, PA, WI	CW Lotic Lentic	Omnivore Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103689/Cottus_ricei			

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Appendix E: Calculation of Composite Trophic Transfer Factors

Derivation of Trophic Transfer Factor (TTF) Values

The parameter $TTF^{composite}$ (composite trophic transfer factor) in Equation 1 quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system and the proportion of species consumed. It is possible to differentiate bioaccumulative potential for different predator species and food webs by modeling different exposure scenarios. For example, where a fish species of interest is a trophic level 4 predator that primarily consumes trophic level 3 fish, the term $TTF^{composite}$ can be represented as the product of all *TTF* parameters that includes the additional trophic levels given as:

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$

(Equation 1)

where:

TTF^{TL2}	=	the trophic transfer factor of the trophic level 2 species
TTF^{TL3}	=	the trophic transfer factor of the trophic level 3 species
TTF^{TL4}	=	the trophic transfer factor of the trophic level 4 species
$TTF^{composite}$	=	the product of all the trophic transfer factors

The consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* at a particular trophic level as the weighted average of the *TTF*s of all species consumed given as:

$$\overline{TTF}^{TLx} = \sum_{i} \left(TTF_i^{TLx} \times w_i \right)$$

(Equation 2)

where:

TTF_i^{TLx}	=	the trophic transfer factor of the i^{th} species at a particular trophic level
w_i	=	the proportion of the i^{th} species consumed

Figure 1 below describes five example food web scenarios and the formulation of $TTF^{composite}$ to model selenium bioaccumulation in each of them.

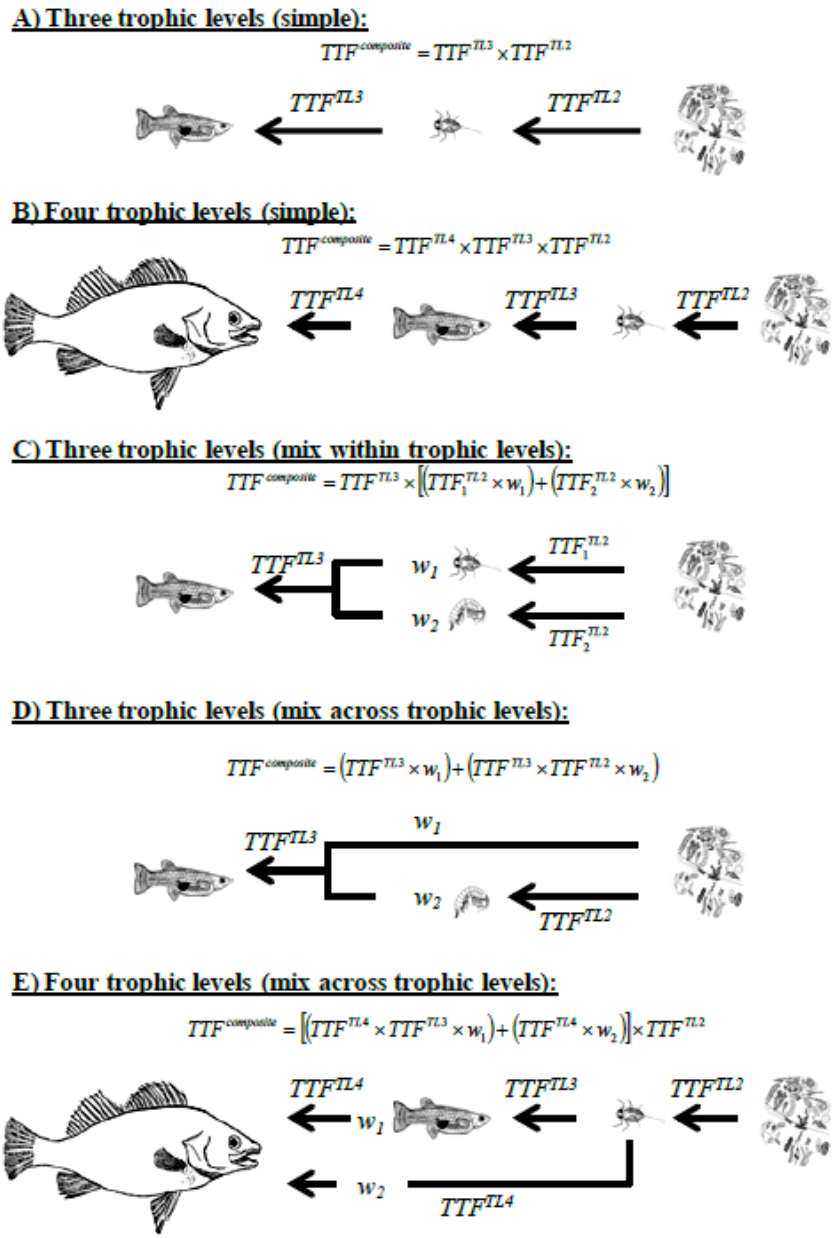


Figure 1. Example aquatic system scenarios and the derivation of the equation parameter $TTF^{composite}$.

Appendix N

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Critical Review

Guidance on assessing the potential impacts of selenium in freshwater ecosystems

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EDITOR'S NOTE:

This article is part of the special series "Regulatory issues surrounding the management of selenium." This series presents a collection of views from North American experts on the aquatic behavior of selenium and environmental, physiological, and operational factors that must be considered in Se regulatory frameworks.

Abstract

Despite decades of fate and effects studies, environmental selenium (Se) contamination and management remain an issue for many freshwater systems in North America. Several regulatory bodies have promulgated updated targets or management levels for Se; however, additional guidance on best practices for monitoring Se to protect freshwater aquatic life is warranted. In this article, we describe current approaches to assessing the ecological risks of Se in impaired freshwater systems and outline recommended methods for collecting and analyzing biological and abiotic samples and interpreting data. Because reproductive impairment of fish populations is most commonly used to determine the potential impacts of Se, several biological factors that could affect Se toxicity are explored, including diet, trophic positions, reproductive biology, body size and maturity, migratory movements, and use of seasonal habitats. Measuring Se concentrations in mature eggs is the most reliable metric for estimating potential reproductive impairment in fish populations because the range of toxicity thresholds is relatively narrow for all but a few tolerant fish species. In situations where collecting mature eggs is not feasible, we review the use of alternative fish tissue for estimating potential effects. Factors affecting Se uptake from freshwater are also considered with guidance on collecting abiotic (e.g., water and sediment) and biotic components of aquatic food webs (e.g., macroinvertebrates, biofilm). *Integr Environ Assess Manag* 2024;00:1–16. © 2024 SETAC

KEYWORDS: Environmental assessment; Freshwater; Invertebrates; Selenium; Toxicity threshold

INTRODUCTION

The issue of environmental selenium (Se) pollution in North America first emerged in the 1960s (Lemly & Skorupa, 2012) and is now known to result from activities related to the irrigation of seleniferous soils, the extraction and preconsumption processing of metals and fossil fuels, coal combustion, and the management of associated waste streams, including their storage and disposal (Chapman et al., 2010). Ecosystem-scale case studies of Se contamination in the 1970s and 1980s motivated decades of field- and laboratory-based research that has contributed to a strong understanding of Se's environmental fate and distribution, biogeochemistry, environmental persistence, and toxicity (see Chapman et al., 2010; Lemly, 2002, for reviews). This research has since informed environmental regulatory structures, including the British Columbia Ministry of Environment (BC MoE), ambient water quality guidelines (Beatty & Russo, 2014), and the USEPA aquatic life ambient water quality criteria (USEPA, 2016). These approaches are distinct from those focused on other contaminants because they reflect that Se readily bioaccumulates in aquatic food webs and exposure occurs primarily through dietary intake. Accordingly, regulatory guidelines prioritize fish tissue-based Se concentration data for aquatic life protection.

Improved waste management has resulted in less environmental Se loading from some major sources, such as effluents from coal-fired power plants; however, Se contamination continues to be a pressing problem for aquatic systems in North America with active mining and irrigation of seleniferous soils, and due to legacy effects at historically contaminated sites. Habitats affected by elevated

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concentrations of Se include the Clark Fork River ecosystem (Montana, USA; Gruen, 2020), the Upper Colorado River Basin, (USA; Brandt et al., 2021; Day et al., 2020), the Elk Valley (BC, Canada; Storb et al., 2023; Wellen et al., 2015), the Upper Columbia River Basin (USA; Gruen, 2020), and the Salton Sea (USA; Hennequin et al., 2022). Therefore, the consequences of Se loading on aquatic ecosystems remain a substantial and widespread ecological concern.

In this article, we argue that there is a need for updated guidance on best practices for environmental Se monitoring for aquatic life protection. Such guidance would provide a timely response to regulatory changes aimed at applying improved Se science to managing persistent environmental Se problems in Canada and the United States. For example, Environment and Climate Change Canada (ECCC) has recently revised the Environmental Effects Monitoring (EEM) requirements for metal and diamond mines regulated under the *Metal and Diamond Mining Effluent Regulations* to include the monitoring of Se in fish tissue. In Canada, EEM is a cyclical and mandatory regulatory program that assesses the potential effects of industrial effluents, including metal and diamond mines and with recent recommendations for coal mines, on aquatic receiving environments. Environmental Effects Monitoring includes a fish survey that assesses potential effects on growth, population structure, and reproduction in resident, effluent-exposed fish compared with fish from reference areas. Environment and Climate Change Canada also proposes to develop the *Coal Mining Effluent Regulations* under the *Fisheries Act*, which will include Se effluent standards for 28 existing mines, as well as all future coal mines and coal mine expansions (ECCC, 2020a, 2020b). In the United States, Montana recently adopted an updated Se standard for Lake Koocanusa and the Kootenai River mainstem (US Geological Survey [USGS], 2022), and the USEPA is proposing distinct criteria for California's San Francisco Bay and Delta that consider unique ecological factors influencing Se uptake and toxicity (USEPA, 2022).

This article synthesizes what has been learned about assessing ecological Se risks in impaired freshwater systems in North America over more than 60 years, focusing primarily on freshwater systems. Recommended monitoring practices reflecting the current state of science are also outlined. Because many management efforts center on the need to protect sensitive fish species, we emphasize issues specific to fish biology while highlighting considerations for monitoring Se in abiotic compartments and lower trophic levels. Benthic invertebrates are not only dietary Se exposure vectors for fish but also sensitive indicators of overall aquatic environmental quality. Therefore, considerations for including invertebrates and sediment sampling in environmental monitoring programs for Se are also provided. Additionally, we review issues related to physicochemical factors that can affect Se bioaccumulation, choices of sampling locations, and sampling considerations for benthic invertebrates and sediment, as well as analytical approaches to determining Se in abiotic and biological samples. We consider methods for assessing exposure to mine effluents, appropriate sampling effort and analytical methods, and data analysis and reporting.

BACKGROUND ON THE ROLE OF DIETARY EXPOSURE IN SE TOXICITY

Dietary exposure is the primary route of Se exposure for fish species. It is widely accepted that Se enters aquatic food webs via concentration by primary producers, is subsequently biotransformed to organic selenide compounds, and transfers through dietary pathways from prey to consumer species (Ponton et al., 2020; Presser & Luoma, 2010). Selenium is an essential micronutrient for all vertebrates and is incorporated in many selenoproteins that support antioxidant function, thyroid hormone regulation, cell proliferation, and muscle metabolism (Beatty & Russo, 2014). However, at concentrations only four- to sevenfold higher than those required in the diet, Se negatively affects reproduction in vertebrates that lay eggs, including fish, which are more sensitive to the potential negative effects of Se than other aquatic organisms (Beatty & Russo, 2014; USEPA, 2016). The primary adverse effects are mediated by the dietary intake of Se by female fish and maternal transfer to their eggs (DeForest et al., 2012). Impaired population recruitment resulting from elevated exposure to Se occurs within a relatively narrow range of Se concentrations in eggs among all fish species studied to date (Janz, 2011). During embryonic development, Se is assimilated from the yolk and can replace sulfur in the amino acid methionine, yielding selenomethionine during embryonic protein synthesis. Although the underlying mechanisms are not completely understood, it is thought that higher rates of Se substitution in proteins and oxidative stress resulting from excess Se metabolism can lead to decreased hatching, elevated rates of mortality, deformities, edema, and mortality that can affect population recruitment (Janz et al., 2010; Kupsco & Schlenk, 2016).

CONSIDERATIONS FOR SELECTING SENTINEL FISH SPECIES

Elevated environmental Se concentrations in North America have spurred a critical reevaluation of how best to monitor ecological impacts and determine which wild species should be selected as sentinels for monitoring efforts. Because dietary factors are most important for mediating the effects of Se in fish, exposure to an effluent or other point source is less important than understanding dietary exposure pathways for determining risk and selecting sentinel fish species. Established or conceptualized dietary pathways of exposure can be estimated based on knowledge of prey organisms and analysis of Se concentrations in those taxa. Participation in the local food web is the first gatekeeper function that must be considered to establish a list of suitable sentinel fish species, likely in consultation with a local fish biologist (ECCC, 2012; Figure 1). Sufficient abundance at the exposed and reference sites will also need to be verified before selecting a sentinel species. If a species

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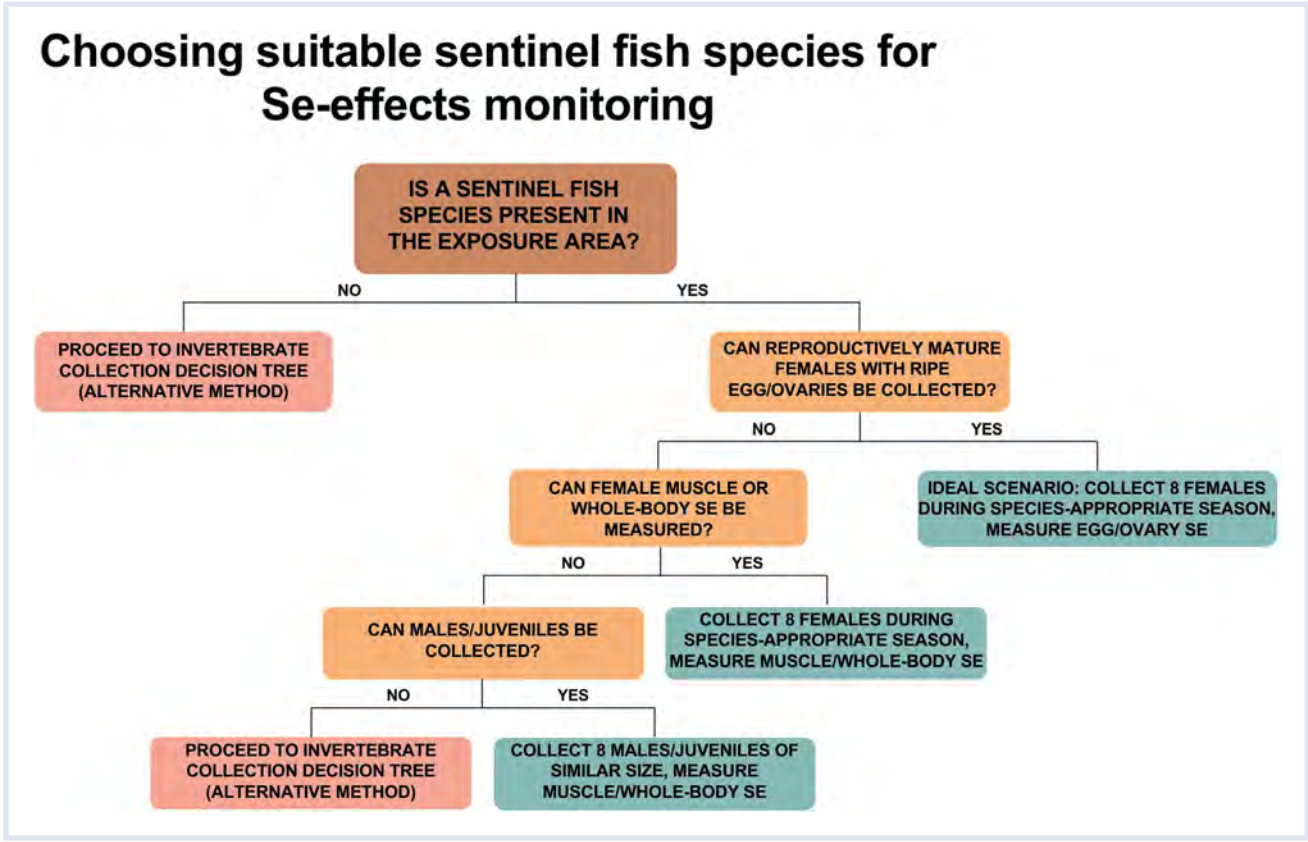


FIGURE 1 Decision tree for choosing suitable sentinel fish species for selenium (Se)-effects monitoring

has been used in prior studies, this may provide some context for temporal data comparisons and the relative abundance of the species (ECCC, 2012). Where a point source discharge is evident, immediate exposure at the discharge location is less important than residence and feeding in habitats influenced by that discharge and where conditions are favorable for Se to be incorporated into the food web. In other words, dietary Se concentrations are not necessarily greatest when they are closest to the discharge; instead, they may be elevated at sites downstream where accumulation in the local food web is facilitated (e.g., depositional zones; Brandt et al., 2021). Finally, sampling eight fish is often sufficient to detect differences between affected and reference locations, but sample sizes increase at higher Se thresholds because of heterogeneity among individual fish at higher Se exposures (Hitt & Smith, 2015).

Several fundamental tools can be used to assess the exposure of fish to potentially elevated Se concentrations in downstream habitats. To assess exposure pathways, it is often useful to construct a conceptual model of exposure that outlines potential routes of exposure to dietary Se for fish in downstream and reference habitats (Presser & Luoma, 2010). The conceptual site model should consider, for example, potential depositional zones where hotspots of Se accumulation may develop. Historical data from previous studies should be reviewed, if available, and if warranted, the conceptual site model could be refined by assessing Se concentrations in primary producers, biofilm, periphyton, algae, bacteria and fungi, and secondary consumers (i.e., benthic invertebrate taxa) commonly the prey of fish. Determining exposure also requires understanding the residence times of a given fish species in specific habitats. Quantitative and seasonal fish population and community surveys should be conducted to determine fish species assemblages, abundance, and habitat use patterns (e.g., via telemetry studies; ECCC, 2012; Cope et al., 2016). Once fish assemblages and potential exposure routes have been determined at the exposed and reference sites, candidate fish species for further exposure monitoring can be identified. In many cases, these initial steps are adequate to refine the conceptual site model so that Se exposure pathways are understood. More detailed studies may seek additional refinement, specifically for dietary exposure routes.

To further refine the conceptual site model, typical dietary items for fish species that are screened-in as potential monitoring candidates can be predicted using resources such as FishBase (https://fishbase.se/search.php). Diet items identified in the conceptual site model can then be verified using gut content analysis of fish sampled from the area. For large-bodied fish, gut contents can be obtained nonlethally using nonlethal gastric lavage techniques (Hartleb & Moring, 1995), but for small-bodied fish it may be necessary to lethally sample fish to obtain gut contents for visual identification of forage items. Analysis of $\delta^{15}N$ stable

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isotope values in representative components of the food web and in fish tissue can be used to further verify trophic relationships (e.g., Brandt et al., 2021). Finally, analysis of Se concentrations in annual growth zones of otoliths can provide additional information regarding the residence patterns of fish at a given site and the relative changes in Se exposure over time (Johnson et al., 2020). Although microchemical analyses of Se in annual growth zones are more technically challenging, they have recently become commercially available.

Trophic position and trophic transfer

Dietary exposure of a given fish species to Se is affected by several factors, including its trophic position. The most important step for determining Se accumulation in aquatic food webs is ascertaining Se uptake from water by primary producers and microbes (i.e., the particulate fraction). However, this step is mediated by species- and habitat-specific factors referred to as enrichment functions (EF), which can vary over several orders of magnitude and are difficult to predict (Ponton et al., 2020; Stewart et al., 2010). The secondary concentration of Se is described by trophic transfer factors (TTFs) that apply to specific consumer species feeding in a given environment. Trophic transfer factors incorporate the ingestion rates and the assimilation efficiency of Se (Stewart et al., 2010). Trophic transfer factors derived for fish feeding on invertebrates, and for top predator level fish feeding on forage fish, have been derived empirically for many North American fish species (Presser & Luoma, 2010). Compared with EFs, TTF values are far lower and less variable, with values for fish feeding on invertebrates generally ranging from 0.5 to 1.8 (Presser & Luoma, 2010). Fewer studies have examined TTFs for upper trophic level piscivorous fish, but the available data indicate similar values for fish feeding on invertebrates (Stewart et al., 2010). Trophic transfer factors as high as 10 have been estimated for benthivorous fish such as spottail shiner, white sucker, stickleback, and burbot, but TTFs vary depending on the specific prey consumed (Muscatello et al., 2008, Muscatello & Janz, 2009). If they were identified, higher TTFs among upper trophic level fish would indicate Se biomagnification. Historically, there has been some scientific discord about the ability of Se to biomagnify (Beatty & Russo, 2014, and references therein), but data from field studies indicate that biomagnification (i.e., TTFs consistently >1) from forage fish species to piscivores is uncommon (Brandt et al., 2021; Day et al., 2020). In other words, fish feeding at a higher trophic level do not typically have higher tissue Se concentrations than those feeding at lower trophic levels. Furthermore, in their thorough evaluation of aquatic food web data, the USEPA (2016) found little variation in Se across all trophic levels of fish, except for fish consuming mollusks. This is relevant to sentinel species selection because fish that consume bivalves as a primary prey item may accumulate higher concentrations of Se than species feeding on other organisms. Mollusks, including bivalves, have proportionally greater uptake and up to 10 times slower elimination rates for Se than other organisms (Stewart et al., 2010). Finally, if bivalves are used as a surrogate for fish, an accepted alternative in Canada's EEM for metal mines, relatively high estimates of Se bioaccumulation may be derived.

We examined the relationship between trophic status and TTFs for freshwater fish species used for EEM studies in Canada. A numeric value for trophic status was retrieved from FishBase (http://www.fishbase.org), a database of more than 8300 fish that has compiled dietary records to assess trophic level based on weighted calculations of all food items consumed by a species. These estimates agree closely with the trophic positions determined by stable isotope studies (Romanuk et al., 2011). Species-specific TTFs were obtained from a list published in USEPA (2016). It is important to recognize that the analysis is based on a single median TTF for each fish species (USEPA, 2016) despite understanding that TTFs are dynamic ranges and are influenced by context-dependent factors (Graves et al., 2019; Presser & Naftz, 2020) and that uptake functions (e.g., EFs and TTFs) are inversely related to Se exposure concentrations in aquatic systems (DeForest et al., 2017). These inverse relationships reflect that receptor-mediated uptake of selenocysteine dominates at low Se concentrations but that selenomethionine uptake increases (i.e., the proportion of Se as selenomethionine increases) as receptors become saturated, resulting in lower relative TTFs (Janz et al., 2014; Ponton et al., 2020). Paired TTF and trophic status assignments were available for 28 species that included varied feeding strategies (i.e., benthivorous, invertivorous, piscivorous, and carnivorous) and a range of TTFs (i.e., 2.4–4.7). There was no significant relationship between species-specific TTF and trophic status (Figure 2), suggesting that Se does not biomagnify and that diet type has a greater influence on Se uptake than trophic status.

Reproductive biology

After assessing dietary exposure pathways and confirming sufficient abundance within a study area, understanding a species' reproductive biology is the next most important factor in selecting a sentinel species. The adverse consequences of elevated Se exposure are associated primarily with Se assimilation from the yolk during embryological development (Janz et al., 2010). This early life exposure is a consequence of Se substitution for sulfur during vitellogenin (an egg yolk precursor phospholipoglycoprotein) synthesis in the maternal liver and subsequent transfer from maternal fish to ovary/egg tissue during the final oocyte maturation phases of egg development (Janz et al., 2010). The resulting enrichment of Se in egg yolk leads to elevated exposure during yolk resorption by embryos after egg fertilization (Janz et al., 2010). As such, Se concentrations in egg/ovary tissue—and more specifically, eggs collected from gravid (i.e., ripe and running) females after the vitellogenic phase of development—are the most reliable measure of potential reproductive effects in fish populations (Beatty & Russo, 2014; Brandt et al., 2019; Janz, 2011; USEPA, 2016).

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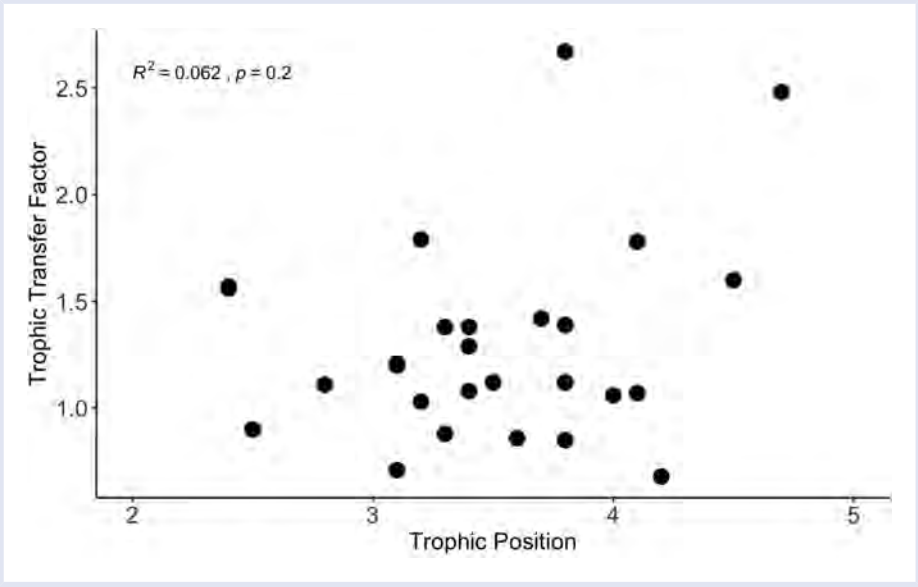


FIGURE 2 Trophic transfer factors (TTFs) vs. trophic position for fish used for Environmental Effects Monitoring (EEM) studies in Canada

Because they relate to the amounts of Se available for maternal transfer to eggs, factors related to egg development and adult spawning are important considerations when selecting sentinel species for monitoring purposes. The processes of vitellogenesis and oogenesis, and when they occur relative to spawning, vary considerably among species (Osmundson et al., 2000; Tyler et al., 1990), and maternal Se burdens immediately preceding these processes determine Se loading in eggs (Golder Associates, 2018). Furthermore, some small-bodied fish spawn several times in each reproductive cycle; therefore, clutches of eggs derived across spawning events may have varying Se concentrations reflecting maternal tissue stores and/or recent dietary intake (Driessnack et al., 2011). Egg sampling should be conducted either immediately before or during spawning for single spawning species and before the first spawning event for multiple spawners.

The selection of sentinel species should also consider the resources invested in eggs (ECCC, 2012). For a species to be a viable sentinel species candidate, it should be practical to obtain sufficient eggs for Se analysis and to determine other reproductive metrics, including the gonadal somatic index (GSI). The GSI is the proportion of a fish's weight contributed by reproductive tissue and is used to measure reproductive potential. Numbers of eggs and egg sizes may also be considered indicators of energy investment. The ecological importance and evolutionary factors governing egg numbers and sizes in fish have been linked to habitat suitability, spawning season, parental size, and energy investment (Kolm & Ahnesjö, 2005; Sargent et al., 1987). It has been suggested that species with relatively large eggs and yolk contents could deposit more selenium in their eggs than species with smaller eggs and yolks, and this may be related to developmental Se sensitivity (Osmundson & Skorupa, 2011). However, we found no significant relationships between the existing Se-toxicity thresholds (i.e., EC_{10} values) and egg volume (Figure 3A), yolk volume (Figure 3B), or the ratio of yolk:egg volume (Figure 3C) in mature eggs spawned from gravid females of 12 species previously used for EEM studies in Canada (range in *p*-values = 0.39–9.94).

Selenium developmental toxicity may have less to do with egg size or yolk:egg ratio than with Se transport between tissues during egg formation and/or the use of vitellogenin during early embryological development. For example, DeForest et al. (2017) suggested that fish with greater sensitivity to Se (i.e., low EC_{10} values) also partition more Se to eggs from muscle or whole-body Se concentrations relative to species with lower Se sensitivity. Variability in vitellogenin synthesis may also play a role in determining how Se is mobilized from the muscle and/or whole body and in differences in species-specific sensitivity to Se. There are differences among fish species in the number of VTG protein sequences produced during vitellogenesis. The protein sequence is a major factor in determining other constituents that may bind to VTG and are delivered to the egg along with the lipoprotein (Riddle & Hu, 2021). Differences in the vitellogenin sequences may dictate how strongly Se binds to the lipoprotein during egg maturation and how efficiently Se is mobilized from the tissue to the developing oocyte. Mutations in some vitellogenin subtypes can even increase the rates of edema and spinal deformities (Riddle & Hu, 2021), both hallmarks of Se developmental toxicity. Additional research is needed to examine the potential role of vitellogenin subtypes to determine differences among species in the mobilization of Se from tissue to egg and relative sensitivities to the effects of Se on embryological development.

Understanding the temporal linkages between maternal dietary Se intake, tissue deposition and oogenesis, and spawning is a key element for selecting appropriate sentinel

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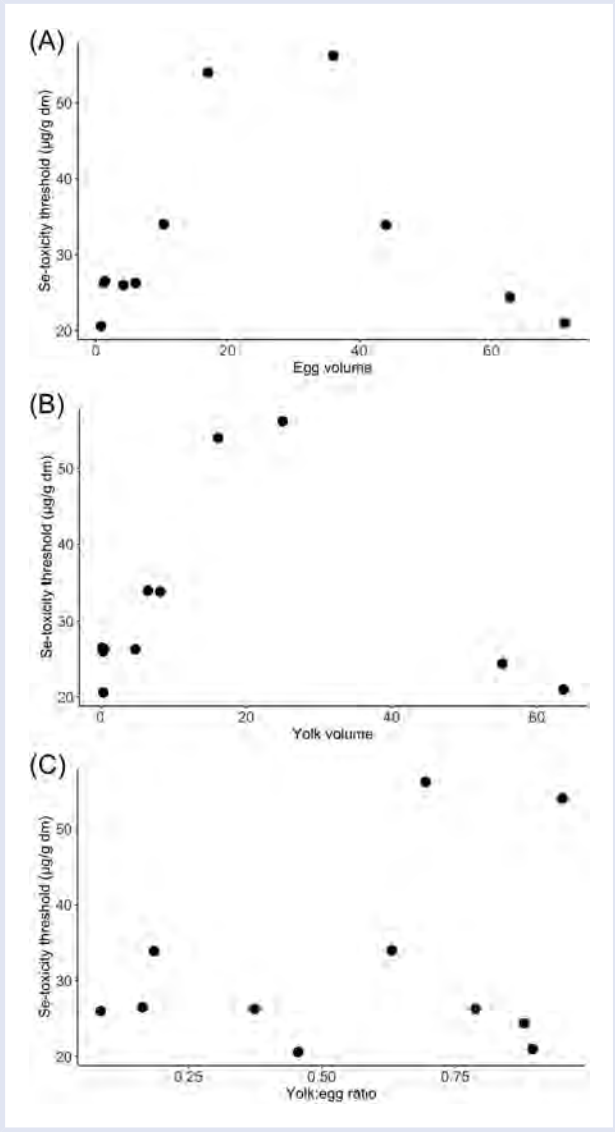


FIGURE 3 Selenium toxicity threshold concentrations µg g^{-1} dw) vs. egg volume (A), yolk volume (B), and yolk:egg volume (C) for fish species previously used for metal mining Environmental Effects Monitoring (EEM) studies in Canada

fish species to assess the potential effects of Se. Therefore, it is advantageous to select sentinel species whose reproductive timing is well characterized and for which Se-dietary exposure is also linked to the local food web before or during gonad maturation. In many cases, this may mean selecting large-bodied fish species that spawn once per year (USEPA, 2016). There are some added advantages to this approach. For example, there is more information regarding Se-toxicity thresholds for large-bodied fish, and it is more practical to estimate GSI from spawning fish using nonlethal methods. Specifically, the expressed egg volume and mass of large-bodied fish can often be measured to provide a sufficiently reliable measure of GSI in lieu of lethal sampling of gonad tissue (Bromage et al., 1990). Small-bodied fish with known reproductive biology should also be considered. For example, fathead minnow eggs can be collected on artificial spawning substrate deployed at spawning locations (Masson et al., 2006). Establishing field methods to collect eggs from other small-bodied species with less information regarding reproduction requires significant investment in field resources and time (De Bruyn et al., 2023). However, the effort is warranted if these species benefit the study design via smaller home ranges and participation in local food webs.

Environmental monitoring programs have established relatively wide sampling windows for determining GSI in many species of North American fish (ECCC, 2012; USEPA, 2016), but these times may not be suitable for determining the potential for Se exposure and effects. Sampling within larger windows of ovarian development may not impair the ability to detect relative differences in GSI for large-bodied species (ECCC, 2012), but sampling eggs before full maturation does not allow meaningful comparisons of Se concentrations in eggs with existing toxicity thresholds (USEPA, 2016). For example, it has been recommended to sample ovary tissue and assess GSI in late autumn for species such as rainbow trout that spawn synchronously in spring (ECCC, 2012). However, this sampling period occurs well before the peak of vitellogenesis when the oocyte diameter is only 40%–50% of the expected size at ovulation (Tyler et al., 1990) and before a significant portion of maternally deposited Se would be delivered to the eggs (Janz et al., 2014). Because toxicity thresholds are established based on concentrations measured in eggs, it is imperative to perform Se analysis of mature eggs to allow comparisons with egg Se guidelines or criteria or Se EC_{10} values (USEPA, 2016). Although final egg maturation before ovulation can increase the diameter and weight of each oocyte caused by hydration significantly (Milla et al., 2006), these increases will not affect reported egg Se concentrations, which are provided on a dry-weight basis (i.e., after freeze drying; Beatty & Russo, 2014; ECCC & Health Canada, 2017; USEPA, 2016).

Body size and maturity

As discussed above, trophic position is not a reliable predictor of a fish's Se burden. Accordingly, we did not find a significant relationship between maximum body size and Se TTFs for the same list of species noted above ($R^2 = 0.035$, $p > 0.33$; data not shown). Previous studies have also reported that, among 20 freshwater and marine species, size variations were not a significant factor in determining fish muscle or whole-body Se concentrations (Burger & Gochfeld, 2011; Cianciolo et al., 2020; Stewart et al., 2010). It is possible to explore whether this trend also applies to juvenile life stages. A small set of relevant studies have contrastingly reported (a) significantly higher uptake and assimilation by relatively large larval fathead minnows than by smaller fish of the same age (Bennett et al., 1986), and (b) lower feeding rates and Se bioaccumulation by larger juvenile black seabream despite higher Se assimilation efficiency (Zhang & Wang, 2007). Finally, it has been demonstrated that rapid growth does not dilute whole-body Se concentrations in fish, as it does for some bioaccumulating contaminants, because greater food

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intake during growth phases can counteract growth dilution effects (Luoma & Rainbow, 2005; USEPA, 2016).

On the other hand, dietary shifts that correspond to size transitions (e.g., ontogenetic shifts) can affect Se accumulation. Changes in fish Se concentration will be context dependent and reflect whether the predator species transitions to a diet with relatively higher or lower Se concentrations (Stewart et al., 2004). In conclusion, body size should not be a factor in selecting an appropriate sentinel fish species. However, dietary shifts resulting from size transitions must be considered, especially among juvenile fish. This is considered in more detail in the Monitoring Se in juvenile life stages section.

Migratory vs. nonmigratory fish species

Federal guidance in both Canada and the United States recommends against selecting highly mobile or migratory species for Se-impact studies because their exposure to areas influenced by the effluent is uncertain (ECCC & Health Canada, 2017; USEPA, 2016). The main reason for this is that dietary intake of Se can vary significantly among habitats, and migratory species could be exposed to areas of low or elevated Se concentrations, confounding source allocation and assessment of Se risk in a given area of influence (Beatty & Russo, 2014). If migratory species are selected, it is important to understand the dietary exposure pathways, including the timing of diet exposure relative to egg yolk deposition for that species (USEPA, 2016).

Localized movement by mobile fish species has also been shown to influence dietary Se exposure. Prior research has identified the confounding effects of seasonal or biological patterns in lentic versus lotic habitat occupancy, within-habitat use patterns, and interannual variability in habitat use on Se exposure (Friedrich et al., 2011; Orr et al., 2006; Palace et al., 2007). Collectively, these studies indicate that mobile species can complicate efforts to assess environmental Se impacts on fish. Sedentary and small-bodied fish (adult size $\leq$150 mm) may be preferred choices for environmental Se monitoring studies, especially if their food chains are well characterized (Palace et al., 2005, and references therein). However, these species may be relatively understudied and incomplete understanding (e.g., about their reproductive cycles) can therefore compromise their use as sentinels (Barrett et al., 2015).

Sensitivity and species of conservation concern

The relative sensitivities among different fish species to the reproductive effects of Se have informed several recent regulatory documents (Beatty & Russo, 2014; ECCC & Health Canada, 2017; Jenni et al., 2017; USEPA, 2016, 2018). Species sensitivity distribution (SSD) curves, based on effects linked to Se concentration in eggs, were used to model the relative sensitivity to Se among different fish. Depending on the approach used to set target tissue concentrations, slightly different thresholds can be derived. For example, the USEPA (2016) calculated a chronic criterion of 15.1 mg kg^{-1} (dry weight [dw]) based on the 5th percentile of a distribution of EC_{10} values compiled from 10 fish genera. The EC_{10} estimates the concentration of Se in eggs that results in a less than or equal to 10% effect among all fish species for which data are available (USEPA, 2016). The BC Ministry of the Environment derived a final threshold value 11 mg kg^{-1} dw by incorporating a safety factor of 2 from the geometric mean of EC_{10} values from the two most sensitive resident species in the province: rainbow trout and Westslope cutthroat trout (Beatty & Russo, 2014). In Canada, a predicted no-effect concentration (PNEC) for Se in fish eggs was developed (14.7 mg kg^{-1} dw). The PNEC estimates the hazardous concentration to 5% of fish species (i.e., = HC_5; ECCC, 2022; ECCC & Health Canada, 2017). Additional models have advocated SSD approaches using only the data for species that are relevant to a given study area (DeForest et al., 2012). Regardless of the refinements included in the applied models, there is a relatively narrow range in toxicity thresholds based on egg Se concentrations for most freshwater fish species (i.e., 16.2–25 mg kg^{-1} dw for relatively sensitive species and >54 mg kg^{-1} dw for relatively tolerant species including dolly varden and mountain whitefish), although the number of species for which established toxicity thresholds based on Se concentrations in eggs is still limited (De Bruyn et al., 2023, Brix et al., 2021).

Fish species with established chronic toxicity thresholds based on Se concentrations in eggs are preferred for studies of Se effects. However, this may not always be practical given the limited number of species for which information is available. For example, species for which egg thresholds have been established may not overlap with an assessment area of interest. Ultimately, it is more important to select monitoring species based on knowledge of their dietary exposure pathways and participation in the local food web than to select only species with established Se-toxicity thresholds. Again, this is because the range of Se-toxicity thresholds in eggs is relatively narrow. Therefore, if species without known toxicity thresholds but with high site fidelity and established dietary exposure pathways available, then current toxicity thresholds derived from the SSD of EC_{10} values for eggs from all species can be conservatively applied to estimate risk based on Se concentrations in their eggs.

It is important to note that the presence of species of conservation concern may serve as specific motivation for monitoring efforts in Se-impaired habitats (i.e., where there is contaminant impairment of established critical habitat; see Brandt et al., 2021, for example). Here, the research context at hand will determine whether monitoring efforts prioritize generating data for the susceptible species in place of an alternative sentinel species. In cases where it is not feasible or advisable to sample the susceptible population, a similar species should be identified as a proxy for Se concentration monitoring. Ideally, proxy species will be like the species of conservation concern in aspects that influence their Se exposure, as described above (i.e., diet, reproductive biology, and habitat use) so that concentrations measured in the proxy species provide reliable estimates.

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It is often difficult to identify a sentinel species that meets all the criteria described above, and research managers must consider the trade-offs during species selection. When possible, monitoring several species can help account for shortcomings presented by the use of any one species. Thorough and transparent justifications of the species selected for any given monitoring effort are important for contextualizing the results of a study as they relate to the associated contamination context.

Monitoring Se in other tissue

Mature eggs remain the best tissue to sample for evaluating potential reproductive effects arising from Se exposures. Because there is a strong positive relationship between concentrations of Se in ripe ovary tissue and eggs, the toxicity values based on residues in either tissue are often assumed to be equivalent (ECCC & Health Canada, 2017). However, the log–log regression equations for Se measured in the ovary and egg vary between 0.57 and 0.97 among species (reviewed in ECCC & Health Canada, 2017), indicating that egg concentrations can sometimes be higher than those in the ripe ovary and that there are differences in the slopes of the relationship among species. Recent work also reveals that Se concentrations can be higher in ovary tissue after spawning than in ovulated eggs (De Bruyn et al., 2023). As discussed above, vitellogenin and the associated Se are incorporated during the final stages of oocyte maturation, so Se concentrations in ovary tissue will not always be comparable with those in mature eggs. Therefore, only ovary tissue containing mature eggs should be considered equivalent to eggs when comparing Se concentrations with established toxicity thresholds (USEPA, 2016).

Conversion factors for relating egg/ovary Se concentrations to muscle or whole-body Se concentrations have been established for numerous species. Such conversion factors are not only species specific but are only intended for application to adult fish (ECCC & Health Canada, 2017; USEPA, 2016). When supported by appropriate species-specific statistical models, conversion factors should be applied to tissue collected during periods appropriate to that species' corresponding reproductive cycle and phase (Casey & Siwik, 2000; Presser & Naftz, 2020; North American Metals Council [NAMC], 2009). Muscle tissues should be collected from gravid females for the best modeling of Se concentrations in eggs. Muscle tissue samples should not be collected immediately after spawning because of reproductive depuration of Se (Day et al., 2020; USEPA, 2021). The predictive relationships between egg and muscle or whole-body Se concentrations are less certain in species that do not spawn annually because tissue Se mobilization will not be synchronized across the population (Rideout & Tomkiewicz, 2011).

Whole-body samples (i.e., complete intact fish, including ovaries and eggs) can be the preferred sample type when monitoring small-bodied fish that spawn multiple times during the year. When samples are collected outside the active spawning season, this strategy can be used to avoid the inherent variability of Se concentrations in ovaries among actively spawning fish (USEPA, 2016). In Canada, there are at least 15 multiple spawning species that have been used for previous EEM studies; however, established whole-body to egg Se concentration relationships have only been established for creek chub and fathead minnows and, of these, toxicity thresholds have only been described for fathead minnows. Because females can accumulate significant concentrations of Se in eggs, it is recommended to separate whole-body tissue by sex before analysis. If male and female fish muscle samples must be pooled, the practice should be confined to periods outside oogenesis for that species (Mo et al., 2020).

Asynchronous spawning species represent a distinct challenge in establishing models of Se concentrations in eggs versus muscle. This is because ovaries contain oocytes at various stages of development, and each egg stage might contain different Se concentrations. For these species, comparisons of contaminant levels should be made between mature spawned eggs, collected separately from ovary tissue and muscle tissue obtained during the spawning period (USEPA, 2016). The relationship between ovary and egg concentrations has been established in only two asynchronous spawning species in Canada, the fathead minnow (*Pimephales promelas*) and, more recently, redside shiners (*Richardsonius balteatus*; De Bruyn et al., 2023).

Obtaining samples from gravid female fish is especially difficult for some species like burbot and shorthorn sculpin that spawn in winter under the ice (Froese & Pauly, 2021) and other early spring spawners including northern pike and pearl dace (Eklöf et al., 2023; Timlick et al., 2022) that spawn when ice conditions are unsafe. In these instances, it may be necessary to assess the risks of Se toxicity using the analysis of other tissues (i.e., muscle or whole body), obtained at times outside the spawning window.

When Se concentrations cannot be measured in eggs or reliably extrapolated from muscle tissue, assessment using generic tissue guidelines is an accepted approach to screening level assessments. This method is often used at sites that lack species with established Se-toxicity thresholds or well characterized spawning strategies and peak spawning times. Predicted no-effect concentrations have been established for egg, muscle, and whole-body tissue based on multi-SSD for reproductive toxicity thresholds in eggs (ECCC & Health Canada, 2017). Most of the species used to develop the PNEC are relevant to North America, providing a high degree of confidence in the derived PNEC. The generally applicable PNEC for egg/ovary tissue (=14.7 mg kg^{-1} dw) is based on the modeled concentration of Se in eggs that is expected to present a hazard to less than 5% of fish species. Therefore, in the absence of species-specific information, Se concentrations in eggs or ovaries can be compared with PNEC to estimate potential Se effects. Similarly, a whole-body PNEC (=6.7 mg kg^{-1} dw), developed by converting egg:ovary to whole-body concentrations using established relationships gleaned from the scientific literature, can also be used for screening purposes

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(ECCC & Health Canada, 2017). The ECCC and Health Canada (2017) PNECs align well with the egg:ovary (15.1 mg kg^{-1}) and whole body (8.5 mg kg^{-1}) criterion for Protection of Aquatic Life, prescribed by the USEPA (2016), who also specified a criterion for muscle (11.3 mg kg^{-1}). Comparisons against these nonspecies-specific threshold values should be considered a screening level assessment only and not a substitute for subsequent collection of more empirical data to establish species-specific, Se-toxicity thresholds. Considerable variability in tissue–tissue relationships between fish species (Janz et al., 2010) should motivate additional data collection to refine risk assessment estimates for Se.

Monitoring Se in juvenile life stages

When adult female fish are either not appropriate for monitoring because they do not participate in the local food web or are absent (e.g., the affected site is a nursery habitat), whole-body samples of juvenile fish may be collected. These can then be analyzed for screening level comparisons with whole-body threshold concentrations of Se for the same species (ECCC, 2012; USEPA, 2021). Nonlethal surveys of juvenile life stages can also provide information about population recruitment and complement our understanding of the potential reproductive effects of Se in the study context (Gray et al., 2002).

It is most appropriate to collect whole-body samples when assessing the potential effects of Se on the growth of juvenile fish. Dietary thresholds for effects on growth among juvenile fish are less well established and less certain than for reproductive effects of Se but appear to occur at similar concentrations to those described for reproductive effects (e.g., 4–10 mg kg^{-1}, reviewed in Beatty & Russo, 2014). Based on their review of these data, the authors recommended a whole-body tissue guideline of 4 mg kg^{-1} (dw) to be protective of effects to juvenile fish growth. Study design considerations, including pooling fish of the same species, sex (where possible), and size, should also apply to juvenile collections. Juveniles of many small-bodied fish species have strikingly similar appearances, so rigorous methods must be applied to verify closely related species before pooling (ECCC, 2012). Additionally, although trophic position and body size do not appear to greatly affect the potential for Se accumulation, gape size can vary among juvenile fish of different sizes, affecting their access to certain prey items, which can significantly affect their Se accumulation potential (Bennett et al., 1986; Zhang & Wang, 2007). For this reason, an accompanying analysis of Se concentrations in dietary items of different sizes can facilitate the interpretation of juvenile whole-body Se concentrations and potential effects on their growth.

ASSESSING ECOLOGICAL SE EFFECTS WITH ALTERNATIVE ENVIRONMENTAL MEDIA

As described above, fish tissue integrates the influence of abiotic and lower trophic level variation and should be prioritized for ecosystem assessments. In cases where it is not possible to collect sufficient fish, abiotic (i.e., water and sediment) and lower trophic level sample types can be analyzed as the basis for inferring Se exposure in fish (Figure 4). Several studies have illustrated the value of such approaches (e.g., deBruyn & Chapman, 2007; Graves et al., 2021), and robust, context-dependent Se bioaccumulation models can be developed from data representing Se cycling from the water column to particulate, macroinvertebrate, and fish compartments (Brandt et al., 2021; Presser & Luoma, 2010; Presser & Naftz, 2020). Subsequent use of

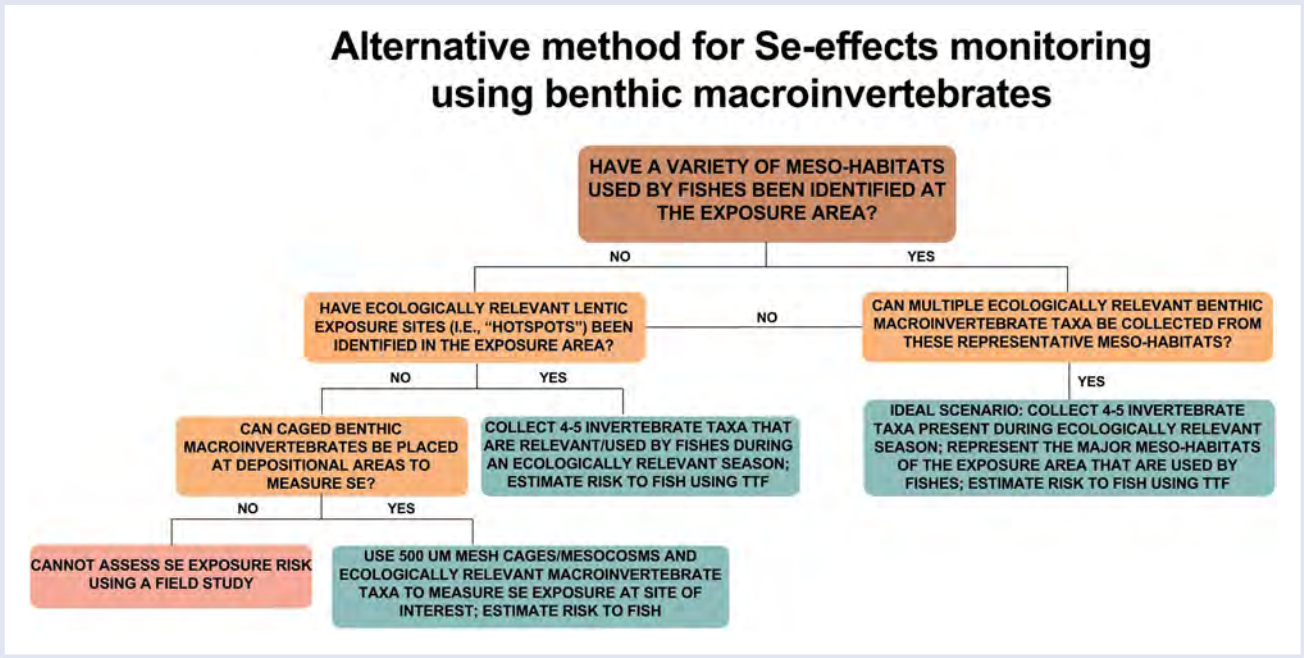


FIGURE 4 Decision tree for assessing potential reproductive effects of selenium (Se) in fish using invertebrate collections

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these models can make use of established concentration relationships to predict fish tissue Se concentrations if the accompanying uncertainty is appropriate to the monitoring effort. This section provides an overview of the sampling approaches used for these alternative sampling types, beginning with macroinvertebrates as the prey of lower trophic level fish species. Additional details are provided in Supporting Information S1 to guide sampling efforts in cases where macroinvertebrates collections are in place of, rather than complementary to, fish sampling.

Macroinvertebrates

Se variability and taxonomic resolution. Selenium concentrations in macroinvertebrates reflect the influence of abiotic and particulate Se concentrations on the food web and represent dietary exposures to resident insectivorous fish. Bioaccumulation of Se by benthic macroinvertebrates depends on both physiological factors, such as assimilation, ingestion, and efflux rates, and ecological factors such as habitat type, habitat use, and diet (reviewed in Presser & Luoma, 2010). We assessed these relationships using Se concentration data for macroinvertebrates and associated diet items collected from both lentic and lotic systems in Canada and the United States (see Supporting Information S1 for details). Relationships between Se concentrations in macroinvertebrates and their associated diets (i.e., TTFs) are highly variable both within and among taxonomic groups (Figure 5, Supporting Information Figure S2; Table 1, Supporting Information Table S1). Therefore, taxonomic, habitat, and diet-related variations in Se uptake must be considered when assessing Se exposure to higher trophic levels. It is also advised to measure macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values to characterize the food web structure and assess the relative contribution of various prey to the fish species of interest. Sorting invertebrates to the level of family or functional feeding group before Se analysis provides a means of understanding the relative input and/or use of different habitats and different invertebrate types to fish. Including four to five representative taxa will further our understanding of the risk of exposure to fish based on invertebrate diet items and mesohabitat use.

Site selection and sample timing. Site selection and the timing and/or seasonality of sample collection should be designed to best represent food web exposure to fish. As such, a sampling design to collect representative invertebrate taxa from both erosional and depositional zones of affected and reference sites is preferred, although sampling efforts can be designed such that proportionately greater efforts are placed on potential hotspot mesohabitats. Sampling should be performed when it represents the most relevant exposure to fish relative to effluent exposure, gonad maturation, life-history characteristics, or feeding patterns most relevant to Se accumulation. Note that the relevant dietary Se exposure period for many fish species can occur well before gonad maturation. If aqueous and biotic Se concentrations are temporally consistent, one sampling event at a time relevant to fish Se exposure should suffice. If there are large seasonal fluctuations in aqueous Se concentrations (caused by seasonal changes in water flow and resulting dilution and/or concentration of effluent), a seasonal study design should be considered.

Sampling and processing. Standard operating protocols for the collection and processing of macroinvertebrates can be followed to collect organisms from a variety of habitats (ECCC, 2012; USEPA, 2016). The main adjustments and/or

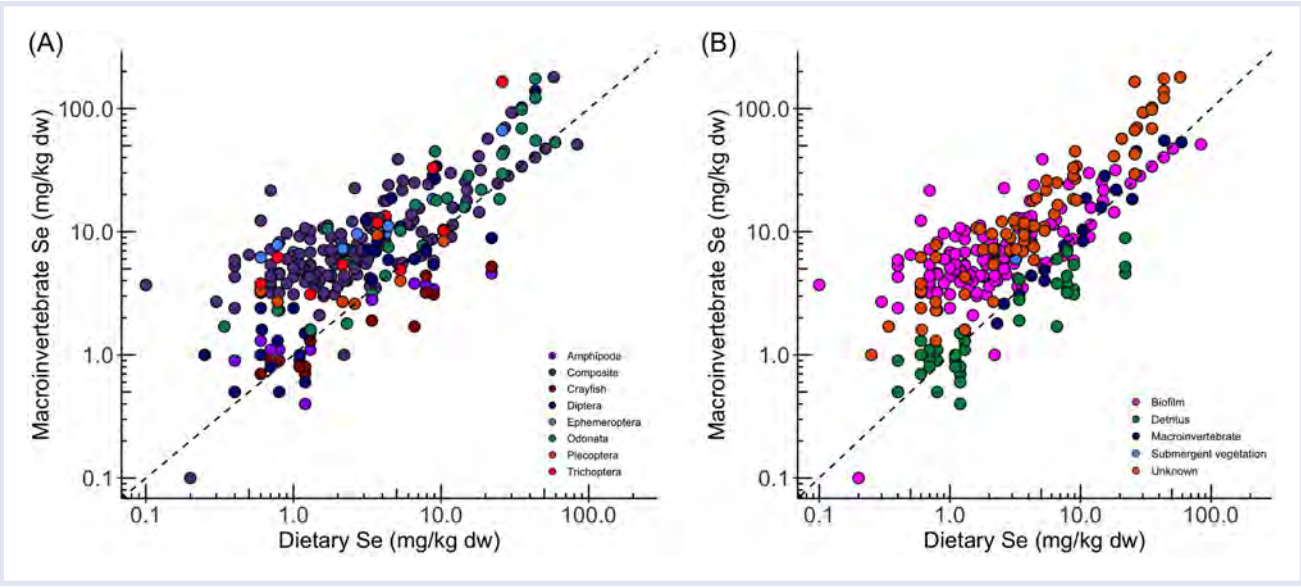


FIGURE 5 Macroinvertebrate vs. dietary selenium (Se) concentrations for several taxonomic groups collected from both lotic and lentic environments throughout Canada and the USA. Colors indicate taxonomic group (A) or diet item (B). Dashed lines mark the 1:1 concentration relationship between diet and invertebrate Se (i.e., trophic transfer factor [TTF] = 1)

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TABLE 1 Summary of trophic transfer factors (TTFs) for various taxonomic groups of macroinvertebrates collected from lentic and lotic habitats throughout the USA and Canada

Invertebrate (order)	Average TTF (±SD)	Minimum TTF	Maximum TTF
Amphipoda	1.0 ± 0.6	0.2	2.2
Composite (unknown)	4.3 ± 4.6	0.5	37
Diptera	2.0 ± 1.1	0.4	4.1
Ephemeroptera	4.5 ± 2.9	2.0	10
Odonata	2.1 ± 1.4	0.7	6.2
Plecoptera	2.0 ± 1.7	0.8	5.5
Trichoptera	3.8 ± 2.4	0.9	7.9

Note: TTFs are based on assumed diet (generally detritus or biofilm Se measured from the same site). All data were obtained from previous literature reviews (DeForest et al., 2017; Presser & Luoma, 2010). See Supporting Information S1 for methods.

considerations specifically for the collection of macroinvertebrates for Se determination are avoiding contamination by using acid-cleaned plastic materials and obtaining sufficient mass of four to five taxonomic groups for Se analysis. Three replicate composite samples of each taxon collected from different subsites in a site are recommended to characterize the variation in Se (ECCC, 2012; USGS, 2008). Details on sample processing and analysis of tissue Se concentrations by ICP-MS are provided in USEPA (2014, 2015) and Vacchina and Dumont (2018).

Statistical analyses. To assess whether Se exposure raises concerns in a particular area, tissue Se concentrations in macroinvertebrates can be compared with predetermined guidelines or alert levels (for instance, the BC MoE [Ministry of Environment] interim guideline for macroinvertebrates is 4 mg kg^{-1} dw). This is the preferred method over strictly comparing means between the exposed and reference sites. It is also useful to compare affected sites with the distribution of concentrations at reference sites to understand the relative change in Se exposure between impact and reference areas. As such, an effect size is less important than establishing whether an appropriate alert and/or trigger level is exceeded. Thus, macroinvertebrate sampling plans should be designed to ensure that several reference sites are sampled and that affected site samples can be compared with the distribution of reference samples and the alert and/or trigger level of Se.

To estimate fish tissue Se concentrations from macroinvertebrate Se when fish cannot be collected, a known TTF value for a particular fish species can be applied if the data are available. If no relevant TTF data exist for a particular species, a mean TTF of 1.2 (the mean of all fish TTFs examined through meta-analysis, data from DeForest et al., 2017; Presser & Luoma, 2010) can be used to estimate fish muscle Se. Then, this estimated concentration can be compared with the fish tissue Se guidelines established by the USEPA (11.3 mg kg^{-1} dw) or BC MoE (4 mg g^{-1} dw; interim; Beatty & Russo, 2014) as described above.

Water, sediment, and particulate samples. The biogeochemical cycling of Se among abiotic and particulate compartments, including uptake by primary producers as the largest bioaccumulation step in aquatic food webs, is highly variable both within and among study sites (Maher et al., 2010; Ponton et al., 2020; Presser & Luoma, 2010). As a result, Se concentrations in these sample types have been shown to be unreliable predictors of Se concentrations in upper trophic levels (Brandt et al., 2021; Ponton et al., 2020; Presser & Luoma, 2010). However, the inclusion of water, sediment, and particulate samples is valuable for broader assessments of Se-impaired ecosystems. Furthermore, federal criteria in Canada and the United States include dissolved Se concentrations considered protective of aquatic life, such that concentrations measured above these thresholds indicate the potential for adverse environmental health consequences. Monitoring of dissolved Se concentrations can therefore help determine whether monitoring of upper trophic levels is indicated; however, the USEPA and BC MoE recommend that fish tissue data take precedence over water column data (Beatty & Russo, 2014; USEPA, 2016). There has been considerable research into the influence of abiotic and context-specific factors on Se biogeochemical cycling in aquatic systems (Besser et al., 1993; Franz et al., 2011; Ponton et al., 2020; Simmons & Wallschläger, 2005). Here, we summarize the factors that should be considered when sampling water, sediment, and particulates to determine selenium exposure for fish.

Water. Selenium speciation significantly influences Se uptake at the base of aquatic food webs (reviewed in Ponton et al., 2020, alongside other mediators of Se partitioning). However, due to analytical costs and challenges, few field studies have reported on dissolved Se speciation. Therefore, the influence of aqueous Se speciation on bioaccumulation is not completely understood. Se concentrations in surface water are generally reported as total dissolved Se, and aquatic life criteria are also based on total Se concentrations. Water collection protocols developed by the USEPA and the International Organization for Standardization (ISO) allow for

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standardized approaches and data comparison across studies (e.g., filtration at 0.45 μm and acidification).

Factors related to water column Se dynamics, including Se speciation and the hydrology of the impaired system, inform study design. Selenate (SeO_4^{2-}; SeVI) is the fully oxidized form of Se in water and is typically the predominant form of Se in fast-flowing (i.e., lotic) and oxidizing systems such as rivers and streams. The reduced forms of Se, including selenite (S; SeO_3^{2-}; SeIV) and organo-Se species, are more abundant in slow-moving waters (i.e., lentic) with higher residence times in lakes and wetlands (Sharma et al., 2015). Dominant Se species can also depend on contamination sources because coal-fired power plant and oil refinery effluents are typically dominated by selenite, whereas mine effluent and agricultural drainage tend to be mostly selenate (Maher et al., 2010; Young et al., 2010). Laboratory studies have shown that organo-Se forms are the most bioavailable, followed by selenite and selenate (Besser et al., 1993; Franz et al., 2011; Simmons & Wallschläger, 2005), suggesting that greater Se bioaccumulation is expected in environments that favor larger proportions of reduced Se species.

In general, lentic environments are associated with greater Se accumulation than lotic environments because of greater biological activity, larger proportions of reduced Se forms, and greater potential for Se remobilization and recycling from sediments (Hillwalker et al., 2006; Orr et al., 2012; Simmons & Wallschläger, 2005). Longer water residence times in lentic environments can also facilitate Se bioaccumulation (Luoma & Rainbow, 2005; Orr et al., 2006; Sharma et al., 2015). Reducing the conditions in lentic waters can lead to greater Se accumulation in sediment and greater bioavailability in water (Luoma & Rainbow, 2005; Simmons & Wallschläger, 2005; USEPA, 2016). Greater partitioning of Se in sediment and detritus in lentic systems also appears to support food web Se accumulation in lentic systems so that benthic invertebrates and their consumers in lentic systems may be exposed to higher Se concentrations (Hillwalker et al., 2006; Orr et al., 2006; Simmons & Wallschläger, 2005) As discussed above with respect to invertebrate and fish collection, the observation that habitat type (i.e., lentic vs. lotic systems) influences Se accumulation should be a key consideration in the design of monitoring studies aimed at estimating Se exposures (Martin et al., 2008, 2011). Finally, Se speciation can be useful for identifying potential sources of Se uptake into food webs. For example, anomalously high Se concentrations of organo-Se species in lotic systems can indicate influences from nearby ponds or oxbows not previously identified as contributing to the local environment.

Sediment, particulates, and biofilm. Partitioning Se to sediment and particulates, and enriching Se into the base of the food web, dictates exposure to higher trophic level organisms (Presser & Luoma, 2010). As such, colocated sediment, particulate, and/or biofilm samples collected alongside macroinvertebrates and fish are important to fully characterize Se bioaccumulation in the food web and can aid the interpretation and understanding of Se exposure in higher trophic level organisms (Luoma & Presser, 2009). Selenium concentrations in these compartments can be highly variable both spatially and temporally. Consideration of the factors influencing binding and resuspension of Se to, and from, sediment and particulates. The relevance of the compartments being sampled to the species of interest should be considered during sampling to reduce variation and understand food web Se exposure.

Selenium binds to organic matter in sediment and particulates, making these compartments an important sink for Se in the aquatic environment. Furthermore, depositional zones with greater concentrations of organic matter are probably associated with greater Se deposition. The potential for this bound Se to be resuspended from organic matter into water depends on the sediment oxidation-reduction status (Maher et al., 2010; Martin et al., 2008, 2011). Lentic environments generally have longer residence times and are associated with greater biological activity and lower oxygen, leading to reducing conditions (Simmons & Wallschläger, 2005). Reducing conditions promote the release of Se previously bound to organic carbon in sediments and kinetically favor the reduction in selenate to more bioavailable forms of Se such as selenite and organo-Se (Masscheleyn & Patrick, 1993; Masscheleyn et al., 1991), leading to greater bioavailability of Se in lentic or depositional environments.

In addition to reducing conditions that favor the remobilization of Se from sediments, greater residence times in lentic systems also promote recycling of Se and greater opportunity for Se uptake relative to systems with high flow that are constantly flushed (Sharma et al., 2015). Once Se is assimilated by primary producers, it may continue cycling through biological compartments through trophic transfer to higher trophic level organisms or by settling into sediment in dead and decaying organisms (Maher et al., 2010; Sharma et al., 2015). From there, detritivorous or microbial communities in the sediment may assimilate or biotransform Se, facilitating reuptake and trophic transfer through the food web (Orr et al., 2006). Microbial activity is a main driver of Se bioaccumulation because this biological activity leads to the biotransformation of Se to more bioavailable forms, increasing the transport of Se to sediments in bioavailable forms where benthos is exposed (Orr et al., 2006; Simmons & Wallschläger, 2005). Additionally, Se that is taken up by organisms will eventually become detritus or sediment after the organisms die, decompose, and settle out of the water column. Therefore, higher productivity and/or biomass results in more Se accumulation in bottom sediments (Maier & Knight, 1994).

Due to the influence of environmental conditions on Se binding and/or uptake in sediment, particulates, and biofilm, particular emphasis should be placed on collecting samples that represent exposure pathways most relevant to the fish species of concern. Standardizing sample collection to lentic or depositional environments within sites of interest

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is advised to isolate high Se exposure samples. Collecting a consistent and/or relevant size fraction of sediment and particulates (<63 μm), and collecting biofilm taxa that are relevant to the food web are also important (Beatty & Russo, 2014). In addition to focusing on depositional zones where higher Se accumulation is expected, sampling microhabitats relevant to the fish species of interest is recommended.

CONCLUSION

- Decades of research and environmental fate and effects data have resulted in updated regulatory frameworks to manage Se in aquatic systems, but impaired systems still exist across North America.
- Selenium is incorporated into aquatic food webs by primary producers, with subsequent exposure to higher trophic levels occurring primarily via dietary intake.
- Fish are more sensitive to the potentially negative effects of Se than other aquatic organisms, and the primary negative effects are mediated by dietary intake of Se by female fish, maternal deposition into her eggs, and impaired embryo development.
- Conceptualized pathways of dietary exposure can be estimated for a given fish species based on knowledge of their prey organisms and analysis of Se concentrations in those taxa.
- Fish feeding at a higher trophic level and with greater maximum body size typically do not accumulate more Se than those feeding at lower trophic levels, suggesting that Se does not biomagnify.
- Ontogenetic diet shifts can affect the potential for fish to accumulate Se.
- Determining concentrations of Se in the water column, particulate and macroinvertebrates can augment our understanding of fish tissue concentrations or serve as proxies in cases where collection of fish tissue is not possible. Collection of these samples should be designed to best represent food web exposure to fish, including several habitat types and affected and reference sites.

AUTHOR CONTRIBUTION

Vince Palace: Conceptualization; data curation; formal analysis; funding acquisition; project administration; writing—original draft; writing—review and editing. **Stephanie Graves**: Conceptualization; data curation; formal analysis; visualization; writing—original draft; writing—review and editing. **Jessica Brandt**: Conceptualization; formal analysis; methodology; validation; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Cited data are available in publicly available formats or contained in the Supporting Information.

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SUPPORTING INFORMATION

Files ancillary to the main document but pertinent to the central theme of the discussion are provided.

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Supporting Information for

Guidance for assessing potential impacts of selenium in freshwater ecosystems

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SI part a: Considerations for selecting sentinel fish species

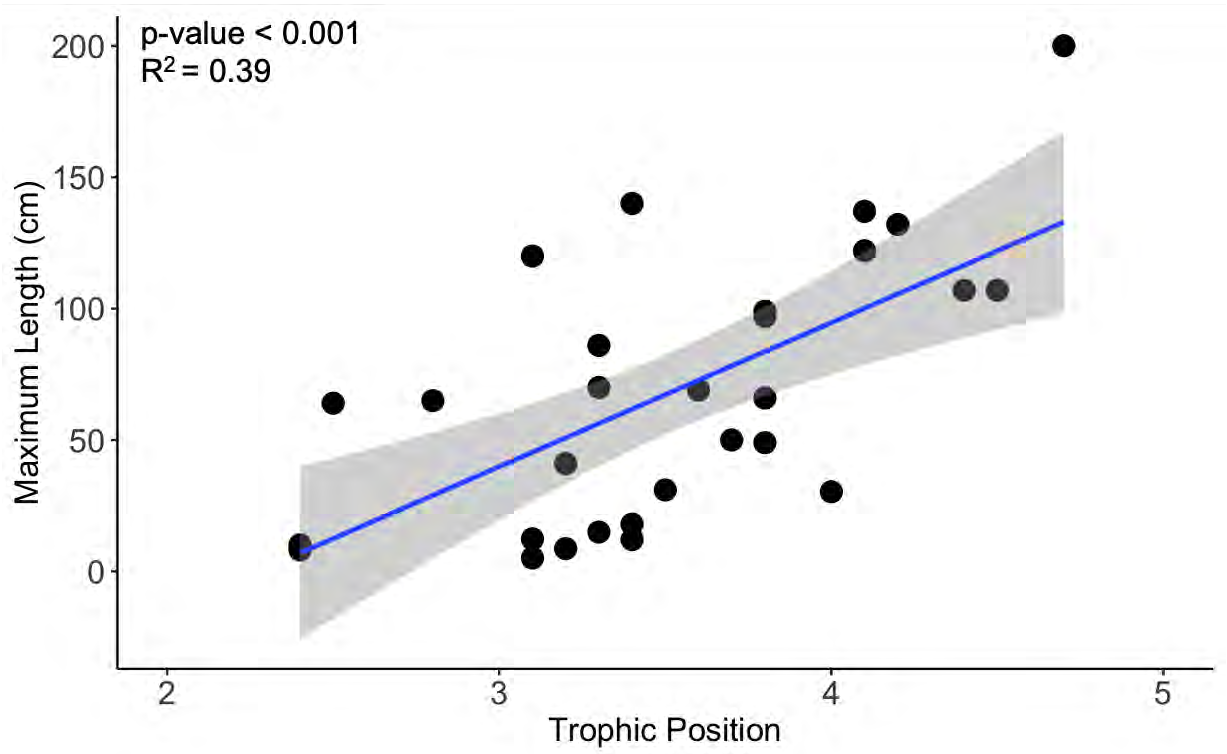


Figure S1: Maximum body length (cm) versus relative trophic position for fish species previously used for metal mining EEM studies in Canada.

Table S1: Egg and yolk volumes and egg:yolk volume ratios for fish species with known selenium toxicity thresholds that have previously been used for Environmental Effects studies in Canada.

Family Common Name *(Scientific Name)*	Established Se Toxicity Thresholds Egg/ovary (dw)	Egg Volume mm^3	Yolk volume mm^3	Ratio yolk:egg volume	Reference and Notes
Dolly varden (Salvelinus malma)	56.2 egg (a)	36	25	0.694444	Galagher et al. (2019)*
Cutthroat trout (Salmo clarki)	26.3 egg (a)	6.03	4.75	0.787728	Carim et al. (2021)**
Brown trout (Salmo trutta)	21.0 egg (a)	71.1	63.6	0.894515	Bonisławska et al. 2001
Rainbow trout (Oncorhynchus mykiss)	24.4 egg (a)	62.8	55.2	0.878981	Bonisławska et al. 2001
Arctic grayling (Thymallus arcticus)	>33.9 (b)	44	8.18	0.185909	Bonisławska et al. 2001
Mountain whitefish (Prosopium williamsoni)	>54 (d)	17	16.15	0.95	Wydoski et al. 2001, Mitz et al. 2019 and Sreethran et al. 2015
Northern pike (Esox lucius)	34.0 (E)	10.3	6.5	0.631068	Bonisławska et al. 2001
Fathead minnow (Pimephales promelas)	<26.5 egg (a)	1.4	0.23	0.164286	Wang et al. 2014, Scahill 2008
White sucker (Catostomus commersoni)	26	4.2	0.36	0.085714	Munkittrick and Dixon 1989, Fuiman and Trojnar 1980,
Bluegill (Lepomis macrochirus)	20.6 egg (a)	0.79	0.36		Oplinger and Wahl 2015
Largemouth bass (Micropterus salmoides)	26.3 ovary (a)	1.23	0.46	0.373984	Sepúlveda et al. 2003

**Proportional yolk volume estimated from other Salvelinus species from Bonisławska et al. 2001*

*** Yolk voluome estimated from average of 4 other salmonids from Bonisławska et al. 2001*

SI part b: Assessing ecological Se effects with alternative environmental media

a. Meta-analysis methods

To understand how habitat type, diet, and taxonomy influence Se trophic transfer in benthic macroinvertebrates, we conducted a meta-analysis using data reported by Presser and Luoma (2010) and by DeForest et al. (2017). Using analysis of covariance (ANCOVA), with the covariate "Order" included, selenium concentrations in benthic macroinvertebrates were related to those in diet across six orders (n=277 individual data points; Supporting Information 2, Table 1). For data points that listed diet items (biofilm, detritus, invertebrate, submergent vegetation or unknown) and habitat (lentic or lotic), ANCOVA was used to assess how diet and habitat influenced the relationship between BMI and diet Se. Trophic transfer factors (BMI Se concentration/dietary Se concentration) were calculated for the orders Amphipoda, Diptera, Ephemeroptera, Odonata, Plecoptera, Trichoptera.

b. Meta-analysis results

We observed significant positive relationships between dietary Se and invertebrate Se concentrations for several taxonomic groups (Figure 5A; Table S1). The slopes of these relationships differed among taxonomic groups, indicating differences in the trophic transfer of Se among taxa; Diptera, Odonata and Trichoptera had the highest slope estimates, while Amphipoda, Ephemeroptera, Plecoptera and Crayfish had relatively lower slope estimates (Figure 5A; Table S1). Trophic transfer factors derived from these data ranged from 0.2 in Amphipoda to 37 in a composite sample, illustrating the range in TTFs that can be observed in BMIs. Though these estimated TTFs are dependent on an assumed dietary Se concentration, it is evident that taxonomic variation in Se uptake is high and needs to be considered when assessing Se exposure to higher trophic levels. The greater variation in composite samples also demonstrates the need to sort and analyze invertebrates by finer taxonomic groups.

Benthic macroinvertebrate feeding strategies are diverse and due to the variation in Se exposure among food web pathways (for instance, greater exposure from benthic-detrital pathways versus particulate TSe in the water column), feeding habits can have a significant impact on invertebrate Se concentrations (Orr *et al.*, 2006; Muscatello *et al.*, 2008; Graves *et al.*, 2019, 2021) Invertebrate feeding strategies are classified by FFGs. The main FFGs are: collector-gatherers (feed on fine particulate organic matter (FPOM) in detritus/sediment), collector-filterers (filter FPOM from the water column), scrapers (scrape algae from surfaces), shredders (feed on coarse

particulate organic matter (CPOM) i.e., leaf litter), and predators (feed on other invertebrates). To date, no known studies have thoroughly investigated the variation in Se bioaccumulation among these FFGs, but previous studies have observed overall greater accumulation of Se in collector-gatherers, and lesser accumulation of Se in collector-filterers (Orr *et al.*, 2006; Muscatello *et al.*, 2008; Graves *et al.*, 2019, 2021). We were not able to separate taxa by functional feeding groups (FFGs) due to the coarse taxonomic identification herein, but we did observe that the slope of the relationship between invertebrate Se and dietary Se differed according to reported diet; invertebrates feeding on detritus had the lowest slope estimates, followed by biofilm, while those feeding on other invertebrates had greater slope estimates.

In contrast to the effect of receiving environment on Se uptake at the base of the food web, there was no significant difference in slopes or intercepts of the relationship between invertebrate Se and dietary Se in lentic versus lotic environments (ANCOVA, $p=0.713$, t-value=-0.368, Table S1), suggesting that the receiving environment does not impact TTFs in the same way that it affects EFs. This is unsurprising, since the majority of Se accumulated by benthic macroinvertebrates is attributed to diet.

Overall, our meta-analyses confirmed that taxonomic group and diet both influence Se bioaccumulation among macroinvertebrate BMI taxa, suggesting that these factors need to be considered in the sampling of macroinvertebrate BMI to infer Se exposure to fish.

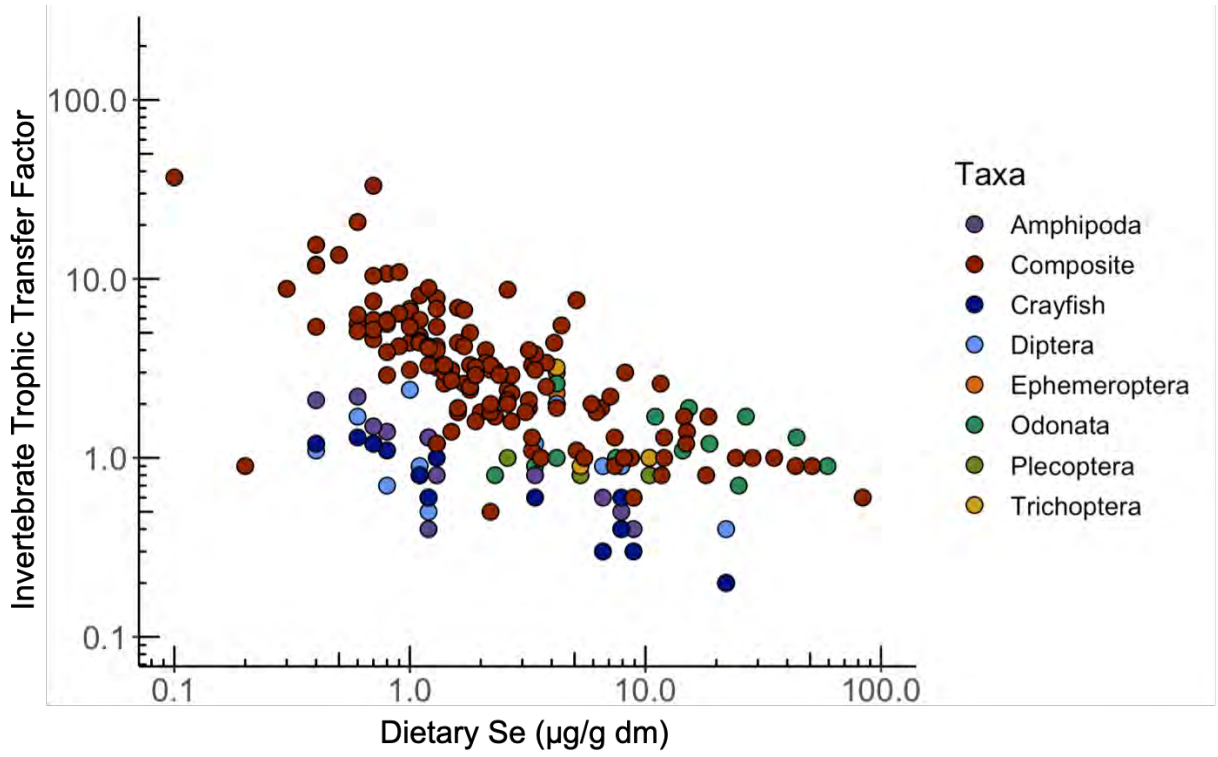


Figure S2: Invertebrate trophic transfer factors for various taxonomic groups plotted against dietary Se concentrations. Invertebrate TTFs vary widely among taxa and decrease with increasing dietary exposure.

Revised May 22, 2024

Table S2: Statistical analyses of relationships between invertebrate diet Se and invertebrate Se body burdens

X variable	Y variable	Covariate	Statistical test	p-value	F-statistic	df	R2	Slope	Intercept
log Diet Se	log Invert Se - Amphipoda	N/A	LR	<0.001	33.04	1,12	0.71	0.501	0.056
log Diet Se	log Invert Se - Composite	N/A	LR	<0.001	212.7	1153	0.58	0.587	0.04
log Diet Se	log Invert Se - Crayfish	N/A	LR	<0.001	120	1,12	0.9	0.579	-0.053
log Diet Se	log Invert Se - Diptera	N/A	LR	<0.001	129.2	1,30	0.81	1.056	0.177
log Diet Se	log Invert Se - Ephemeroptera	N/A	LR	<0.001	42.81	1,12	0.76	0.529	0.782
log Diet Se	log Invert Se - Odonata	N/A	LR	<0.001	124.7	1,27	0.82	0.886	0.365
log Diet Se	log Invert Se - Plecoptera	N/A	LR	0.036	7.23	1,6	0.47	0.419	0.468
log Diet Se	log Invert Se - Trichoptera	N/A	LR	0.004	15.37	1,8	0.62	0.81	0.585
log diet Se	log invert Se	Taxa	ANCOVA	<0.001	59.54	15,260	0.76	0.501 (amphipoda)	0.056
		logdiet:Composite	ANCOVA	0.515					
		logdiet:Crayfish	ANCOVA	0.659					
		logdiet:Diptera	ANCOVA	<0.001					
		logdiet:Ephemeroptera	ANCOVA	0.886					
		logdiet:Odonata	ANCOVA	0.01					
		logdiet:Plecoptera	ANCOVA	0.727					
		logdiet:Trichoptera	ANCOVA	0.128					
log diet Se	log invert Se	Lentic vs Lotic	ANCOVA	<0.001	95.95	5 and 270	0.63	0.629 (lentic)	0.36
		logdiet:lotic	ANCOVA	0.713				-0.0448	0.143
log diet Se	log invert Se	Diet	ANCOVA	<0.001	120.5	10 and 265	0.81	0.493 (Biofilm)	0.66
		logdiet:BMI	ANCOVA	0.0017				0.619	-0.756
		logdiet:Detritus	ANCOVA	0.056				0.143	-0.655
		logdiet:unknown	ANCOVA	<0.001				0.412	-0.119
		logdiet:waterboatmen	ANCOVA	0.005				0.504	-0.596

SI part c: Detailed information on the sampling and analysis of macroinvertebrates to determine Se exposure in aquatic ecosystems:

In this section we provide important details on the aspects of study design and sample collection that are necessary to determine Se concentrations in macroinvertebrates for the purpose of estimating Se exposure in higher trophic level organisms. At the end of this section we provide a table (Table S2) summarizing the sampling considerations for collecting macroinvertebrates to estimate Se exposure.

a. Taxonomic resolution

Invertebrates should be live-sorted at least to coarse taxonomic levels in the field. Once sorted, invertebrates should be stored at -20°C for transport and until further processing. Given the amount of variation in TSe among invertebrate taxonomic groups and functional feeding groups, we recommend that invertebrates be sorted to Family prior to TSe analysis. In addition, grouping invertebrates by size/similar instar stage will reduce variation caused by changes in feeding habits. Invertebrate samples should be limited to one thawing event to minimize protein breakdown and loss of Se from the sample. Collected, sorted, and identified invertebrates should then be freeze-dried for 48-72 hours (until a constant mass is obtained) and ground to a homogenous powder using an acid-cleaned mortar and pestle. Ground samples are transferred to acid-cleaned vials or containers and transported to the laboratory responsible for the analysis of TSe.

b. Site selection

Streams and rivers can be divided into types of habitats that are more, or less, likely to be subject to high Se accumulation. These habitat characteristics may be used to focus on sampling sites and invertebrate taxa that would be expected to accumulate the most Se. Lotic environments can be subdivided into erosional and depositional habitats. Erosional habitats are defined by fast-flowing water with coarse sediments like cobble, gravel, and boulders . “Riffle” and “run” meso-habitats are considered erosional where sediments do not accumulate. In these well-oxygenated meso-habitats, Se is expected to exist mainly as selenate and Se bioaccumulation is expected to be lower. Erosional habitats are characteristic of head-water and medium-sized streams but are less common in large rivers. In contrast, depositional habitats have slower-moving water where sediments accumulate. They are characterized by finer substrates like sand, silt and clay. Due to these characteristics, depositional habitats tend to be associated with greater Se accumulation.

However, the decision to focus strictly on studying Se accumulation in invertebrates from depositional habitats is complicated because generally, erosional habitats such as riffles support greater invertebrate diversity and may represent a higher proportion of a fish's preferred diet items, even though Se concentrations are expected to be lower. To best represent food web exposure to fish, a sampling design to collect representative invertebrate taxa from both erosional and depositional zones of a site is preferred, though sampling efforts can be designed such that proportionately greater efforts are placed on potential "hot-spot" meso-habitats.

c. Timing and Seasonality of sample collection

In contrast to collections of macroinvertebrates for community composition analysis, collecting invertebrates to estimate dietary exposure risk to fish does not need to coincide with the presence of the greatest number of taxa or the largest instars. Instead, sampling should be performed when it represents the most relevant exposure to fish relative to effluent exposure, gonad maturation or life history characteristics or feeding patterns most relevant to Se accumulation.

If there are large seasonal fluctuations in aqueous Se concentrations (for instance, due to seasonal changes in water flow and resulting dilution/concentration of effluent), a seasonal study design should be considered. Based on previous studies - temporal variation of Se in biota is site-specific and should be evaluated on a case-by-case basis. Golder (2018) assessed temporal variation of Se in macroinvertebrates by collecting composite samples from May to October in mine-influenced streams of the Elk River watershed. They found no seasonal differences but Se concentrations among sites within each sampling time were highly variable. This variation was likely caused by compositing many taxonomic groups and functional feeding groups into one sample.. In the same investigation, there was no evidence that accounting for lag in accumulation of Se in invertebrates relative to concentrations in water would improve performance of models relating water to invertebrate Se.

In contrast to the work by Golder (2018), Brandt *et al.* (2021) found that throughout the Lower Gunnison River, Hydropsychidae TSe concentrations were lower in October than April or August. This may be related to seasonal fluctuations in aqueous Se concentrations, or seasonal energy dynamics in the river. In fall and winter, when days are shorter and biofilm growth is slow, allochthony represents a relatively larger energy source to streams and rivers. Greater activity of shredders during this time period is expected, with terrestrial organic matter presumably

contributing significantly to Se accumulation in invertebrates. During spring and summer when autochthony dominates energy production, activity of scrapers and collector-gatherers is expected to increase, and Se concentrations may be subsidized during these times of year. No studies have investigated the impact of seasonal changes in energy sources and activity levels of different invertebrate groups in relation to Se accumulation patterns, but it should be considered in the selection of invertebrate sampling times.

d. Sampling methods

Standard protocols for the collection of macroinvertebrates in wadeable or non-wadeable streams, wetlands, or lakes have been prescribed (BC MoE, 2006; ECCC, 2012). Collecting invertebrates from natural substrates is always preferred, however, if standard collection techniques are not feasible, or if increased standardization of collection is needed for the purposes of seasonal/repeated sampling, artificial substrates such as rock baskets or Hester Dendy Samplers can be used (BC MoE, 2006). This will limit the diversity of invertebrate taxa being collected, but will increase consistency and decrease variation among sites and among sampling times (BC MoE, 2006). Collecting invertebrates for Se analysis requires enough mass from 4-5 different Families and/or FFGs). Three replicate samples of each taxa should be collected from different sub-sites within a site to characterize variation in TSe concentrations (i.e. a total of 15 invertebrate samples will need to be collected from each site) (USGS, 2008; ECCC, 2012). Due to the small size/mass of most benthic invertebrates, many individuals need to be pooled to obtain sufficient mass for Se analysis. Compositing several species and measuring the average concentration of the pooled individuals will decrease the number of samples needed within a site (by decreasing variation in results), but it should be noted that information about variability and the range of concentrations among individuals will be lost (US EPA, 2016). Composite samples are acceptable when the objective of the study is to determine if a reference site is different from an impacted site but when variation of Se among taxonomic groups is needed, compositing should be limited to genus. If the site is large and variable and there is a need to characterize Se bioaccumulation across larger spatial scales, more effort can be directed to multiple sites, rather than collecting replicate samples within one site. This recommendation is based on research by Cianciolo et al. (2020) and Brandt et al. (2021) showing that Se bioaccumulation does not necessarily decrease as the distance from a point source increases. Rather, Se can be efficiently transported downstream and there is often no

difference in bioaccumulation at sites immediately downstream of a source compared with sites as far as 20 km downstream.

e. Quality Assurance/Quality Control and Analytical Considerations

Commercial analytical laboratories typically require large dry masses for sample analysis but, samples of 0.01 to 0.1 g dry weight are often sufficient for TSe analysis using ICP-MS techniques. Analytical results for the same samples can differ between analytical laboratories due to different instruments and calibration standards. Variation in results needs to be considered during study design, the selection and analysis of calibration standards and reference materials, and in the interpretation of results (US EPA 2021). The consistent use of standardized methods for collection, handling, preservation, and analysis are recommended. With respect to analytical accuracy and precision, sufficient sensitivity in the analysis is necessary to reduce variability. For instance, analytical methods with detection limits sufficiently lower than the lowest expected concentrations are needed. For animal tissue such as invertebrates and fish, this should be achievable since most instrument detection limits are around 0.1 ppb, and most animal tissue Se concentrations are > 1 ppm.

Measuring and reporting tissue concentrations in dry weightmass is the standard and preferred method for Se analysis. Freeze-drying is preferred, rather than a drying oven, to reduce potential of Se loss by evaporation. To prepare the tissues for analysis, dried tissue is homogenized and then digested in strong acid (HNO_3) using a microwave digestion (closed-vessel) or heated digestion (open-vessel) procedure. Reporting requirements for analysis of Se concentrations should include: 1) limit of quantification, 2) limit of detection, 3) means of method and instrumental blanks, 4) percent recovery of instrumental standards and certified reference materials, and 5) variability in duplicates.

f. Power Analysis, Statistical Analysis and Considerations

Guidance for each specific sample type (compartment) may vary, but overall, the statistical design that best suits current understanding of Se accumulation and variability in aquatic ecosystems is one where concentrations in compartments (eg. macroinvertebrates, sediment, fish) at impacted sites are compared to a guideline or to a distribution of concentrations at reference sites, rather than strictly comparing means between exposed and reference sites (Figure 7.1). As such, an effect size is less important than establishing whether an appropriate alert/trigger level is exceeded.

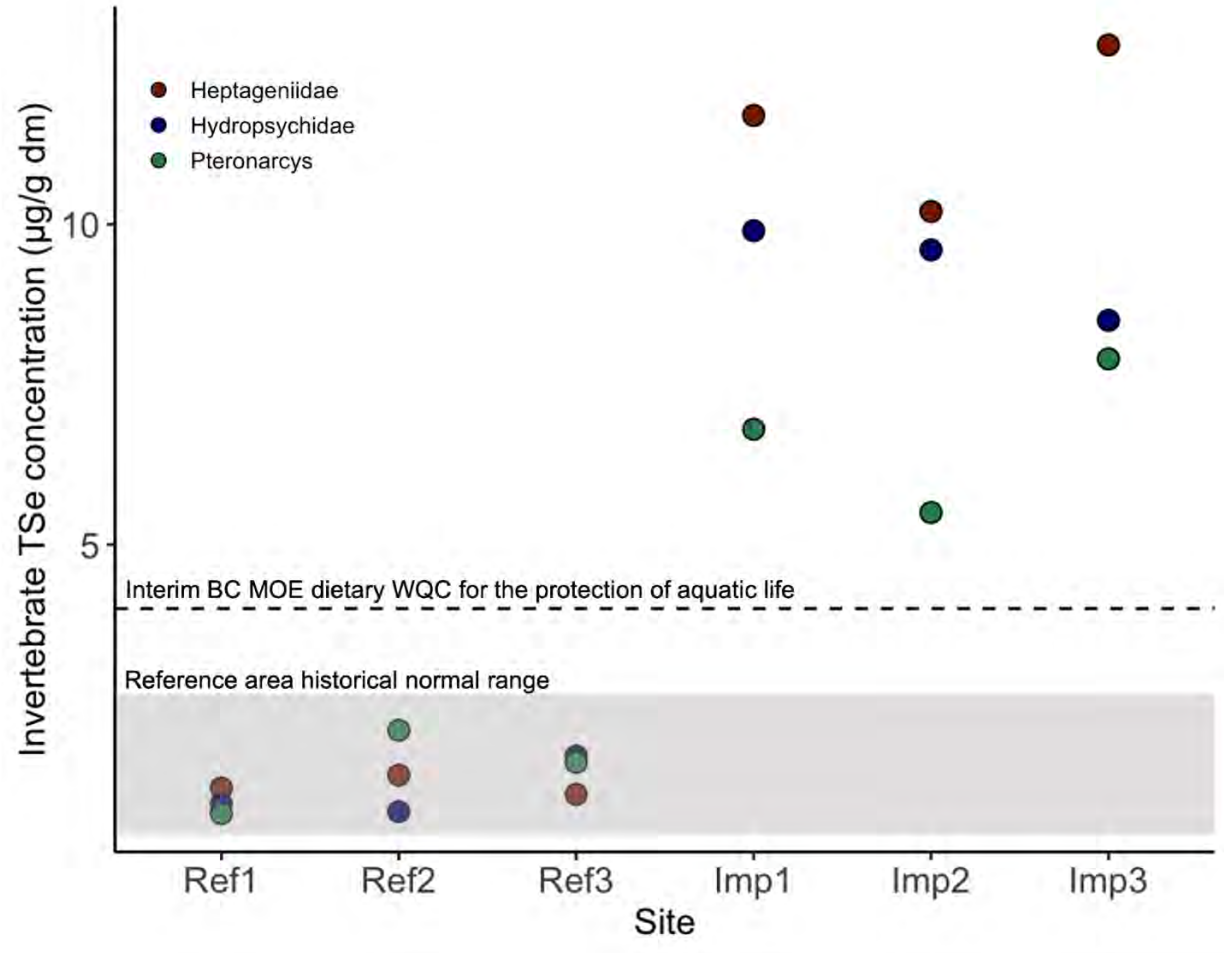


Figure S3: An example of data comparisons for the benthic invertebrate compartment from a site being investigated for potential Se effects. The BC MoE interim guideline for the protection of aquatic life was used in this case.

The sampling design for the collection of benthic invertebrates will depend on the goal of the study. Two main scenarios for sampling are presented below, because the sampling design will differ based on the goal:

A. *Characterizing invertebrate Se based on the potential diet of fishes - i.e. collection of invertebrates from all mesohabitat types.*

   This is the ideal study design because it allows the characterization of Se across all relevant areas where fish may accumulate Se. This would include riffles, runs and pools. In this

scenario, the objective would be to target invertebrate taxa that are relevant to fish diet at the site of interest, and that represent each of the five main functional feeding groups. This includes collecting scrapers, collector-gatherers, collector-filterers, shredders, and predators. If fish study species are decided *a priori*, the invertebrate taxa selected can be chosen based on the known diet of the fish of interest. Through this approach, the different pathways of food web exposure to fish and the relative importance of different dietary items to fish at the site can be evaluated.

B. *Characterizing invertebrate Se in the "worst-case scenario" - i.e. collection of invertebrates from lentic/depositional habitats only.*

   In this scenario, the focus of the study is sampling areas where Se bioaccumulation is expected to be highest. In this case, pools, backwaters, and main channel margins should be sampled for invertebrates that are expected to contain greater Se concentrations due to their location and feeding strategies (collector-gatherers feeding on fine particulate organic matter, detritivore shredders feeding on biofilm-conditioned leaf litter, and predators feeding on other invertebrates).

Though collecting three replicate samples within a site is generally recommended, power analysis can be performed using previous data from other reference and impacted sites to determine the appropriate sample size in a particular scenario. An example is presented here: The statistical power to detect changes in benthic invertebrates will be based on 1) variability in Se concentrations among sites, 2) the size of change being detected, and 3) the number of samples collected. Variability in Se concentrations among sites can be estimated using previously collected data. For instance, we can use Muscatello *et al.* (2008) data from reference lakes and lakes impacted by mining activity to estimate variability in invertebrate Se. For detritivores, mean TSe concentrations (± standard deviation) were 12.39 ± 10.89 mg kg^{-1} dm at an impacted site and 0.93 ± 0.49 mg kg^{-1} dm at a reference lake. Predator TSe was 12.74 ± 2.0 mg kg^{-1} dm at the impacted lake and 1.23 ± 0.96 mg kg^{-1} dm at the reference lake. To determine the number of samples needed to detect the increased Se in an impacted lake, the critical effect size is calculated using the mean and SD of each invertebrate type using equation 1.

Equation (1) $CES = mean_{impacted}\text{-}mean_{reference}/SD_{reference}$

For detritivores, the CES is 1.05 and for predators, the CES is 5.8. Using this information and a t-test power calculator in the "pwr" package in RStudio software environment, a sample size of 15 would be needed to detect a difference in detritivore Se and a sample size of 2 would be needed to detect a difference in predator Se.

g. Se-Toxicity to invertebrates and influence on fish Se

deBruyn and Chapman (2007) reviewed sublethal and lethal toxicity of Se to a range of invertebrate taxa, and found that lethal concentrations ranged from 10 to 1000 μg Se/g dm, while sublethal effects occurred in the range of 1 to 80 μg Se/g dm. In multiple laboratory experiments using the mayfly *Centroptilum triangulifer*, Conley et al. (2011, 2013) demonstrated that relatively low dietary Se exposure (12.8 μg/g dm) results in decreased fecundity, and that adult mayfly survivorship decreased significantly when Se body burdens were 31.7 μg/g dm. In limnocorral experiments where Se was added as selenite and the community-level effects were monitored for 63 days, zooplankton showed adverse effects at whole-body Se concentrations of 11 μg/g dm, while macroinvertebrates invertebrates showed decreased survivorship at dietary Se concentrations of 30 μg/g dm (Graves et al. 2022). These recent studies have demonstrated that invertebrate Se toxicity and community-level adverse effects can be observed at concentrations similar to those affecting fish health (Conley et al. 2011, 2013; Graves et al. 2019, 2022). In addition, similar to egg-laying vertebrates, early life-stages of invertebrate taxa appear to be more sensitive to elevated Se exposure. As a result, the toxic effects of Se on lower-trophic level organisms (i.e, zooplankton, macroinvertebrates) should be considered in the experimental design and interpretation of Se exposure data.

The toxicity of Se to invertebrates at relatively low, environmentally relevant levels is important to consider in the estimation of Se exposure to fishes. First, changes in the invertebrate community at an impacted site can change fish Se exposure due to changes in food availability, and shifts in diet to more available species. For instance, at sites where the density of preferred food items such as Ephemeroptera have decreased due to Se toxicity, the relative density of more tolerant taxa such as Chironomidae may increase. According to previous studies, detritivorous taxa such as many Chironomids may accumulate greater Se concentrations due to their habitat and

feeding preferences, and this could lead to elevated exposure in fish. In addition to the influence of Se bioaccumulation, Se toxicity to invertebrates may negatively impact fish health if preferred food sources are unavailable.

Table S3: Sampling considerations for study design and sample collection to determine Se concentrations in benthic macroinvertebrates at Se-impacted sites.

Sampling aspect	**Highlights of study design and sample collection**
Taxonomic resolution	● 4-5 Representative taxa at each site ● Identified and sorted at least to Family level prior to analysis ● Representation of multiple functional feeding groups ● Relevant to higher trophic levels
Site selection	● Ideally both erosional (lotic) and depositional zone sampled to best represent food web exposure to fish ● Sampling effort can be higher in "hot spot" meso-habitats - depositional or lentic environments generally associated with greater Se accumulation ● 3 replicates within a site x
Sampling effort	● 4-5 representative taxa x 3 replicates per site – need to pool individuals to obtain minimum sample mass for Se analyses (~0.1 g dry mass). ● If TSe variation among taxonomic groups is not known, finer taxonomic resolution is helpful (Genus level) ● At larger, more variable/diverse sites multiple sites is better than replicates from fewer sites
Sample collection - timing	● One sampling season likely sufficient but need to consider the dynamics of the system and seasonality of exposure ● Choose timing relevant to fish exposure (time of recent/significant effluent exposure, ecologically relevant to fish gonad maturation)
Sample collection - techniques	● Established SOPs for quantitative collection of macroinvertebrateBMIs can be followed ● Common methods: kick net/D net, grab samples (Ekman, dredges), sweep nets, hand picking from rocks
Sample collection - storage and preservation	● Sort to Family and group by size, freeze at -20℃ ● Freeze-dry 48-72h ● Ground to homogenous powder for TSe analysis
Analytical and QA/QC considerations	● Consistent and standardized methods of collection, handling, preserving, and analyzing samples ● ICP-MS is most common methods ● Typical instrument detection limit for animal tissue = 0.1 ppb ● Measure/report tissue Se in dry weight as mg kg^{-1} dw (or µg g^{-1} dw)
Statistical analysis considerations	● Se concentrations at impacted sites compared to guidelines – has an alert or trigger level been exceeded?

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Appendix O

Selenium Assessment Method for Lake Koocanusa and the Kootenai River in Montana

Public Comment Draft
July 2024

WQDMASWQA-01, Version 1

Approvals:

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Signatures on file

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Revision Date	Version number	Summary of change(s)	Revised sections(s)	Revised by

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ACRONYMS

ARM	Administrative Rules of Montana
AU	assessment unit
BC	British Columbia
BPJ	best professional judgement
CFR	Code of Federal Regulations
CWA	Clean Water Act
CWAIC	Clean Water Act Information Center
°C	degrees Celsius
DEQ	Montana Department of Environmental Quality
DQA	data quality assessment
EPA	Environmental Protection Agency
EQuIS	Environmental Quality Information System
FWP	Montana Fish, Wildlife and Parks
HDPE	high-density polyethylene
HNO_3	nitric acid
MCA	Montana Code Annotated
mg/kg dw	milligrams per kilogram dry weight
µg/L	micrograms per liter
MT-eWQX	Montana EQuIS Water Quality Exchange
MWQA	Montana Water Quality Act
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
TMDL	Total Maximum Daily Load
US	United States
USACE	United States Army Corps of Engineers
USFWS	United States Fish and Wildlife Service
WARD	Water Quality Assessment and Reporting Documentation
WQD	Water Quality Division
WQPB	Water Quality Planning Bureau
WQS	Water Quality Standard

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1.0 INTRODUCTION

This document details the Montana Department of Environmental Quality's (DEQ) site-specific selenium assessment method for the Lake Koocanusa reservoir and the Kootenai River. DEQ assesses surface water quality pursuant to Title 75, Chapter 5, Part 7 of the Montana Water Quality Act (MWQA) and Section 303(d) of the federal Clean Water Act (CWA) (See Section 75-5-702, Montana Code Annotated [MCA] and 33 US Code § 1251). Water quality standards (WQS) must protect the beneficial uses of a given waterbody. The state of Montana classifies waterbodies based on their designated beneficial uses, as found in the Administrative Rules of Montana (ARM) sections 17.30.621-629. All assessment units (AUs) on Lake Koocanusa and the Kootenai River have a B-1 use classification. State law requires waters classified as B-1:

> *"Are to be maintained suitable for drinking, culinary, and food processing purposes, after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply"* (ARM 17.30.623).

This document will be used to assess the aquatic life beneficial use for all AUs within Lake Koocanusa and the Kootenai River in Montana. The most sensitive beneficial use to selenium in these waters is the growth and propagation of salmonid fishes and associated aquatic life. Selenium is a pollutant for which Total Maximum Daily Loads (TMDL) can be developed.

1.1 BACKGROUND INFORMATION

Selenium is a member of the chalcogen group (Group 16) on the periodic table of elements. Classified as a nonmetal, selenium has properties of both metals and nonmetals. It is a naturally occurring chemical element that is nutritionally essential in small amounts, but toxic at higher concentrations (EPA, 2021).

Selenium appears in the CWA Section 307(a)(1) list of toxic and priority pollutants. It bioaccumulates in tissues, predominantly through dietary exposure, with egg-laying vertebrates determined to be the most sensitive. Selenium is transferred to the eggs of adult female fish during vitellogenesis where it replaces the sulfur in proteins (Janz *et al.*, 2010). During development of Montana's site-specific selenium standards in ARM 17.30.632, fish were determined to be the most sensitive ecological endpoint in Lake Koocanusa and the Kootenai River (DEQ, 2020).

Selenium toxicity in fish manifests through reproductive impairment, via maternal transfer to eggs, resulting in embryo toxicity and teratogenicity (i.e., deformities) (Chapman *et al*., 2010). Extremely high concentrations of selenium can be lethal to adult fish, but this is not common. More frequently, fish populations are affected by chronic low dose exposure to selenium. Refer to DEQ (2020) for additional details on selenium toxicity and the toxicological exposure pathway in Lake Koocanusa and the Kootenai River.

1.2 APPLICABILITY

This assessment method applies only to Lake Koocanusa from the US-Canada international boundary to Libby Dam and the mainstem Kootenai River from the outflow below Libby Dam to the Montana-Idaho border (ARM 17.30.632).

Lake Koocanusa is a 90-mile-long transboundary reservoir in northwest Montana and southeast British Columbia (BC) (DEQ, 2020). The reservoir was formed by the impoundment of the Kootenai River

following the construction of Libby Dam in 1972. Lake Koocanusa was created under the Columbia River Treaty between the United States (US) and Canada to provide hydroelectric power and flood control (Storm *et al*., 1982). The total surface area of Lake Koocanusa at full pool within Montana's borders is 28,994 acres.

The Kootenai River is 485 miles long. It originates in southeast BC, flows across the US-Canada border via Lake Koocanusa, extends through Montana and Idaho, then returns to BC where it flows through Kootenay Lake, and terminates upon reaching the Columbia River in Castlegar, BC. The segment within Montana's state border is approximately 50 miles long and is entirely within the Northern Rockies Level III Ecoregion (Woods *et. al*, 2002).

1.3 Common Sources, Pathways, and Factors Influencing Occurrence

Selenium is a naturally occurring element found in sedimentary rocks, shales, coal and phosphate deposits, and soils (EPA, 2021); weathering of natural sources can release selenium into surface water. Selenium can also come from anthropogenic sources. Data collected by DEQ and investigations by McDonald *et. al* (2009) and Storb *et. al* (2023) suggest there are no identified significant sources of selenium (natural or anthropogenic) to Lake Koocanusa or the Kootenai River that originate in Montana.

The primary source of selenium to Lake Koocanusa and the Kootenai River is the anthropogenic release into the environment from historic and present-day mining operations in the Elk Valley of BC (DEQ, 2020). Coal in the Elk Valley belongs mainly to the Mist Mountain Formation of the Jurassic-Cretaceous Kootenay Group and is part of the East Kootenay coalfields (Grieve, 1952). Bedrock in the Elk Valley is excavated to access coal seams underneath for metallurgical steelmaking coal production. The excavation process creates a by-product called overburden (waste rock) which becomes exposed to oxidation, increasing selenium mobilization through infiltration and runoff to nearby groundwater and surface water including the Elk River in BC, a major tributary to Lake Koocanusa (DEQ, 2020).

Diet is the dominant pathway of selenium exposure for aquatic life, with the largest step in accumulation occurring at the base of the food web. Selenium is assimilated into the tissue of primary producers, then transferred to primary consumers, and then further transferred to predators (EPA, 2021; **Figure 1-1**). Selenium bioaccumulation and transfer through the food web can be influenced by the hydrology, biogeochemistry, and food web dynamics of the ecosystem (EPA, 2021).

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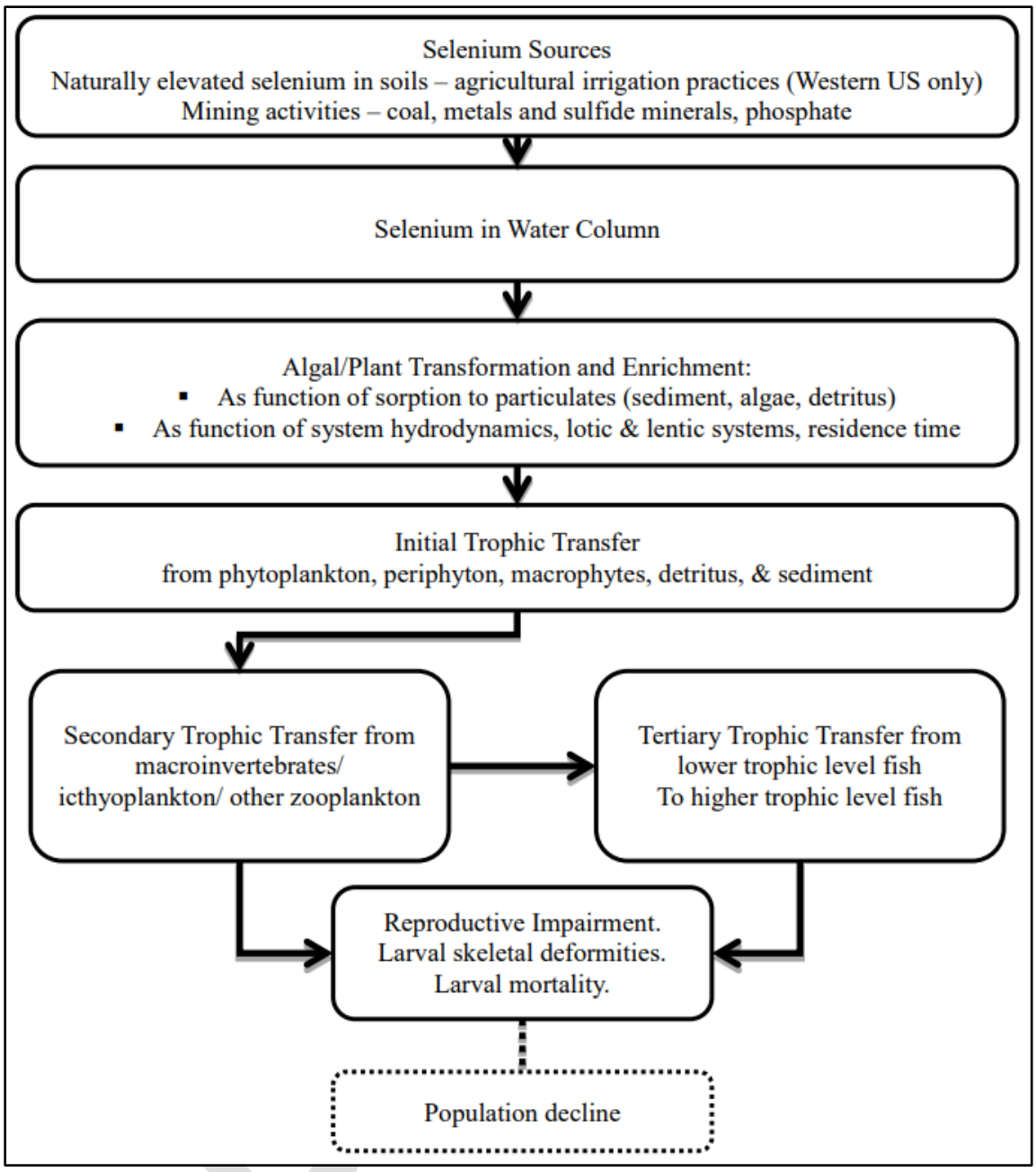


Figure 1-1. Diagram of selenium partitioning, bioaccumulation, and effects in the aquatic environment (EPA, 2021).

2.0 Lake Koocanusa and Kootenai River Selenium Water Quality Standards

Montana WQS designate the allowable concentration of pollutants, or the condition of a waterbody that will protect the beneficial uses. Beneficial uses and water quality criteria provide the framework for achieving federal CWA (40 CFR 131.2) and MWQA (75-5-102, MCA) goals and protections for Montana's water resources.

Montana is required under Section 303(c)(2)(B) of the CWA to establish water quality criteria for toxic pollutants; selenium is a toxic and priority pollutant under Section 307(a)(1) of the CWA. Montana adopted selenium standards into state law on December 25, 2020 to protect the beneficial uses of Lake Koocanusa and the Kootenai River at ARM 17.30.632. These WQS were approved by the Environmental Protection Agency (EPA) on February 25, 2021. The selenium standards for Lake Koocanusa and the Kootenai River (**Table 2-1**) are expressed as fish tissue and water column (dissolved selenium) concentrations.

Table 2-1. Selenium standards for Lake Koocanusa and the Kootenai River.

Media Type	Fish Tissue			Water Column
Criterion Element	**Egg/Ovary**	**Muscle**	**Whole Body**	**Monthly Average Exposure**
Magnitude	15.1 mg/kg dw	11.3 mg/kg dw	8.5 mg/kg dw	0.8 µg/L in Lake Koocanusa 3.1 µg/L in Kootenai River
Duration	Instantaneous measurement	Instantaneous measurement	Instantaneous measurement	30 days
Frequency	Not to be exceeded	Not to be exceeded	Not to be exceeded	Shall not be exceeded more than once in 3 years, on average

mg/kg dw = milligrams per kilogram dry weight, µg/L = micrograms per liter.

DEQ followed the 2016 EPA selenium criteria guidance presented in Appendix K to develop the site-specific chronic water column standard for Lake Koocanusa (EPA, 2016). DEQ adopted the national recommended 304(a) criteria for fish tissue in Lake Koocanusa and the Kootenai River as well as the monthly average exposure water column criteria for the Kootenai River (EPA, 2016). The water column standards are chronic values with no acute selenium standard included since the greatest toxicity risk to aquatic life is from chronic dietary exposure.

3.0 Monitoring Methods and Data Quality Considerations

Waterbody condition must be evaluated based on all existing and readily available data and information (§75-5-702, MCA; 40 CFR 130.7(b)(5)). This section describes several considerations for monitoring and assessing data quality when performing selenium assessments pursuant to this assessment method.

3.1 Parameters Required for Assessment

Data considered for assessment purposes must be either dissolved selenium water column concentrations or fish tissue selenium concentrations (egg/ovary, whole body, or muscle). Montana's numeric WQS concentrations are provided in micrograms per liter (µg/L) for water column concentrations and milligrams per kilogram dry weight (mg/kg dw) for fish tissue samples. All data should be converted to these unit conventions during assessments.

3.2 Field Monitoring Protocols

Procedures used when collecting and analyzing selenium samples vary by sample media. Water column samples are to be collected on-site in high-density polyethylene (HDPE) bottles and preserved with nitric acid (HNO_3). The samples are then stored on ice (≤6°C) until delivery to the laboratory for analysis

(Makarowski, 2020). DEQ advises recording the depth at which each water column selenium concentration sample was taken for Lake Koocanusa.

Fish tissue samples are to be collected and processed by qualified personnel with appropriate training. Close coordination with Montana Department of Fish, Wildlife, and Parks (FWP) fisheries biologists during sampling is highly recommended. Measurements and observations associated with sampling (fish species, length, and weight) should be taken in the field, or as required by a sampling and analysis plan. Detailed descriptions of fish capture methods (electrofishing, gillnetting, etc.) are beyond the scope of this assessment method.

3.3 Monitoring Locations and Spatial Independence

This section includes factors that may affect where sampling should occur, as well as factors to be considered (e.g., depths, distance between sites, zones) when evaluating spatial independence of samples. For attainment decisions, all areas of an AU are expected to meet WQS.

3.3.1 Water Column

Water column sampling considerations relevant to monitoring locations and spatial independence differ between Lake Koocanusa, a reservoir, and the Kootenai River, a large river. While any location may be sampled, it is preferred to have data that represent the entire AU. At a minimum, it is recommended that locations expected to have greater selenium concentrations be targeted.

Lake Koocanusa

Several sampling sites have been established on Lake Koocanusa for collection of long-term water quality data including the International Boundary, where selenium concentrations may be higher. Continued sampling of these established sites is encouraged to strengthen long-term data sets. It is understood that individual sampling locations may vary slightly depending on reservoir levels and other variables (Presser and Naftz, 2020). The assessor may decide to visualize and describe data by zones, but it will not affect how the data are analyzed for beneficial use assessment.

Samples should be collected from multiple depths based on DEQ's current understanding that dissolved selenium concentrations may vary by depth throughout the reservoir. Selenium concentration data collected within the epilimnion (surface), metalimnion (middle), and hypolimnion (deepest) layers should be aggregated separately by layer. Vertical profile measurements of depth and temperature that accompany selenium concentration data can be used to distinguish between these layers.

The assessor must carefully consider all data within the AU and decide if and how the data should be aggregated or segregated for statistical analysis. To maintain spatial independence, if multiple sites are located within a one-mile radius from one another and data are from the same depth layer (epilimnion, metalimnion, or hypolimnion), the assessor should combine those data into a single site.

Kootenai River

Water column samples collected from the Kootenai River will be considered spatially independent if they are collected at least one mile apart. Samples from sites closer than one mile may be considered spatially independent if there is a major tributary inflow or other substantial change between them. Only data collected from within an AU may be applied to assessment decisions for that AU. As mentioned above for Lake Koocanusa, it is recommended that well-established sampling sites be revisited to bolster long-term understanding of selenium in the system.

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3.3.2 Fish Tissue

Considerations pertaining to fish tissue monitoring locations and spatial independence are the same for Lake Koocanusa and the Kootenai River. When selecting species to sample, considerations include, but are not limited to, their sensitivity to selenium, bioaccumulation potential, and diet (**Appendix A**). Fish are mobile; therefore, it is appropriate to aggregate data from sites across an entire AU. However, if analysis shows significant differences within species at various sites, the assessor may evaluate fish tissue data by site rather than combining them across the AU. Fish tissue data must be segregated by species and tissue type within an AU. Generally, individual fish samples are preferred over composite samples to capture the full range of concentrations. However, composite samples are acceptable and are particularly appropriate for small-bodied fish. Only data from the same fish species and the same fish tissue type (egg/ovary, muscle, or whole body) are to be aggregated for comparison to the applicable standard. Additionally, to avoid survivor-bias where only surviving adult fish are sampled, if logistics allow, it is recommended that both adult and juvenile fish be sampled to deepen understanding of selenium bioaccumulation in fish in this system.

3.4 Monitoring Timeframe and Temporal Independence

This section includes factors that may affect when sampling should occur and factors to be considered (e.g., frequency, time of year, spawning periods) when evaluating temporal independence of samples.

Frequency and duration are components of all WQS. For selenium in Lake Koocanusa and the Kootenai River, these are defined in ARM 17.30.632 and differ between water column and fish tissue standards. Water column values are computed as a 30-day average (duration) not to be exceeded more than once in three years, on average (frequency). Fish tissue values are instantaneous measurements (duration) not to be exceeded (frequency) (**Table 2-0**). Fish tissue data reflects integrative accumulation of selenium over time and space in the fish population(s) at a given site (EPA, 2021); therefore, an instantaneous measurement is applied.

3.4.1 Water Column

For the purposes of this assessment method, the 30-day average concentration may be interpreted as a monthly average. The selenium WQS for Lake Koocanusa and the Kootenai River are applicable year-round; therefore, water column selenium data may be collected during any time of the year. To ensure data are collected when selenium concentrations may be greatest, and to strengthen an overall understanding of selenium in this system, it is recommended that monitoring efforts include data collection year-round.

While the magnitude of water column selenium standards differs between Lake Koocanusa (0.8 µg/L) and the Kootenai River (3.1 µg/L), the duration and frequency are calculated the same. The 30-day average will be calculated per site and depth for Lake Koocanusa, and per site for the Kootenai River using discrete samples collected during a single month. If the assessor receives high quality data reported as a daily or weekly composite for which there is no record of the individual samples comprising the composite, then the assessor will use the reported composite value as part of the calculation for the 30-day average. If individual samples are collected at the same site and depth for Lake Koocanusa, or at the same site for the Kootenai River, then each sample should be treated as an individual discrete sample to be included in the 30-day average calculation. This approach includes the full range of concentrations recorded.

To determine when to sample Lake Koocanusa or the Kootenai River, it may be important to consider how each waterbody is managed. Lake Koocanusa is managed for hydroelectric power and flood control. Water levels in the reservoir are lowest in late winter and early spring (i.e., February through April) and highest in summer and early fall (i.e., August through October) (USFWS, 2020). Kootenai River flows are managed by the United States Army Corps of Engineers (USACE) Libby Dam operations. The release of water from Libby Dam into the Kootenai River considers spring white sturgeon spawning and fish migration while mitigating floods and generating power (USFWS, 2020). Kootenai River flows are variable and do not follow a traditional hydrograph. Higher flows may occur in the spring (i.e., May-June) and in the late fall (i.e., November-December). However, Kootenai River flows do not always follow this pattern and they are not consistent from year to year because flows are dependent on annual water supply forecasts and Variable-Flow Flood Control, in conjunction with management goals for Lake Koocanusa (USFWS, 2020). To capture when concentrations may be greatest in Lake Koocanusa, sampling efforts may consider monitoring both during spring freshet when selenium loading is expected to be higher and during the winter/early spring when reservoir levels are the lowest. If possible, sampling year-round for both waterbodies is recommended.

3.4.2 Fish Tissue: Egg/ovary

The type of fish tissue (egg/ovary, whole body, or muscle) targeted for collection will depend on the time of year and the species being sampled. Egg/ovary concentrations have the greatest correlation to reproductive harm in fish, and therefore are the preferred fish tissue to be sampled.

DEQ uses a table prepared by FWP that identifies appropriate egg/ovary sampling times based on spawning periods for fish species known to inhabit Lake Koocanusa and/or the Kootenai River (**Appendix B**). Water temperature is a key factor affecting fish spawning times (Hoffman *et al*., 2002); therefore, there may be instances when fish spawn outside of the periods defined in **Appendix B**. Egg/ovary data from fish sampled outside of the spawning period shown in **Appendix B** may be considered appropriate to use upon the evaluation and direction of FWP fisheries biologists. Egg/ovary tissue data from across the spawning period as defined in **Appendix B** may be aggregated.

3.4.3 Fish Tissue: Whole Body or Muscle

For selenium assessments, egg/ovary is the preferred fish tissue to be sampled. However, whole body and muscle tissues may be sampled as an alternative if reasonable attempts to obtain egg/ovary data are unsuccessful. Selenium concentrations in these tissues will provide representative information on selenium bioaccumulation and ecological exposure at almost any time of the year (except during, and/or immediately before or after, spawning periods for females). It is expected that selenium concentrations may vary across species (EPA, 2021). Recognizing the logistical challenges associated with fish tissue sample collection it is recommended, but not required, to use male fish for muscle samples because their selenium levels are more stable than female fish. Additionally, female fish should not be sampled during, and/or immediately before or after, their respective species' spawning period. Whole body or muscle tissue data from across the same calendar year of collection may be aggregated.

3.5 Minimum Data Requirements

This section describes minimum data requirements that are necessary to perform statistical analyses for making selenium impairment decisions for the specific waters this method pertains to. **Sections 3.5.1 and 3.5.2** outline the data requirements for listing an AU as impaired, and **Section 3.5.3** outlines the requirements for delisting an AU. **Table 3-1** summarizes all data types and their respective minimum and preferred data requirements.

Table 3-1. Minimum and preferred data requirements.

Media Type	Data Type	Allowable Exceedance Frequency	Required Minimum # of samples	Preferred # of samples
Water column	30-day averages, within 3-year period	Shall not be exceeded more than once in 3 years, on average	2	≥ 4
	Samples per 30-day average, spaced one week apart	N/A	1	≥ 2
Fish Tissue	5 individual fish **or** a composite of 5 fish	Not to be exceeded	1	≥ 4

3.5.1 Water Column

Minimum requirements for selenium water column data are provided to guide assessors when performing quality assessments of readily available data. For Lake Koocanusa and the Kootenai River, at least two 30-day averages are required per three-year period to make WQS attainment decisions. These 30-day averages may be from the same year or separate years. At least one 30-day average per calendar year is recommended. When planning for a monitoring project to address this assessment method, additional 30-day periods of data are strongly encouraged.

It is recommended that each 30-day average should include at least two samples spaced one week apart. However, if a site is sampled only once per month, that data can be used to represent a 30-day average in attainment decisions. Further considerations for calculating water column 30-day averages are included in **Section 4.3**.

3.5.2 Fish Tissue

The species list in **Appendix A** is to be used as a guide to assist fish tissue sampling. However, the WQS were developed to protect all fish species. Therefore, any fish species data can be used for WQS attainment decisions as long as the minimum data requirements are met. Fish tissue concentrations for multiple species of fish may be determined and considered when making assessment determinations. An AU can be listed as impaired if one species shows an exceedance.

Fish tissue sample results shall be reported as a single value representing an average of individual fish samples or a composite sample, each option requiring a minimum number of five individuals from the same species (ARM 17.30.632). One composite sample (comprising at least five individuals) meets the minimum data requirements for this assessment method.

All fish samples used during assessments must meet the following specifications:

- All samples that are aggregated or composited to represent an average result must be of the same fish species and tissue type combination.
- All samples must be from the same AU.
- All aggregated egg/ovary samples must be collected within the same spawning period as outlined in **Section 3.4.2**.
- All aggregated muscle and whole body samples must be collected within the same year as outlined in **Section 3.4.3**.

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3.5.3 Delisting

Due to the bioaccumulative and persistent properties of selenium in aquatic systems, DEQ will delist an impaired AU after three consecutive years of fish tissue and water column data show zero exceedances of the corresponding WQS.

If any AU is listed because of exceedances in a specific species' fish tissue, those same species and tissue types must be targeted during the delisting process. Whichever fish species and tissue types were initially used to list the waterbody as impaired must show zero exceedances for three consecutive years, in addition to all other species and tissue types. Yet, all applicable readily available fish tissue results must be used for all listing decisions and must meet standards.

3.6 DATA QUALITY

Data must meet quality requirements to be included in the attainment decision-making process. DEQ's quality assurance/quality control (QA/QC) protocols are provided in the Beneficial Use Assessment Method (Makarowski, 2020 - Sections 5.0 and 6.0) and the Quality Assurance Project Plan (QAPP) for Water Quality Planning Bureau (WQPB) Environmental Data Operations (DEQ, 2022 - Section 2.0). These documents also include information regarding the programmatic approach to assessments, approved monitoring methods, and data quality objectives aimed at protecting and improving water quality.

Many other organizations also collect water quality data and information which may be useful for DEQ's selenium assessments. DEQ will accept credible data related to the current condition of Lake Koocanusa and the Kootenai River from external entities in accordance with the guidelines from Section 5.0 of the Beneficial Use Assessment Method (Makarowski, 2020). Data quality requirements apply to all data incorporated while making assessment decisions, whether collected internally (by DEQ) or externally. Data that do not meet DEQ's data quality objectives will not be included in the assessment but may be used to supplement the assessment determination (Makarowski, 2020).

3.7 DATA QUALITY ASSESSMENT OVERVIEW

Data quality assessment (DQA) is the scientific and statistical evaluation of data to determine whether data obtained from monitoring operations are of the right type, quality, and quantity to support water quality assessments (EPA, 2002). Assessors use DEQ's Water Quality Assessment and Reporting Documentation (WARD) system to document the DQA outcomes (pass or fail) for each parameter group being assessed per beneficial use. All data quality indicators must be met to pass the DQA; if a single indicator is not met, the DQA fails for that parameter group. An assessor may override-pass or override-fail a DQA, but they must accompany this override with adequate justification.

Additional data quality screening may be necessary before the data set is ready to support attainment decisions (EPA, 2002), for example:

- Handling non-detects
- Evaluating database flags
- Evaluating QC samples (e.g., field duplicates and field blanks)
- Verifying holding time and incubation times were adhered to
- Reviewing QA/QC control reports
- Investigating errors in collection or analysis
- Addressing missing data
- Reviewing deviations from project documents

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Once DEQ determines the data meet basic documentation requirements, the data are ready to be analyzed to support WQS attainment decisions (EPA, 2002).

3.8 DATA CURRENCY

DEQ requires that data used in making assessment decisions be representative of current conditions; typically, this means data must be less than 10 years old (Makarowski, 2020). If at any time significant changes in sources are documented, the assessor should use best professional judgement (BPJ) to determine which data are appropriate to include in an assessment. The assessor will document the specific changes and identify data that best represent current conditions for use in the selenium aquatic life beneficial use assessment.

4.0 DATA ANALYSIS TO SUPPORT WATER QUALITY STANDARDS ATTAINMENT DECISIONS

This section describes how the data will be prepared and analyzed for assessment. DEQ's preferred approach for completing assessments is to incorporate both water column and multiple fish tissue data types. However, it is possible to complete assessments with only one existing and readily available data type. Because the water column standards are derived from modeling selenium bioaccumulation in fish tissue and reflect criteria that protect the aquatic life beneficial use (ARM 17.30.632), either data type is acceptable to complete assessments.

4.1 ASSESSMENT PROCEDURE

The assessment procedure is the same for both steady state and non-steady state conditions. The procedure is as follows:

1. For each AU, gather all current and readily available data.
2. Perform data quality assessments to identify the usable data set.
    a. Note all data excluded from the assessment and justify the exclusion. Document this in each AU's assessment record.
3. For each AU, organize fish tissue data first by year or spawning period (fish tissue only).
    a. Sort fish tissue data by type (egg/ovary, muscle, whole body).
    b. Sort each tissue type by year for muscle and whole body tissues and spawning season for egg/ovary.
    c. Sort fish tissue data types by species.
4. Determine if minimum data requirements (five individual fish) are met for each species.
    a. Calculate the average tissue concentration for each species according to **Section 4.2**.
        i. If composite samples are present in the data set, use weighted averages to calculate fish tissue averages.
5. Compare fish tissue data to the WQS and document any exceedances.
    a. If there are any fish tissue exceedances from an acceptable data set (five or more individual fish), document them in each AU's assessment record.
6. Sort water column data by site, and depth if applicable.
7. Sort water column data by month.
8. Determine if minimum data requirements for calculating 30-day water column averages are met for each month.

   a. Calculate the 30-day average(s) according to **Section 4.3**.
9. Compare monthly average(s) to the WQS and document any exceedances.
   a. If there are any 30-day average exceedances at a site, document them and carry this information forward into each AU's documentation.
10. Proceed to **Section 4.4** and interpret all findings into a decision.

4.2 Calculating Fish Tissue Concentrations

Fish tissue sample results shall be reported as a single value representing an average of individual fish samples or a composite sample (ARM 17.30.632). Only data from the same fish species and tissue type combinations will be averaged during assessments. Egg/ovary averages should only include data collected during each species' spawning period in the same calendar year (**Appendix B**). Muscle and whole body averages should only include data collected in the same calendar year.

4.3 Calculating Water Column 30-Day Averages

Preparing water column data for assessments is largely the same between Lake Koocanusa and the Kootenai River. The exception is where depth measurements exist for Lake Koocanusa. Samples collected at the same site and depth layer (epilimnion, metalimnion, or hypolimnion) can be averaged if they were collected within the same month. Depth layers per site should be averaged only with samples from the same depth layer. All depth layers of Lake Koocanusa must meet the WQS and support beneficial uses.

Each water column data set that meets minimum data requirements (**Section 3.5**) should go through a process to derive a 30-day average. Following this process for each month of available data ensures that all collection timeframes are represented equally, and no timeframe has stronger representation than another. The calculation process is as follows:

1. All data from an AU should be compiled and sorted by site, date and time, and by depth layers (Lake Koocanusa only).
2. Only one result is required to produce a 30-day average. If monthly data requirements are met, proceed to **Step 3**.
3. To derive a 30-day average, all results are averaged per calendar month. The 30-day average can then be compared against the numeric standard and used for the decision process provided in **Section 4.4**.

4.4 Impairment Listing and Delisting

Before determining impairment listings, the assessor must review the current WQS in ARM 17.30.632 to verify if the waterbody being assessed is in steady state or non-steady state. The minimum data requirements described in **Section 3.5** must also be met to make an assessment decision to "list" or "delist" an AU. Once the data have been evaluated against the numeric WQS for selenium through the assessment process, an attainment decision can be made. When assessment confirms that the AU-cause combination is an impairment that is not currently listed, the AU-cause will be "listed" in Montana's impaired waters list. Once listed, AU-cause impairments will remain on the list of impaired waters unless and until a subsequent impairment indicates delisting is appropriate. When assessment confirms that an AU is attaining WQS for selenium, the assessment decision is either "do not list" if the AU-cause combination is not already listed, or "delist" if the AU-cause combination was listed previously.

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When the aquatic ecosystem is in steady state and selenium data are available for both fish tissue and the water column, the fish tissue standards supersede the water column standard. "Steady state" means, for the purposes of ARM 17.30.632:

> *"Conditions whereby there are no activities resulting in new, increasing, or changing selenium loads to the lake or river aquatic ecosystem, and selenium concentrations in fish living in the aquatic ecosystem have stabilized*" (ARM 17.30.602.32).

Additionally, when in steady state the fish tissue standards have a hierarchy of importance; for a given species the egg/ovary standard takes precedence over the whole body and/or muscle standards.

When the aquatic ecosystem is in non-steady state, both the fish tissue and water column standards apply (ARM 17.30.632). Moreover, for any individual species, exceedance of the WQS for any tissue type indicates impairment.

Tables 4-1, 4-2, and 4-3 provide the framework for attainment decision-making when listing and/or delisting an AU in both steady state and non-steady state conditions. The columns and rows that display the WQS being attained or exceeded assume all minimum data requirements outlined in **Section 3.5** have been met. "Insufficient Data" indicates that minimum data requirements have not been met.

Table 4-1. Decision-making chart for listing when the system is in steady state.

Steady State		**Water Column**		
		No Exceedance	**Exceeded**	**Insufficient Data**
Fish Tissue	**No Exceedance of any tissue**	Not Impaired	Not Impaired	Not Impaired
	E/O Available and Not Exceeded	Not Impaired	Not Impaired	Not Impaired
	E/O Available and Exceeded	Impaired	Impaired	Impaired
	E/O Unavailable, WB or M Exceeded	Impaired	Impaired	Impaired
	Insufficient Data	Not Impaired	Impaired	Insufficient Information

E/O = egg/ovary, WB = whole body, M = muscle.

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Table 4-2. Decision-making chart for listing when the system is in non-steady state.

Non-Steady State		Water Column		
		No Exceedance	Exceeded	Insufficient Data
Fish Tissue	No Exceedance	Not Impaired	Impaired	Not Impaired
	Exceeded	Impaired	Impaired	Impaired
	Insufficient Data	Not Impaired	Impaired	Insufficient Information

Fish tissue includes egg/ovary, whole body, and muscle tissue.

Table 4-3. Decision-making chart for delisting after an AU has been declared impaired.

Steady State or Non-Steady State		Water Column		
		No Exceedance in Three Years	Any Exceedance in Three Years	Insufficient Data
Fish Tissue	No Exceedance in Three Years	Delist	Keep as Listed	Keep as Listed
	Any Exceedance in Three Years	Keep as Listed	Keep as Listed	Keep as Listed
	Insufficient Data	Keep as Listed	Keep as Listed	Keep as Listed

Fish tissue includes egg/ovary, whole body, and muscle tissue.

4.5 Documenting Assessment Decisions and Review

The assessor must document all data and decisions pertaining to selenium impairment and aquatic life use support determinations. Assessment outcomes for individual AUs, including data summaries, impairment decisions, and beneficial use support determinations are documented by assessors using Montana DEQ's WARD system and Clean Water Act Information Center (CWAIC). Assessment decisions are reviewed by the Monitoring and Assessment Section Supervisor and may be reviewed by DEQ's Water Quality Division (WQD) Quality Assurance Manager and managers or staff from other DEQ programs. Impaired waterbodies are included in Montana's biennial Water Quality Integrated Report and list of impaired waters. A public comment period is provided for each of Montana's Integrated Reports.

5.0 Threatened Status

Threatened waters (75-5-103, MCA) are AUs for which sufficient data and information exist to determine that designated uses are being attained, but non-attainment is predicted within the next two Integrated Report cycles (approximately four years). This type of listing should include trend analysis and could also include modeling of sources over time. Waterbody-pollutant combinations that are threatened are reported as causes of impairment, they appear on the 303(d) list of pollutant-impaired waters in need of a TMDL, and the associated use is considered not fully supported (Makarowski, 2020).

If none of the water column or fish tissue standards are exceeded in Lake Koocanusa or the Kootenai River AUs during assessment, then DEQ will reevaluate the data and observable trends in the system. Trends that show imminent exceedances in the near future will trigger a "Threatened" listing for the given AU. Source analysis will also help DEQ accurately define the sources of the potential impairments identified in each waterbody.

6.0 Probable Sources

Probable sources of impairment are the activities, facilities, or conditions that generate the pollutants that prevent AUs from meeting WQS. Two major anthropogenic activities are known to cause increased selenium mobilization and introduction into aquatic systems:

1) mining of metals, minerals, and refinement and use of fossil fuels, and
2) irrigation of selenium-rich soils (EPA, 2021).

Additional source selections are available in the WARD system if needed. BPJ will be used to determine any other significant sources on a site-by-site basis. If water quality data are available that suggest a probable source is likely contributing to impairment, the assessor should check the "Source Confirmed" box in WARD. If probable sources are present in the watershed but are not confirmed, the assessor should check the "Source Not Confirmed" box. If source data exist, they should be incorporated into the data analysis and data matrix within WARD. The assessor may also include a brief description of sources in the overall condition of the waterbody summary in WARD.

7.0 Assessment Documentation

Selenium data collected by DEQ are stored in the Montana Environmental Quality Information System (EQuIS) Water Quality Exchange (MT-eWQX) database and are uploaded regularly to the Water Quality Portal. Assessment outcomes for individual AUs, including data summaries, impairment decisions, and beneficial use support determinations, are documented via DEQ's CWAIC (available at https://clean-water-act-information-center-mtdeq.hub.arcgis.com).

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APPENDIX A – RECOMMENDED TARGET FISH SPECIES FOR MONITORING

This table is intended as a guide for monitoring design. It includes fish species in Lake Koocanusa and the Kootenai River that have characteristics that make them desirable for monitoring efforts. However, the selenium WQS outlined in **Section 2.0** apply to all fish species. Therefore, data from any fish species must be included in assessments. This table was developed in consultation with MT FWP biologists and using information described in EPA (2021).

Species	Location		Native	Game Species	Known sensitivity to selenium	Known selenium bio-accumulator	Diet
	Lake Koocanusa	Kootenai River					
Rainbow Trout *Oncorhynchus mykiss*	X	X		X	X		Insectivore, piscivore
Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi*	X	X	X	X	X		Insectivore
Peamouth *Mylocheilus caurinus*	X	X	X			X	Insectivore
Redside Shiner *Richardsonius balteatus*	X	X	X			X	Insectivore
Mountain Whitefish *Prosopium williamsoni*	X	X	X	X		X	Insectivore
Northern Pikeminnow *Ptychocheilus oregonensis*	X	X	X				Insectivore (juvenile); piscivore (adult)
Longnose Sucker *Catostomus catostomus*	X	X	X				Benthic algae, Insectivore
Largescale Sucker *Catostomus macrocheilus*	X	X	X				Benthic algae, Insectivore
Kokanee Salmon *Oncorhynchus nerka*	X	X		X			Planktivore
Brown Trout *Salmo trutta*	X	X		X	X		Insectivore, piscivore
Burbot *Lota lota*	X	X	X	X			Planktivore (juvenile); piscivore (adult)

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Species	Location		Native	Game Species	Known sensitivity to selenium	Known selenium bio-accumulator	Diet
	Lake Koocanusa	Kootenai River					
Columbia Slimy Sculpin *Cottus cognatus*		X	X				Insectivore, piscivore
Rocky Mountain Sculpin *Cottus bondi*		X		X			Insectivore
Torrent Sculpin *Cottus rhotheus*		X	X				Planktivore (juvenile); insectivore, piscivore (adult)
White Sturgeon * *Acipenser transmontanus*		X	X	X	X		Detritivore, molluscivore, insectivore, piscivore
Bull Trout * *Salvelinus confluentus*	X	X	X	X	X		Insectivore (juvenile); piscivore (adult)

*Species listed under the Endangered Species Act (1973) require special authorization to catch and/or handle. https://ecos.fws.gov/ecp/report/species-listings-by-state?stateAbbrev=MT&stateName=Montana&statusCategory=Listed

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APPENDIX B – SPAWNING TIMES OF FISH IN LAKE KOOCANUSA AND/OR THE KOOTENAI RIVER

The following table lists the known or expected spawning times for fish in Lake Koocanusa and/or the Kootenai River. It was modified by Jim Dunnigan (FWP Fisheries Biologist) from Skaar (2001) and the Montana Field Guide (https://fieldguide.mt.gov) to display accurate spawning periods for species known to inhabit Lake Koocanusa and/or the Kootenai River.

The code for the table is as follows: **J1, J2, F1, F2,** etc. refer to half month increments (e.g., **J1** = January 1-15, **J2** = January 16-31, **F1** = February 1-14, **F2** = February 15-29, etc.). **S** refers to the spawning period for each species. Spawn timing was based either on area specific observations in the Kootenai drainage (K), statewide periods identified by Skaar (S), or the Montana Field Guide (FG).

Species	J1	J2	F1	F2	M1	M2	A1	A2	M1	M2	J1	J2	J1	J2	A1	A2	S1	S2	O1	O2	N1	N2	D1	D2
White Sturgeon [K]									S	S	S	S	S											
Mtn. Whitefish [K]																		S	S	S	S	S		
Kokanee Salmon [K]																		S	S	S				
Cutthroat Trout [K]							S	S	S	S	S	S	S											
Rainbow Trout [K]					S	S	S	S	S	S	S	S												
Brook Trout [K]																	S	S	S	S				
Bull Trout [K]																	S	S	S					
Lake Trout [S]																			S	S	S	S	S	
Brown Trout [K]																			S	S	S	S	S	S
Redband Trout [K]										S	S	S	S											
Northern Pike [S]					S	S	S	S	S															
Peamouth [K]										S	S	S												
N. Pikeminnow [K]									S	S	S	S	S											
Longnose Dace [S]									S	S	S	S	S	S	S									
Redside Shiner [K]										S	S	S	S	S										

Species	J1	J2	F1	F2	M1	M2	A1	A2	M1	M2	J1	J2	J1	J2	A1	A2	S1	S2	O1	O2	N1	N2	D1	D2
Longnose Sucker [S]						S	S	S	S	S	S	S	S											
Largescale Sucker [K]							S	S	S	S														
Black Bullhead [S]									S	S	S	S	S											
Burbot [S]			S	S	S	S																		
Pumpkinseed [S]										S	S													
Smallmouth Bass [S]									S	S	S	S												
Largemouth Bass [S]									S	S	S	S	S											
Black Crappie [S]									S	S	S	S												
Yellow Perch [K]						S	S	S	S	S	S													
Torrent Sculpin [FG]									S	S	S	S												
Columbia Slimy Sculpin [FG]									S	S	S	S												
Rocky Mountain Sculpin [FG]									S	S	S	S												

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Appendix P

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Original Article

Hazard/Risk Assessment

Relationships in selenium concentrations among fish tissues to support selenium assessments and regulations

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Abstract

Selenium (Se) toxicity to fish is primarily manifested via maternal transfer to the eggs. Several regulatory jurisdictions have adopted Se criteria or guidelines expressed as the Se concentration in fish eggs (typically expressed as egg/ovary Se concentrations, thereby suggesting these are equivalent). Because it is not always feasible to sample fish eggs or ripe ovaries at a site, it can be necessary to sample muscle or whole-body (WB) tissue. This study evaluated whether there were consistent relationships in tissue Se concentration within and across fish species. Additionally, based on findings in a companion study, we evaluated whether ovary Se concentrations are inversely related to oocyte development in a wide range of fish species. Consistent with the companion study, the spawning status of the wide range of fish species had a significant influence on Se concentrations, with Se concentrations in eggs and ripe ovaries typically lower and less variable than those in unripe ovaries. Considering Se data for eggs|ripe ovaries, there was a consistent relationship with muscle or WB Se over a wide range of fish species. Additionally, for a majority of fish species, the relationships between egg|ripe ovary Se concentrations versus muscle and WB Se concentrations had slopes significantly different than 1, indicating that single-tissue Se ratios are not applicable over a broad range of muscle or WB Se concentrations. Considering these findings, we recommend future studies clearly report whether eggs or ovaries were sampled and provide other diagnostic measures of reproductive status such as the gonado-somatic index. We also conclude direct comparison of Se concentrations from unripe ovaries to egg Se thresholds is not appropriate.

Keywords: selenium, bioaccumulation, ecological risk assessment, regulatory guidelines, fish tissue

Introduction

Several regulatory jurisdictions in North America have adopted fish tissue-based selenium (Se) criteria or guidelines. The United States Environmental Protection Agency (USEPA), for example, recommends Se criteria of 15.1 mg/kg dry weight for fish eggs|ovaries, 11.3 mg/kg dry weight for fish muscle, and 8.5 mg/kg dry weight for whole-body (WB) fish tissue (USEPA, 2016, 2021). Several U.S. states have adopted these or comparable criteria into their water quality standards. Similarly, the Canadian federal environmental quality guidelines for Se are 14.7 mg/kg dry weight for fish eggs|ovaries, and 6.7 mg/kg dry weight for WB fish tissue (Environment and Climate Change Canada [ECCC], 2022).

Selenium toxicity to fish primarily results from maternal transfer of Se to the eggs, which can result in larval mortality, deformities, or edema in developing larvae as the yolk sac is absorbed (Janz et al., 2010). As such, Se concentrations in eggs, or by association ripe ovaries, are considered the most reliable indicator of potential Se toxicity to fish (ECCC, 2022; Janz et al., 2010; USEPA, 2016). However, sampling of fish eggs or ripe ovaries can be logistically challenging, because it can be difficult to locate and catch females in spawning condition or it may not be possible to obtain sufficient sample mass for reliable Se analyses. Further, unless eggs can be readily expressed, sampling of eggs or ripe ovaries is not always an option when there is a desire for nonlethal sampling or to coordinate sample collection with fish consumption monitoring when assessing human health risks. For these reasons, regulatory jurisdictions in the United States and Canada also developed Se criteria and guidelines based on muscle and/or WB Se tissue.

There is greater uncertainty when the muscle or WB Se criteria are implemented over the egg|ovary Se criterion, because these tissues provide an indirect measure of exposure relative to the most sensitive endpoints based on maternal transfer to eggs. This is why the USEPA's Se criterion for eggs|ovaries supersedes its recommended muscle and WB Se criteria. Although expressed as an "egg|ovary" Se criterion, it is inferred that the ovary be ripe because the criterion was derived from maternal transfer toxicity

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studies in which Se concentrations were measured in eggs or ripe ovaries. In Appendix K of USEPA's ambient water quality criteria document, it is stated that "ripe or gravid" females need to be collected from a site for comparison to the egg/ovary Se criterion (USEPA, 2016; 2021). Further, the USEPA's technical support document for fish tissue monitoring states that ovaries should be ripe, and that unripe or developing ovaries should not be used for implementing the egg/ovary Se criterion (USEPA, 2024a).

Understanding Se concentration relationships among tissues is important for implementing the USEPA's muscle and WB criteria versus the egg/ovary criterion and managing the associated uncertainty. If we assume that the egg/ovary, muscle, and WB Se criteria are applicable to all fish species, it will not matter which tissue is analyzed for determining compliance with the Se criteria. For this hypothetical assumption, the egg-to-muscle Se concentration ratio would be 1.3 (egg|ovary Se criterion divided by the muscle Se criterion), and the egg-to-WB Se concentration ratio would be 1.8 (egg|ovary Se criterion divided by the WB Se criterion). However, these ratios do not apply to all fish species. If a fish species has an average egg-to-muscle Se concentration ratio >1.3, that species could be in compliance with the muscle Se criterion but not the egg/ovary Se criterion. Conversely, if a fish species has an average egg-to-muscle Se concentration ratio <1.3, that fish species could be out of compliance with the muscle Se criterion but in compliance with the more relevant egg/ovary Se criterion.

Understanding Se concentration relationships among tissues is also important for developing site-specific Se bioaccumulation models to support site-specific Se water quality criteria or site-specific assessments. These site-specific Se bioaccumulation models are often based on WB Se concentration data, either because WB Se data are more readily available for the water body of interest or because the model was developed from literature-based trophic transfer factors that are based on WB Se concentrations (Mendes et al., 2025; Presser & Naftz, 2020; USEPA, 2024a, 2024b). Because these models are typically based on WB Se concentrations, or relationships between WB Se and muscle Se, that are then applied to an egg/ovary criterion, one or more tissue conversion factors are usually required.

The objective of this study is to develop between-tissue Se concentration relationships that can support regulatory and site-specific Se assessment needs. In part, this study represents an update to tissue Se relationships previously compiled by the USEPA, which were used to support development of updated Se criteria (USEPA, 2016). In addition to considering additional datasets that may not have been available to the USEPA at that time, this study is a companion to Brix et al. (2025), which demonstrates that oocyte development stage has an important influence on the magnitude of ovary Se concentrations, using four fish species as an example: northern pikeminnow (*Ptychocheilus oregonensis*), mountain whitefish (*Prosopium williamsoni*), peamouth chub (*Mylocheilus caurinus*), and redside shiner (*Richardsonius balteatus*). Based on the results of that study, the present study provides a meta-analysis to evaluate whether similar observations on Se concentrations relative to oocyte development apply to other fish species, and then to determine the implications of the relationship between oocyte development and Se concentration in developing Se relationships in eggs and ovaries versus muscle and WB tissue. This study also evaluates relationships between Se concentrations in muscle and WB tissue.

The first step of the study was to compile a database of Se concentrations for eggs, ovaries, muscle, and WB tissue for a suite of fish species representing several families and orders. This database was then used to test the following hypotheses: (1) Ovary Se concentrations are inversely related to oocyte development as characterized by gonado-somatic index (GSI) or qualitative descriptions of spawning status; (2) Variability in Se concentration relationships between ovaries and muscle or WB tissue among fish species is reduced when only data for eggs or ovaries of spawning females are considered; and (3) Tissue Se concentration relationships observed across fish species are influenced by phylogenetic relationships.

Methods

Terminology

In compiling data for this study, it became apparent that the terms "eggs" and "ovaries" were sometimes used interchangeably by study authors or in monitoring reports and databases. In the present study, we use the term "eggs" when eggs were clearly sampled and "ovaries" for all other samples of female reproductive tissue. If a study noted that the fish sampled was of spawning status or that the ovary was otherwise ripe, we refer to the sample as "ripe ovaries." If the ovary sample was noted as unripe or if no information on oocyte development was provided, then we refer to the sample simply as "ovaries."

Fish tissue Se database

Fish tissue Se data were obtained by directly contacting potential data holders (e.g., mine operators with Se monitoring programs), querying publicly available online databases, and through searches of peer-reviewed and grey literature. A standard data template was developed that included fields for fish species, tissue sampled, and the water body from which the fish was collected, as well as any available measurements from the sample such as the length and mass of the sampled fish, and the GSI, which can be used to infer oocyte ripeness (Brix et al., 2025; McGarvey et al., 2021). Lastly, when available, qualitative descriptions of spawning condition of the fish were tabulated. If Se tissue concentrations were reported on a wet weight basis (less than 0.01% of the samples), they were converted to dry weight concentrations based on an assumed moisture content of 61% for rainbow trout and brook trout eggs, and 80% and 76% for centrarchid muscle and ovaries, respectively (USEPA, 2016). Data were excluded from the study if there were fewer than two tissue types reported per sample out of egg, ovary, muscle, and WB, or if there were fewer than five samples for a single species. No effort was made to independently review the analytical data quality from these studies; all data were assumed to be of adequate quality.

Hypothesis 1: Ovary Se concentrations are inversely related to oocyte development

In the companion to this study, Brix et al. (2025) observed that in northern pikeminnow and mountain whitefish, two species with synchronous oocyte development, an inverse relationship between ovary Se concentrations and GSI was observed (R^2 = 0.72–0.82). They also observed in redside shiner, a species with asynchronous oocyte development, a relationship between Se concentrations in expressible eggs and the remaining ovary, with egg concentrations on average 59% of those observed in the ovary. In a fourth species, peamouth chub, where it is unclear whether oocyte development is synchronous or asynchronous, a weak relationship (R^2 = 0.08) between ovary Se and GSI was observed. To evaluate whether egg|ovary Se concentrations decrease with increasing egg|ovary ripeness for other fish species, similar analyses based either on GSI data or qualitative measures of egg|ovary ripeness were conducted. Because quantitative GSI data or qualitative information on

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egg|ovary ripeness were not always reported, this evaluation was completed on a subset of species.

For species with GSI data, trends and relationships between tissue Se concentration ratios (e.g., egg|ovary-to-muscle) and GSI were tested using correlation and ordinary least squares (OLS) regression. Because fish samples were potentially collected from a range of Se exposure conditions, the egg|ovary Se concentrations were normalized to corresponding muscle Se concentrations based on the assumption that muscle Se concentrations more directly reflect Se exposure and are less influenced by the spawning status of the fish. Although muscle Se concentrations may theoretically be reduced as Se is mobilized during vitellogenesis, changes in muscle Se concentrations are less responsive to changes in spawning status than ovary Se concentrations (see additional discussion in online supplementary material S1). Correlation and OLS regression were performed for all species that had paired tissue-concentration ratios and GSI measurements for at least 10 samples, and a minimum GSI range of at least fivefold (11 species). These minimum sample size and GSI ranges were selected so that any conclusions regarding the presence or absence of statistically significant relationships were based on adequate data. These minimum sample size and GSI ranges were applied to support this meta-analysis of a suite of fish species; alternative minimum ranges may be appropriate for specific species in support of focused assessments.

For fish species with qualitative data on egg|ovary ripeness, we evaluated tissue Se concentrations for ripe females compared with other pre-spawning categories, which were grouped into a single "unripe" category. Eight species had datasets with Se concentrations for ripe/gravid females and at least one other spawning status. For each of these eight species, egg|ovary Se concentrations in ripe versus unripe females were compared using two methods: nonparametric analysis of covariance (ANCOVA) and nonparametric multiple contrasts. The nonparametric ANCOVA compared egg|ovary Se in the two groups using muscle Se concentrations as a covariate that represented Se exposure. This method was conducted in the R software environment (R Core Team, 2024) using the "fANCOVA" package (Wang, 2020) with significance evaluated using the ANOVA-type statistic ($\alpha = 0.05$; Dette & Neumeyer, 2001). This method is the more robust of the two methods; however, the number of species analyzed using nonparametric ANCOVA was reduced from eight to four as the method required that species-specific datasets have similar range and overlap of muscle Se concentrations in both the ripe and unripe categories. Nonparametric multiple contrasts were performed as a secondary analysis to corroborate the ANCOVA and test the remaining four species. Therefore, for all eight species, egg|ovary-to-muscle Se concentration ratios were compared in the ripe versus unripe spawning statuses within each species using nonparametric multiple contrast tests with simultaneous confidence intervals and the log odds asymptotic approximation method (Noguchi et al., 2020; familywise error rate = 0.05).

Hypothesis 2: Variability in Se concentration relationships between ovaries and muscle or WB tissue is reduced when only data for eggs or ovaries of spawning females are considered

Linear regression was used to compare relationships between egg|ovary Se and muscle or WB Se. All available species-specific datasets were first reduced to only samples qualitatively described as "ripe females." Individual species were first evaluated using both OLS and total least squares (TLS) regression to assess the presence of a linear relationship between egg|ripe ovary Se and muscle or WB Se. Due to concerns that a narrow concentration range in the predictive tissue (i.e., muscle or WB) for a species could interfere with the ability to determine the presence or nature of the relationship with egg|ripe ovary Se, species with a concentration range <10 mg/kg dry weight in the predictive tissue were omitted if the predictive R^2 was <0.35. Species with a significant slope and a linear relationship (qualitatively assessed using residual diagnostic plots) were included in the following multiple linear regression (MLR) models:

$$\log(\text{Egg|Ripe Ovary Se}) = \log(\text{Muscle Se}) + \text{Species} + \log(\text{Muscle Se}) * \text{Species} \quad (1)$$

$$\log(\text{Egg|Ripe Ovary Se}) = \log(\text{WB Se}) + \text{Species} + \log(\text{WB Se}) * \text{Species} \quad (2)$$

Hypothesis 3: Tissue Se concentration relationships observed across fish species are influenced by phylogenetic relationships

This hypothesis was evaluated using tissue concentration regressions tested in Hypothesis 2, and also using unadjusted tissue concentration ratios from samples in each of the three tissue pairings (i.e., egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, and WB-to-muscle). Linear models and tissue ratios were then qualitatively assessed to determine whether similar patterns were observed for fish species within taxonomic levels (genus, family, order).

Based on the conclusion from Hypothesis 1, only relationships between muscle and WB versus eggs or ripe ovaries were included in this evaluation. To evaluate relationships between WB and muscle Se, however, the dataset was not limited to data for ripe females. It has previously been hypothesized that muscle Se concentrations in females may be higher prior to vitellogenesis and lower during vitellogenesis and post-spawning as Se is mobilized to the ovaries, but evaluation of the current dataset does not support this hypothesis (see online supplementary material S1). For WB tissue in females, Se concentrations may be lower post-spawning as Se is depurated from the fish along with the eggs (although the corresponding loss in egg mass may partially mitigate the change in Se on a concentration basis). In the present study, due to paired muscle and WB Se data for ripe females only being identified for three fish species, and because the need to translate between muscle and WB Se concentrations may not be limited to ripe females, we used all paired muscle and WB Se data regardless of spawning status. In addition, this allowed use of muscle and WB Se concentration data for male fish.

Results

Fish tissue Se database

Paired egg|ovary and muscle Se concentrations were compiled for 40 species ($n = 2{,}533$), paired egg|ovary and WB Se concentrations were compiled for 30 species ($n = 428$), and paired muscle and WB Se concentrations were compiled for 24 species ($n = 497$; Table 1). For the paired egg|ovary and muscle data, GSI data were available for 19 species ($n = 1{,}143$) and data for ripe females were available for 21 species ($n = 499$; Table 1). For paired egg|ovary and WB data, GSI data were available for 16 species ($n = 112$) and data for ripe females were available for 7 species ($n = 172$; Table 1). All raw data are provided in online supplementary material S2.

Hypothesis 1: Ovary Se Concentrations are Inversely Related to Oocyte Development

Relationships between egg|ovary Se and GSI

A total of 19 fish species with paired egg|ovary and muscle Se concentrations had GSI data for at least a subset of the dataset.

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Table 1. Numbers of samples and fish species represented for each tissue pairing.

			GSI data		Qualitative "ripe" data	
Tissue pair	**Total no. of samples**	**Total no. of species**	**No. of species**	**No. of samples**	**No. of species**	**No. of samples**
Eggs|ovaries and muscle	2,533	40	19	1143	21	499
Eggs|ovaries and WB	428	30	16	112	7	172
Muscle and WB	497	24	–	–	–	–

Note. GSI, gonadosomatic index; WB, whole body.

Based on our established minimum data requirements, relationships between egg|ovary-to-muscle Se ratios and GSI could be evaluated for 10 species, of which six had significant negative correlations and negative regression slopes with GSI once data were log-transformed (Figure 1). For WB tissue, a total of 17 fish species had paired egg|ovary Se concentrations and GSI data for at least a subset of the dataset, but minimum data requirements were met for only four species. All had negative correlations with GSI once data were log-transformed, but none were significant, and neither were the regression slopes (Table 2).

Qualitative evaluation of the residuals plots from the tested regressions suggested potential relationships between model residuals and GSI, so a follow-up analysis tested the relationship between the absolute value of each model's residuals against GSI as the predictor. Model residuals exhibited significant negative correlations with GSI for two species in the egg|ovary-to-muscle Se models (northern pikeminnow [$p < 0.001$] and Sacramento splittail [$p < 0.001$]), while a negative trend was observed for mountain whitefish ($p = 0.076$). Only Sacramento splittail exhibited a significant negative correlation in the egg|ovary-to-WB Se model residuals ($p = 0.01$; Table 2). The presence of model residuals that are not independent from the predictor variable violates a regression assumption necessary for testing the significance of model coefficients (i.e., slope and intercept) and fitting prediction intervals. Although this occurrence did limit the interpretability of the regressions that were modeled for the GSI analysis, it showed that for several species, as GSI increases, the variability of Se tissue concentration ratios decreases. Thus, as the spawning status approaches ripe, the relationship between egg|ovary Se and muscle or WB Se becomes more predictable.

Overall, the evaluation confirms that spawning status, as inferred by GSI, has an important influence on both the magnitude and variability in egg|ovary-to-muscle and egg|ovary-to-WB relationships for most of the species evaluated.

Egg|ovary Se concentrations as a function of spawning status

In the analysis based on qualitative descriptions of spawning status, 5 of the 8 tested species showed significant differences between egg|ovary-to-muscle Se ratios in ripe versus unripe samples (Table 3). For the four species that could be tested using both the ANCOVA and the multiple contrasts, there was agreement on the conclusion of the tests between the two methods for three species (rainbow trout, brook trout, and mountain whitefish) and disagreement for westslope cutthroat trout (nonparametric ANCOVA $p = 0.01$; nonparametric multiple contrast $p = 1$).

The ANCOVA is a more robust analysis to test this hypothesis and is capable of detecting more nuanced differences between categorical groups than multiple contrasts. A graphical representation of the ANCOVA performed on rainbow trout, a species with agreement between ANCOVA and multiple contrasts, and westslope cutthroat trout, a species with disagreement between the two methods, is provided in Figure 2. For westslope cutthroat trout, nonparametric regressions of the ripe and unripe groups diverge from one another while still exhibiting large overlap (Figure 2B). The divergence was not detected when contrasting ripe versus unripe tissue ratios (Figure 3). However, the corroboration of results for the other three species supports accepting the multiple contrast results for the four remaining species that could not be tested using ANCOVA. As with fish species that had decreased variability in residuals with increasing GSI, there was less variability in egg|ovary-to-muscle Se ratios in samples from ripe females than unripe females (Fligner-Killeen test, $\alpha < 0.05$; Figure 3).

Results of this meta-analysis are consistent with the findings from the focused study reported in the companion study (Brix et al., 2025), which is that spawning status has an important influence on the magnitude and variability in ovary Se concentrations. As such, evaluating relationships between ovary Se concentrations and muscle or WB Se concentrations must be based on Se concentration in ripe ovaries to be meaningful for comparison to egg-based criteria or guidelines.

Hypothesis 2: Variability in Se Concentration Relationships Between Ovaries and Muscle or WB Tissue Is Reduced When Only Data for Eggs or Ovaries of Spawning Females are Considered

Of the 19 species with paired egg|ripe ovary and muscle Se data, datasets for 12 species passed the regression acceptability requirements (i.e., minimum five samples per tissue pair; presence of a linear relationship based on OLS; and predictive $R^2 \geq 0.35$ when the muscle Se range was <10 mg/kg dry wt) and were included in the final pooled MLR model (Table 4; Figure 4a). Of the seven species excluded from the model, five were omitted for not exhibiting a relationship (i.e., slope) significantly different from 0 (largemouth bass, white sucker, walleye, bull trout, and mountain whitefish; see online supplementary material S3A). It is unclear whether the species omitted for lack of a significant slope had no detectable relationship due to limitations of the available dataset or because of a true absence of relationship between tissue concentrations.

Of the 12 species included in the pooled MLR, bluegill had a broad range of muscle Se concentrations and a median slope relative to the other species and was therefore selected as the baseline model species against which the other species were compared in the MLR. The bluegill regression model is:

$$\log(\text{Egg|Ripe Ovary Se}) = 0.86 \times \log(\text{Muscle Se}) + 0.29 \quad (3)$$

Of the remaining 11 species included in the MLR analysis, many did not have significantly different slopes and intercepts relative to bluegill (Table 4). The predictive R^2 for the pooled MLR model with all species retained is 0.95 (Figure 4A), and the predictive R^2 for the OLS models for individual species ranged from 0.61 to 0.94 (median predictive $R^2 = 0.84$; Table 4).

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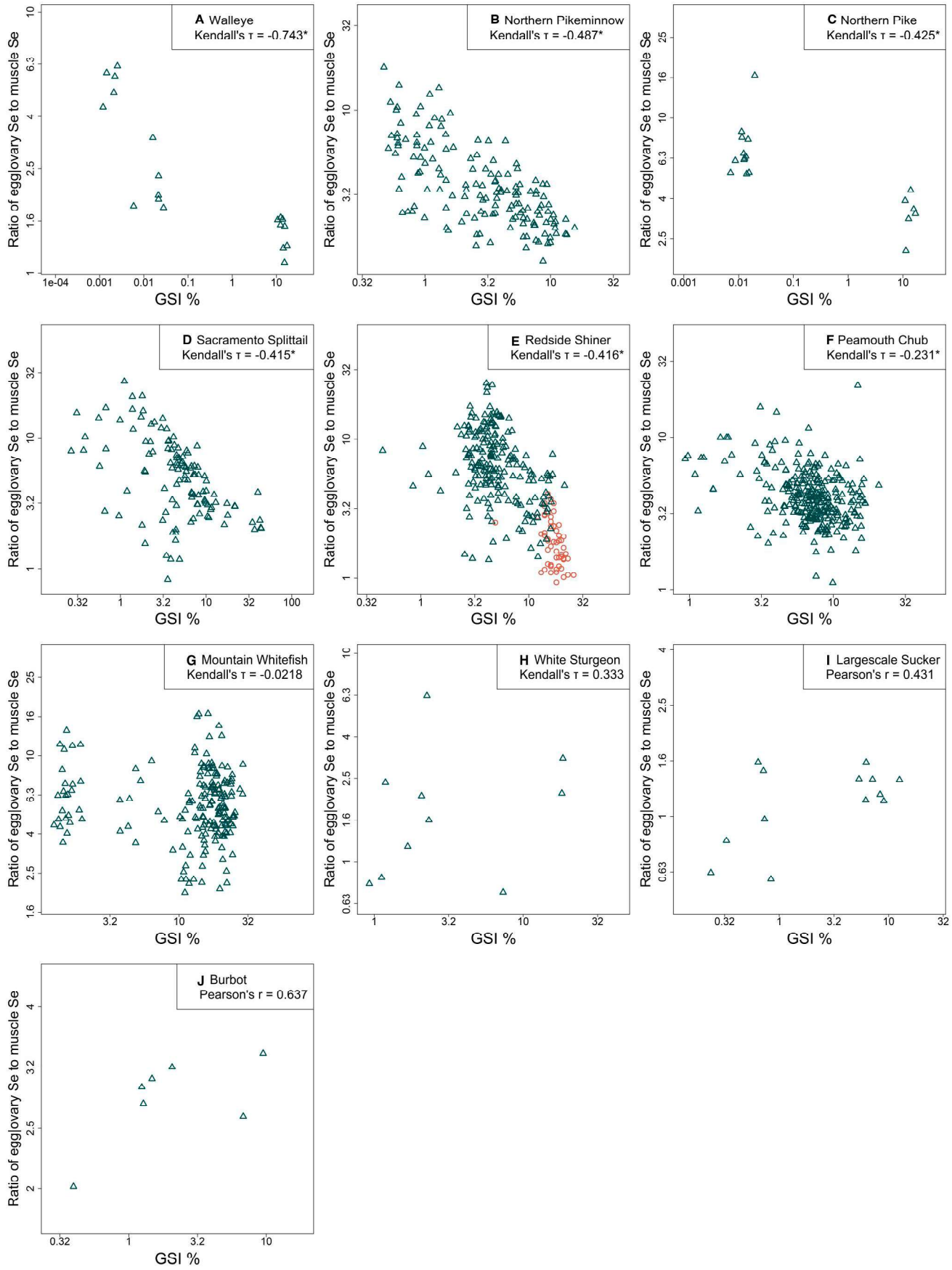


Figure 1. Relationships between egg|ovary-to-muscle selenium (Se) ratios and the gonadosomatic index (GSI) for 10 fish species. Correlation statistics were calculated using log10-transformed values and are represented as either Kendall's τ or Pearson's *r* and are marked with an asterisk (*) when significant ($\alpha = 0.05$). Orange circles used egg Se concentrations from samples from de Bruyn et al. (2023), which also provided paired concentrations of ovary Se.

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Table 2. Summary of correlation and regression statistics between log-transformed selenium (Se) tissue ratios and log-transformed gonadosomatic index (GSI).

Se tissue ratio compared to GSI	Species	Interpretation	Correlation statistic[a]	Correlation *p*-value	Regression slope coefficient	Regression *p*-value	Regression predictive R^2	Regression residuals normality *p*-value[b]	|Residuals| correlation statistic	|Residuals| correlation *p*-value
Log10 ratio of egg|ovary to muscle	Walleye	Significant negative correlation. Nonlinear relationship in primary regression therefore follow-up residuals regression is not interpretable.	−0.743	**9.02E-07**	—[c]	—	—	—	—	—
	Northern Pikeminnow	Significant negative correlation. Significant correlation between absolute value of residuals and predictor show decreasing variability with increasing GSI.	−0.487	**1.35E-18**	−0.39	—	0.457	0.307	−0.195	**4.28E-04**
	Northern Pike	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.425	**0.0137**	−0.102	—	0.489	**0.0267**	−0.137	0.454
	Sacramento Splittail	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.415	**3.38E-11**	−0.348	—	0.245	**7.56E-04**	−0.39	**4.44E-10**
	Peamouth Chub	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.231	**7.33E-09**	−0.272	—	0.125	**6.15E-05**	−0.0226	0.572
	Redside Shiner	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.414	**3.07E-24**	−0.656	—	0.351	**0.0147**	−0.0402	0.323
	Mountain Whitefish	No correlation detected. Passes regression assumptions; slope coefficient is nonsignificant.	−0.0218	0.655	−0.0422	0.219	−0.0111	0.317	−0.0869	0.076
	White Sturgeon	Passes regression assumptions; slope coefficient is nonsignificant.	0.333	0.216	0.162	0.462	−0.316	0.868	−0.244	0.381
	Burbot	No significant correlation. Passes regression assumptions; slope coefficient is nonsignificant.	0.637	0.124	0.0983	0.287	−0.553	0.0553	−0.247	0.593
	Largescale Sucker	Passes regression assumptions; slope coefficient is nonsignificant.	0.431	0.124	0.127	0.124	−0.0453	0.239	−0.231	0.279
Log10 ratio of egg|ovary to WB	Creek Chub	Passes regression assumptions; slope coefficient is nonsignificant.	−0.387	0.092	−0.211	0.092	−0.0142	0.5	−0.0301	0.9
	Central Stoneroller	Passes regression assumptions; slope coefficient is nonsignificant.	−0.364	0.116	−0.338	0.0909	−0.569	0.086	−0.333	0.153
	Bluegill	Passes regression assumptions; slope coefficient is nonsignificant.	−0.286	0.399	−0.314	0.239	−0.26	0.342	−0.357	0.275
	Sacramento Splittail	No correlation detected. Significant correlation between absolute value of residuals and predictor show decreasing variability with increasing GSI.	−0.212	0.261	−0.0553	0.261	−0.0999	0.61	−0.315	**0.0143**

Note. Tests performed on the absolute value of the residuals from the original regression, "|Residuals|," test whether the relationship between the ratio and GSI changes with GSI. The Interpretation information summarizes the findings for each row. Bolded values in the correlation *p*-value columns indicate significant relationships; bolded values in the residuals normality column indicate that assumption failed.

[a] If both the log-transformed ratio and log-transformed GSI for a given species fit a normal distribution (Shapiro-Wilk test, $\alpha = 0.05$), a Pearson correlation was used and the corresponding correlation statistic is reported as Pearson's *r*. If either variable had a non-normal distribution, Kendall's correlation was used instead and the corresponding correlation statistic reported is Kendall's tau.

[b] Regression residuals tested for normal distribution using a Shapiro-Wilk test. Failure of this test ($\alpha < 0.05$) means model coefficients (i.e., slope and intercept) cannot be tested for significance (indicated with "—" in the Regression *p*-value column).

[c] If assumptions were not met to appropriately calculate a statistic, "—" indicates none was calculated.

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Table 3. *p*-values resulting from comparing egg|ovary-to-muscle selenium (Se) concentrations in ripe versus unripe samples within each listed species.

Species	Scientific name	Nonparametric ANCOVA	Nonparametric multiple comparisons
Rainbow Trout	*Oncorhynchus mykiss*	**$p = 0.005$**	**$p = 5E\text{-}05$**
Brook Trout	*Salvelinus fontinalis*	**$p = 0.005$**	**$p = 0.003$**
Westslope Cutthroat Trout[a]	*Oncorhynchus clarkii lewisi*	**$p = 0.02$**	$p = 1$
Mountain Whitefish	*Prosopium williamsoni*	$p = 0.3$	$p = 1$
Bull Trout	*Salvelinus confluentus*	na	$p = 0.2$
Northern Pike	*Esox lucius*	na	**$p = 2E\text{-}05$**
Walleye	*Stizostedion vitreum*	na	**$p = 0.001$**
White Sturgeon	*Acipenser transmontanus*	na	$p = 1$

Note. The nonparametric multiple contrasts compared muscle Se-normalized versus egg|ovary Se concentrations. Bolded *p*-values are significantly different ($\alpha = 0.05$). "na" indicates the test could not be performed for that species (data unsuitable).
[a] The nonparametric nonparametric analysis of covariance (ANCOVA) is a stronger test for this question and is more capable of detecting the subtler differences between ripe and unripe samples of this species (Figure 2B).

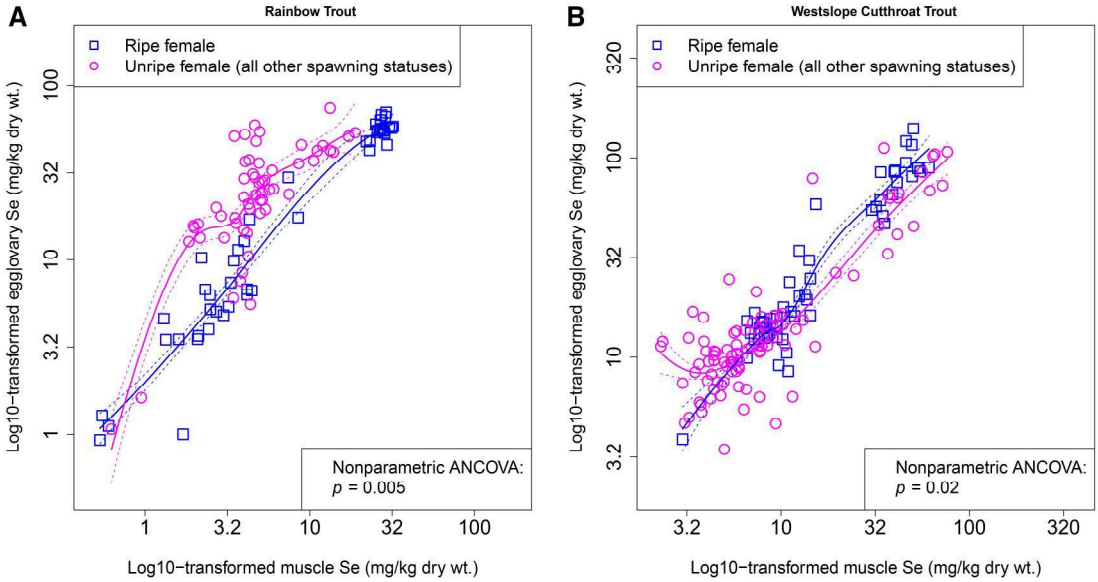


Figure 2. Egg|ovary selenium (Se) versus muscle Se concentrations fitted with weighted smoothing function for samples from ripe and unripe females for (A) rainbow trout and (B) westslope cutthroat trout.

For the pooled MLR model for egg|ripe ovary versus WB Se, all species met the criteria for inclusion in the MLR (Table 4; Figure 4B). As with the muscle Se model, the MLR predicting egg|ripe ovary Se from WB Se was evaluated relative to bluegill. The bluegill regression is:

$$\log(\text{Egg|Ripe Ovary Se}) = 0.91 \times \log(\text{WB Se}) + 0.35 \quad (4)$$

Only brown trout exhibited a significantly different slope from the bluegill model, and brown trout, desert pupfish, and Dolly Varden each had significantly different y-intercepts (Table 4). The predictive R^2 was 0.88 (Figure 4B), and the predictive R^2 for individual species OLS regressions ranged from 0.70 to 1.0 (Table 4), indicating, as for muscle Se, predictable Se tissue concentration relationships when females are in ripe spawning status.

A primary outcome of the MLR modeling analysis is demonstration that much of the variability in egg|ovary-to-muscle Se and egg|ovary-to-WB Se relationships among species is reduced when data for eggs and ripe ovaries are considered. There are, as noted, some species for which intercepts or slopes are significantly different, and some species that did not meet minimum requirements for being included in the MLR analyses, so there is not a "universal model" that can be used to predict egg|ripe ovary Se concentrations from muscle or WB Se concentrations. The primary models, however, provide a useful starting point for evaluating fish species lacking tissue Se relationship data. For species included in the pooled MLR models for predicting egg|ripe ovary Se concentrations from muscle or WB, we recommend using all species-specific model adjustments, whether or not they were significantly different from the pooled model (Table 4). It is not recommended to extrapolate relationships beyond the range of data on which the model is based.

As noted in the *Methods*, TLS regression was also tested and compared to OLS regression (results are reported in online supplementary material S3). Once the dataset was reduced to only samples with eggs|ripe ovaries, the differences between OLS and TLS models were minimal.

Hypothesis 3: Tissue Se Concentration Relationships Observed Across Fish Species are Influenced by Phylogenetic Relationships

Of the 12 species represented in the egg|ripe ovary-to-muscle MLR, five are from the order Salmoniformes, two each are from Hiodontiformes and Perciformes, and one each is from

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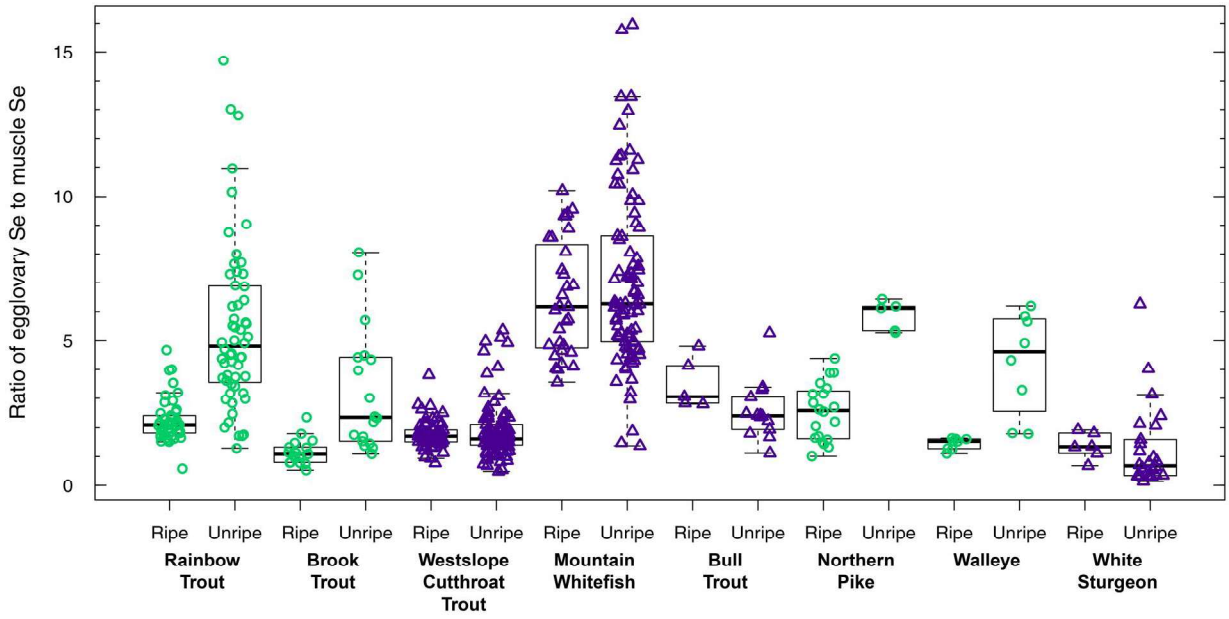


Figure 3. Comparison of egg|ovary-to-muscle selenium (Se) ratios for samples from ripe and unripe females within each species. Boxes denote interquartile range (IQR; 25th–75th percentiles) with horizontal line denoting the median. Lower whisker denotes smallest value greater than 25th percentile − 1.5 × IQR, and upper whisker denotes largest value less than 75th quantile + 1.5 × IQR. Species plotted with green circles exhibited a significant difference between the ripe and unripe samples using nonparametric multiple contrasts.

Acipenseriformes, Cypriniformes, and Esociformes. The slopes of the linear models do not indicate that any groupings or patterns are due to taxonomic relatedness; they are distributed throughout the range of slopes (Table 4). This also applies to mountain whitefish, which is a salmonid that was removed from the MLR model because its tissue concentration relationships did not exhibit a slope different from 0 (see online supplementary material S3A). The family Hiodontidae had two modeled species that also shared the same genus, but, as with taxonomic order, the models for these species did not exhibit similarities.

Because all species in the Salmoniformes order are also in the Salmonidae family, the genera were evaluated for the salmonids instead. In this instance, it was noted that, when multiple species were present within genera (*Oncorhynchus* and *Salvelinus*), they grouped together in the overall ranking of slopes. This suggests a possible phylogenetic effect in the tissue concentration relationships. The two *Salvelinus* species in particular (brook trout and Dolly Varden) have effectively the same slope albeit different intercepts, while the slopes of the two *Oncorhynchus* species (westslope cutthroat trout and rainbow trout) are less similar, differing by 0.1 on a log scale. Overall, though, within-genus comparisons were limited, and data for additional taxa are needed before a phylogenetic pattern is concluded.

The species that passed minimum data requirements for the egg|ripe ovary-to-WB MLR were fewer in number and dominated by the order Salmoniformes, making patterns or groupings difficult to evaluate (Table 4). Unlike the muscle dataset, there were no duplicate families or genera represented across the seven available species. Rather, the overall similarity of slopes exhibited across the available species indicates a possible consistency of Se partitioning.

For relationships between WB and muscle Se, an interesting pattern in taxonomic similarities emerges. The same regression requirements were applied to samples with paired WB and muscle Se concentrations; however, because there is little effect of spawning status on Se concentrations in either tissue, all available samples were included regardless of spawning status (see *Methods*). In addition, the datasets with paired muscle and WB Se concentrations were not necessarily linked only to females, so the issue of spawning status does not apply to the entire dataset. Indeed, all species that had the minimum sample size and range of muscle tissue concentrations required to test in regressions showed linear relationships with log-transformed tissue concentrations with a similar distribution of predictive R^2 as the egg|ripe ovary-to-muscle- and egg|ripe ovary-to-WB regressions (Table 4; Figure 4C).

The regression models for 10 of the 17 fish species did not have y-intercepts that were significantly different from 0 and all were from either the orders Cypriniformes or Perciformes. By contrast, 5 of the 7 remaining species with intercepts significantly greater than 0 were salmonids (0.128–0.917), exhibiting a distinct pattern in model coefficients not shown in either of the two egg|ripe ovary models. This indicates salmonids are generally characterized by greater WB Se concentrations compared to muscle, particularly at lower muscle Se concentrations. This is the opposite of that observed for the orders Cypriniformes and Perciformes, which had intercepts of 0 and slopes between 0.75 and 1, indicating their WB tissue Se concentration is consistently lower than muscle (Sacramento splittail was the only observed exception, showing again that these patterns are not universal). The slopes for the salmonids are diverse with two in the range of 0.75–1 exhibited by the other orders, two significantly lower (0.5–0.65), and one significantly higher (i.e., 1.19). The intercepts of the WB-to-muscle Se relationships for salmonids are consistently greater than for the two non-salmonid orders, but there is still high variability in the magnitudes of both intercepts and slopes among salmonid species.

Some of the same patterns seen in the regression models for each of the three tissue pairing types can also be seen when comparing tissue concentration ratios (Table 5; Figure 5). The two salmonids in the genus *Salvelinus* that had similar egg|ripe ovary-to-muscle slopes also share similar medians, but otherwise orders, families, and genera are distributed without apparent pattern throughout the ratio medians of the available species for both egg|ripe ovary-to-muscle or egg|ripe ovary-to-WB Se ratios. For the WB-to-muscle Se ratios, the taxonomic patterns can also be observed. Salmonids are

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Table 4. Ordinary least squares log-log regression models for egg|ripe ovary selenium (Se) versus muscle Se and whole body (WB) Se, and WB Se versus muscle Se.

Species	Order	Family	Genus	Slope	Intercept	Adj. R^2	Pred. R^2	Muscle or WB Se range (mg/kg dry wt)[a]	Sample size
Egg|ripe ovary Se vs. muscle Se									
Redside Shiner	Cypriniformes	Cyprinidae	Richardsonius	1.27*	0.252	0.869	0.861	0.6–4.6	40
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.22*	0.579*	0.831	0.791	1.07–4.78	19
Mooneye	Hiodontiformes	Hiodontidae	Hiodon	1.12*	0.0902	0.93	0.91	4.48–12.5	12
Westslope Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	1.09*	0.126*	0.905	0.901	3–61	54
Rainbow Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.995*	0.331	0.939	0.936	0.532–32	46
Goldeye	Hiodontiformes	Hiodontidae	Hiodon	0.909	0.188	0.866	0.82	1.88–3.46	8
Bluegill	Perciformes	Centrarchidae	Lepomis	0.862	0.286	0.863	0.853	1.47–55.2	36
Brook Trout	Salmoniformes	Salmonidae	Salvelinus	0.808	0.211	0.866	0.834	1.4–34.7	17
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	0.802	0.411	0.91	0.885	2.2–117	16
White Sturgeon	Acipenseriformes	Acipenseridae	Acipenser	0.789	0.238	0.906	0.822	1.22–15.3	6
Northern Pike	Esociformes	Esocidae	Esox	0.769	0.474*	0.853	0.834	0.9–47.8	20
Yellow Perch	Perciformes	Percidae	Perca	0.517*	0.24	0.634	0.612	0.542–2.24	44
Egg|ripe ovary Se vs. WB Se									
Brown Trout	Salmoniformes	Salmonidae	Salmo	1.27*	−0.0959*	0.832	0.807	4.7–20	23
Slimy Sculpin	Scorpaeniformes	Cottidae	Cottus	1.07	0.229	0.9	0.87	1.55–10.8	20
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	1.05	−0.0186*	0.96	0.953	2.2–108	10
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.02	0.345	0.913	0.9	1.78–11	19
Yellowstone Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.966	0.16	0.725	0.704	1.2–25.7	21
Desert Pupfish	Cyprinodontiformes	Cyprinodontidae	Cyprinodon	0.916	0.126*	0.999	0.999	0.73–26.9	6
Bluegill	Perciformes	Centrarchidae	Lepomis	0.911	0.349	0.844	0.827	0.92–26.8	33
WB Se vs. muscle Se									
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.19*	0.231*	0.905	0.889	1.07–4.78	19
Sacramento Splittail	Cypriniformes	Cyprinidae	Pogonichthys	1.17*	0.0545*	0.65	0.618	1.02–3.15	30
Rainbow Trout	Salmoniformes	Salmonidae	Oncorhynchus	1.02	0.128*	0.817	0.808	0.674–1.97	51
Longnose Sucker	Cypriniformes	Catostomidae	Catostomus	1.01	0.0231	0.819	0.763	0.954–2.39	11
Roundtail Chub	Cypriniformes	Cyprinidae	Gila	1	−0.0599	0.601	0.506	4.34–9.84	10
Common Carp	Cypriniformes	Cyprinidae	Cyprinus	0.977	−0.112	0.823	0.687	5.06–24.2	9
Flannelmouth Sucker	Cypriniformes	Catostomidae	Catostomus	0.955	−0.138	0.561	0.465	3.56–7.28	12
Bluegill	Perciformes	Centrarchidae	Lepomis	0.892	−0.0423	0.928	0.92	1.86–55.2	34
Walleye	Perciformes	Percidae	Stizostedion	0.864	0.0195	0.478	0.451	0.939–2.74	48
Brown Trout	Salmoniformes	Salmonidae	Salmo	0.843	0.159*	0.951	0.933	3.17–25.1	9
Smallmouth Bass	Perciformes	Centrarchidae	Micropterus	0.834	0.00875	0.926	0.917	0.75–11	29
Largescale Sucker	Cypriniformes	Catostomidae	Catostomus	0.832	−0.0477	0.558	0.499	0.55–1.66	32
Bluehead Sucker	Cypriniformes	Catostomidae	Catostomus	0.817	−0.0235	0.942	0.931	1.47–8.57	10
Yellow Perch	Perciformes	Percidae	Perca	0.765	0.0154	0.719	0.639	2.2–3.1	11
Yellowstone Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.653*	0.303*	0.747	0.726	2.44–10.9	43
Burbot	Gadiformes	Lotidae	Lota	0.645*	0.184*	0.645	0.602	0.928–5.92	31
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	0.515*	0.917*	0.784	0.683	19.5–113	10

Note. Slope and intercept values with "*" indicate that the model coefficient was significantly different from the corresponding coefficient of the bluegill model.
[a] Range of muscle or WB Se concentrations in dataset used to develop model.

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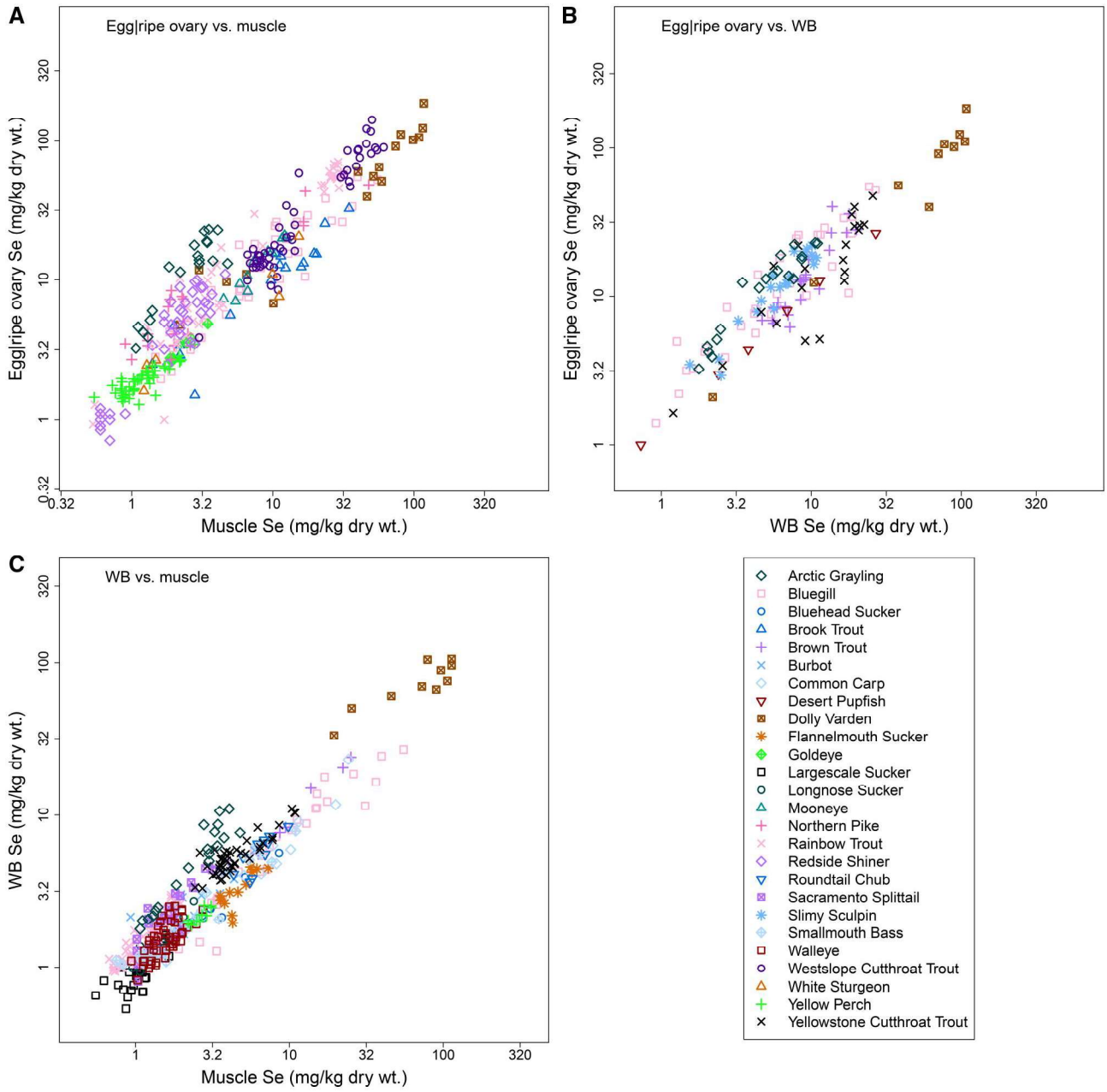


Figure 4. Relationships between egg|ripe ovary selenium (Se) concentrations and (A) muscle Se and (B) whole body (WB) Se, and between (C) WB Se and muscle Se. Multiple linear regression model statistics and coefficients can be found in Table 4.

mostly characterized by median ratios ≥1, and species in the orders Cypriniformes and Perciformes are mostly characterized by median ratios ≤1. The only exceptions are the Sacramento splittail, which was the only Cypriniformes species with either a slope or intercept significantly larger than the other Cypriniformes or Perciformes, and the salmonids Dolly Varden and brown trout, which had median ratios slightly less than 1 rather than greater than 1.

The clearest difference between the regressions and ratios is the high level of precision with which regressions can predict tissue concentrations juxtaposed against the poor predictive capacity of the ratios. The linear regressions that emerge after a log transformation of the tissue concentrations translate to wide ranges of ratios within a species. Several species exhibited as much as a fivefold difference in egg|ripe ovary-to-muscle ratios, whereas their corresponding linear regression had predictive R^2 > 0.85. For prediction of egg Se concentrations, the reported linear regressions are more accurate over a wider range of muscle or WB Se concentrations compared with the use of the tissue concentration ratio.

Discussion

Impact of spawning status on Se tissue concentration relationships

This study presents three analyses that each support the conclusion that spawning status has a significant impact on the relationships between egg|ovary Se and muscle or WB for many fish species. Correlating tissue Se concentration ratios with measured GSI showed that as oocytes develop and the ovary mass increases, the difference between the Se concentration in the eggs|ovaries and the muscle or WB tissue decreases for several species (Figure 1; Table 2). For species where this is observed, the

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Table 5. Tissue concentration ratios for three tissue pairs, separated by species.

Ratio type	Sample spawning status	Species	Order	Family/Genus[a]	Median	Mean	Standard deviation	Sample size	Range of ratios
Egg|ripe ovary vs. muscle	Ripe	Mountain Whitefish	Salmoniformes	Prosopium	6.17	6.47	2	31	3.56–10.2
	Ripe	Arctic Grayling	Salmoniformes	Thymallus	4.62	4.76	1.31	19	2.74–6.85
	Ripe	Bull Trout	Salmoniformes	Salvelinus	3.06	3.53	0.901	5	2.8–4.81
	Ripe	Northern Pike	Esociformes	Esocidae	2.58	2.52	0.99	20	1.01–4.39
	Ripe	Razorback Sucker	Cypriniformes	Catostomidae	2.37	2.37	0.997	34	1.06–5.24
	Ripe	Rainbow Trout	Salmoniformes	Oncorhynchus	2.09	2.23	0.738	46	0.588–4.67
	Ripe	Redside Shiner	Cypriniformes	Cyprinidae	2.07	2.21	0.752	40	1.02–4.05
	Ripe	Northern Pikeminnow	Cypriniformes	Cyprinidae	1.86	2	0.482	43	1.39–3.65
	Ripe	Lake Whitefish	Salmoniformes	Coregonus	1.72	1.83	0.415	9	1.06–2.57
	Ripe	Westslope Cutthroat Trout	Salmoniformes	Oncorhynchus	1.7	1.77	0.509	54	0.78–3.82
	Ripe	Yellow Perch	Perciformes	Percidae	1.63	1.65	0.335	44	1.01–2.66
	Ripe	Mooneye	Hiodontiformes	Hiodontidae	1.61	1.6	0.172	12	1.25–1.85
	Ripe	Walleye	Perciformes	Percidae	1.52	1.44	0.201	8	1.1–1.63
	Ripe	Bluegill	Perciformes	Centrarchidae	1.45	1.55	0.559	36	0.623–3.09
	Ripe	Goldeye	Hiodontiformes	Hiodontidae	1.43	1.43	0.103	8	1.25–1.61
	Ripe	White Sturgeon	Acipenseriformes	Acipenseridae	1.33	1.36	0.456	6	0.686–1.92
	Ripe	White Sucker	Cypriniformes	Catostomidae	1.23	1.28	0.228	6	0.967–1.63
	Ripe	Dolly Varden	Salmoniformes	Salvelinus	1.18	1.45	0.788	16	0.673–3.93
	Ripe	Brook Trout	Salmoniformes	Salvelinus	1.09	1.16	0.445	17	0.536–2.35
	Ripe	Largemouth Bass	Perciformes	Centrarchidae	1.05	1.13	0.339	12	0.769–1.8
Egg|ovary vs. WB	Ripe	Arctic Grayling	Salmoniformes	Thymallus	2.19	2.33	0.468	19	1.72–3.57
	Ripe	Yellowstone Cutthroat Trout	Salmoniformes	Oncorhynchus	2.05	2.75	2.12	37	0.456–10.4
	Ripe	Bluegill	Perciformes	Centrarchidae	1.93	2.04	0.707	33	0.594–3.94
	Ripe	Slimy Sculpin	Scorpaeniformes	Cottidae	1.89	1.93	0.353	20	1.18–2.63
	Ripe	Brown Trout	Salmoniformes	Salmo	1.4	1.18	0.641	31	0.211–2.92
	Ripe	Dolly Varden	Salmoniformes	Salvelinus	1.23	1.21	0.289	10	0.656–1.7
	Ripe	Desert Pupfish	Cyprinodontiformes	Cyprinodontidae	1.16	1.17	0.128	6	0.989–1.37
WB vs. muscle	All	Mountain Whitefish	Salmoniformes	Prosopium	2.73	7.25	12.7	7	0.761–35.7
	All	Arctic Grayling	Salmoniformes	Thymallus	1.84	2.04	0.467	19	1.6–3.1
	All	Kokanee	Salmoniformes	Oncorhynchus	1.36	1.41	0.265	34	0.931–2.08
	All	Rainbow Trout	Salmoniformes	Oncorhynchus	1.33	1.36	0.193	51	0.769–1.77
	All	Lake Whitefish	Salmoniformes	Coregonus	1.28	1.25	0.273	16	0.832–1.79
	All	Sacramento Splittail	Cypriniformes	Cyprinidae	1.25	1.26	0.313	30	0.779–2.03
	All	Yellowstone Cutthroat Trout	Salmoniformes	Oncorhynchus	1.21	1.21	0.251	43	0.885–2.15
	All	Burbot	Gadiformes	Lotidae	1.19	1.24	0.316	31	0.801–2.31
	All	Black Bullhead	Siluriformes	Ictaluridae	1.12	1.11	0.357	14	0.22–1.69
	All	Longnose Sucker	Cypriniformes	Catostomidae	1.04	1.06	0.133	11	0.876–1.29
	All	Walleye	Perciformes	Percidae	0.965	1.02	0.222	49	0.737–1.51
	All	Brown Trout	Salmoniformes	Salmo	0.952	1.05	0.201	9	0.876–1.44
	All	Dolly Varden	Salmoniformes	Salvelinus	0.947	1.14	0.425	10	0.713–1.98
	All	Channel Catfish	Siluriformes	Ictaluridae	0.919	0.92	0.31	8	0.516–1.58
	All	Roundtail Chub	Cypriniformes	Cyprinidae	0.919	0.886	0.143	10	0.653–1.05
	All	Largescale Sucker	Cypriniformes	Catostomidae	0.903	0.901	0.169	32	0.618–1.31
	All	Smallmouth Bass	Perciformes	Centrarchidae	0.888	0.927	0.182	29	0.592–1.48
	All	Yellow Perch	Perciformes	Percidae	0.817	0.83	0.055	11	0.754–0.924
	All	Bluehead Sucker	Cypriniformes	Catostomidae	0.794	0.773	0.091	10	0.585–0.884
	All	Common Carp	Cypriniformes	Cyprinidae	0.784	0.749	0.17	9	0.579–1.04
	All	Bluegill	Perciformes	Centrarchidae	0.767	0.752	0.171	34	0.37–1.05
	All	Flannelmouth Sucker	Cypriniformes	Catostomidae	0.706	0.688	0.113	12	0.456–0.829

[a] All species in the Salmoniformes order are from the Salmonidae family; therefore, genus was substituted instead.

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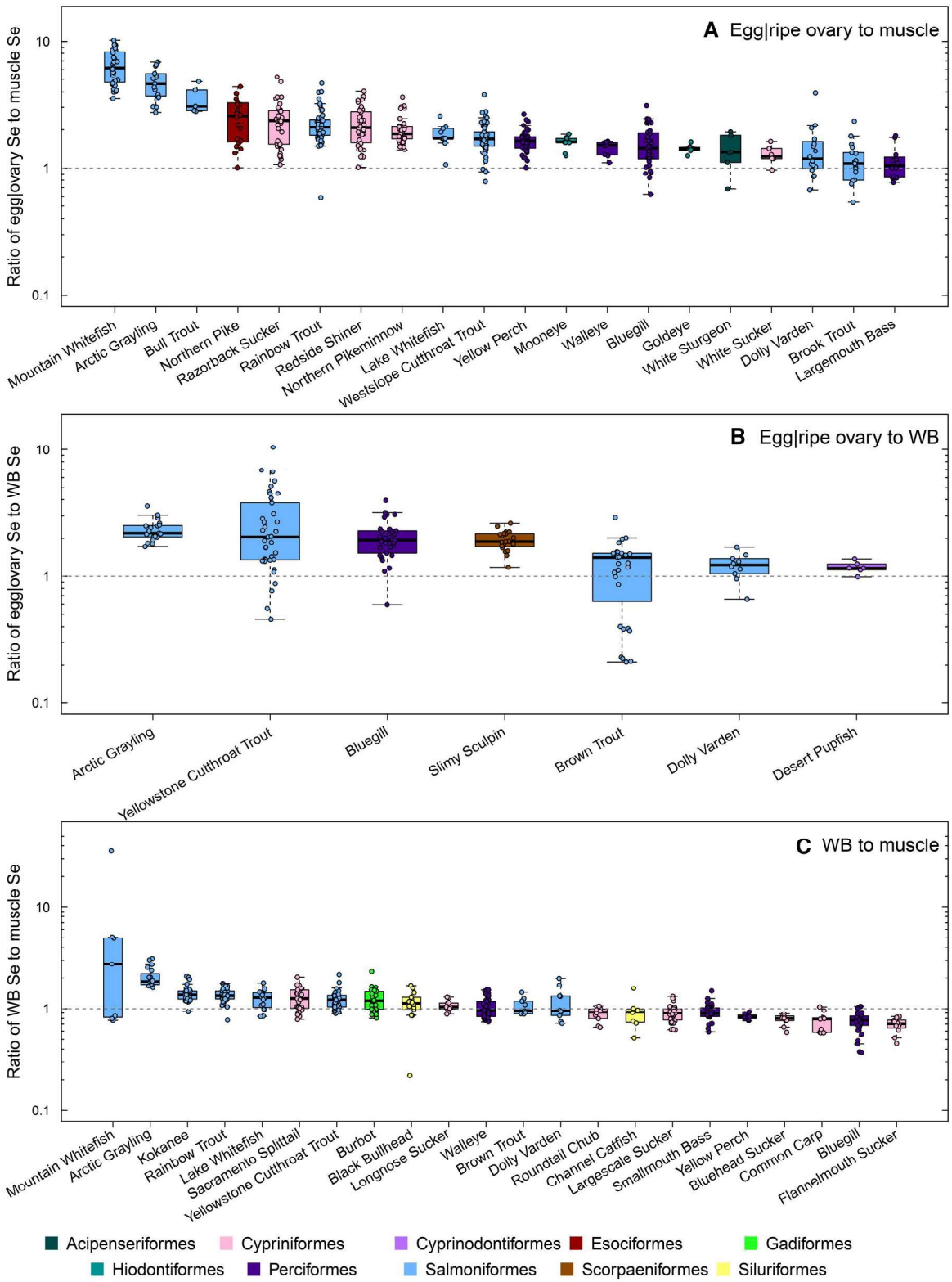


Figure 5. (A) Egg|ripe ovary-to-muscle selenium (Se) ratios; (B) egg|ripe ovary-to-whole body (WB) Se ratios; and (C) WB-to-muscle Se ratios. Box plots are as defined in Figure 3. Points with the same color are in the same taxonomic order.

pattern is driven by decreasing egg|ovary Se concentrations with increasing GSI, as muscle and WB Se concentrations tend not to vary over the GSI range. This pattern is also supported by the analysis comparing egg|ovary-to-muscle Se ratios in ripe and unripe samples (Table 3; Figure 2). Species that exhibited a difference in egg|ovary-to-muscle Se or WB Se ratios between samples from ripe and unripe females consistently had lower tissue Se concentration ratios in the ripe sample group than the unripe group (Figure 3). Although significant patterns were not detected for all species evaluated, either as correlations between egg|ovary-to-muscle Se or egg|ovary-to-WB Se ratios versus GSI or as detected differences between ripe and unripe sample

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populations, for several species it is clear that spawning status influences egg|ovary Se concentrations and subsequent ratios with muscle and WB Se. As a result, analysis of Se concentrations in eggs|ovaries from unripe females or from females of uncertain ripeness may result in erroneous estimates of larval exposure to Se via maternal transfer (i.e., an overestimation of Se concentrations in ripe eggs|ovaries). The data in the present study and the companion study (Brix et al., 2025) support this observation. Possible mechanisms are discussed in Brix et al. (2025).

The analyses using GSI and spawning status both showed a decrease in variability of egg|ovary-to-muscle Se or WB Se ratios once females are ripe—even for species that did not have significantly different ratios in the ripe and unripe sample populations or significant correlations with GSI (Figure 3; Table 2). This finding shows that, not only can the ripe spawning status impact the relationship between Se concentrations in eggs|ovaries versus muscle or WB, it also impacts variability in the relationship and accuracy of predictions made based on the relationship. The relationship between egg|ovary Se for ripe and unripe rainbow trout samples (Figure 2A) demonstrates how the tissue concentration relationships become more predictable once the females become ripe. The samples in the unripe group exhibit high variability that sometimes deviate far from the weighted smoothing line, particularly around 3–5 mg/kg dry weight muscle Se where egg|ovary Se ranges from as low 5.6 mg/kg dry weight to as high as 59.3 mg/kg dry weight. By contrast, samples in the ripe group form a linear relationship.

Based on the high variance that can be introduced by including all stages of oocyte development, and that egg|ripe ovary Se concentrations tend to decrease as the fish approaches the ripe status, only ripe samples were used to generate predictive models for egg|ripe ovary Se based on muscle or WB Se.

Tissue Se ratios versus regression-based relationships

In the present study, we evaluated regression-based approaches for comparing tissue Se relationships among species and concluded that there are significant log-log linear relationships between egg|ripe ovary and muscle Se concentrations for many species. For example, in the MLR evaluation for these relationships, species with a log-log slope <1 meant that modeled egg|ripe ovary Se concentrations increased at a lower rate than corresponding increases in muscle Se concentrations when not log-transformed (Figure 6). For species with a log-log slope >1, the opposite is true: the egg|ripe ovary-to-muscle Se ratios for species in the model were not constant but increased at a higher rate than corresponding increases in muscle Se concentrations. In contrast, evaluations of median tissue Se concentrations do not consider the potential influence of varying Se exposure conditions that could influence the ratio. We evaluated both approaches in the present study, depending on the hypothesis being addressed.

For most fish species in our evaluation, the range and standard deviation of tissue Se concentration ratios (i.e., egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, WB-to-muscle) were high compared to the median or mean ratio (Figure 5; Table 5). For example, coefficients of variation for egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, and WB-to-muscle averaged 29%, 34%, and 28%, respectively. This shows that, even when only examining egg|ripe ovary samples of an individual species, the Se concentration ratio between egg|ripe ovary and muscle or WB tends to have high variability, and a single ratio is therefore an uncertain predictor of egg|ripe ovary Se concentrations over a range of muscle or WB Se concentrations. As noted above, both multiple and species-specific regression models that predict egg|ripe ovary Se from muscle or WB Se have slopes that are significantly different from zero, meaning that relationships between egg|ripe ovary and muscle or WB Se concentrations are concentration dependent. As such, to the extent that data are available for a given species and that significant models can be developed, we recommend the use of regression-based relationships for deriving tissue Se concentration ratios.

The USEPA evaluated regressions versus tissue ratios and recommended the median tissue Se ratio (USEPA, 2016). In calculating tissue Se ratios, the USEPA calculated the median if the linear regression relationship in Se concentrations between the two tissues being evaluated was positive and significant ($\alpha = 0.05$). No tissue Se ratio was calculated by the USEPA if these two requirements were not met. Although the USEPA noted that there may be cases for which the regression-based approach may better capture tissue Se relationships across the fuller range of Se concentrations, they stated that median ratios are simpler to explain and implement, and are not affected by outliers or the distribution of variance over the range of data (USEPA, 2016, 2021). However, we note that developing relationships between egg|ovary Se and either muscle or WB Se when controlling for spawning status (i.e., considering only data for ripe eggs|ovaries) reduces a significant portion of unequal variance across data ranges for many species (Figures 2 and 4). Also, when the log-log slope is not equal to 1, the use of median ratios has increasing

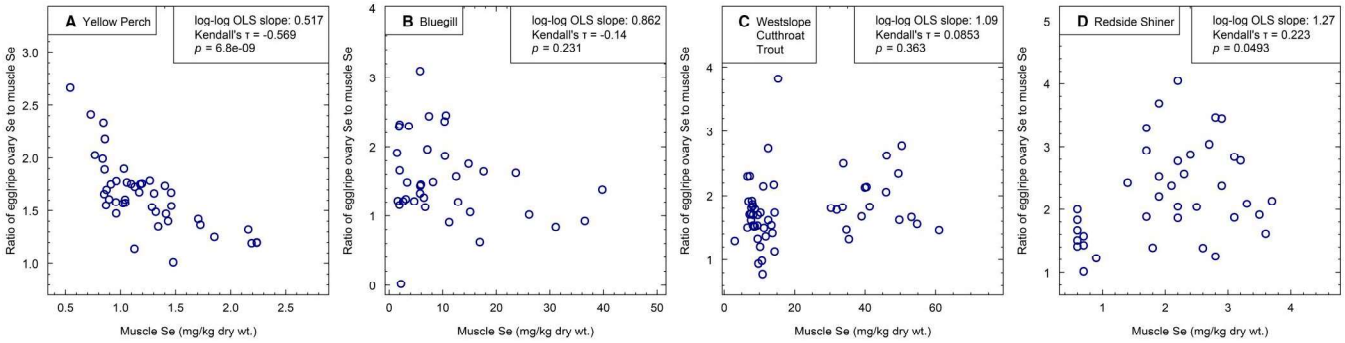


Figure 6. Ratios of egg|ripe ovary-selenium (Se)-to-muscle-Se concentrations plotted against muscle Se for species with egg|ripe ovary-to-muscle log-log slopes that are (A) considerably less than 1 (yellow perch), (B) slightly less than 1 (bluegill), (C) slightly greater than 1 (westslope cutthroat trout), and (D) considerably greater than 1 (redside shiner). The two species with log-log slopes farthest from 1 show the steepest correlations (Kendall's τ) of tissue concentration ratios with increasing muscle concentrations. The two species with log-log slopes closer to 1 (i.e., bluegill and westslope cutthroat trout) show shallower and nonsignificant correlations. Even when there is no relationship with muscle concentration, tissue concentration ratios exhibit a wide range.

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uncertainty as one deviates further from the muscle or WB Se concentration from which the median ratio was derived (Figure 6). For these reasons, we consider a regression-based approach more robust than a ratio approach.

Variability in fish tissue Se relationships among fish populations

While in most cases tissue Se relationships in a species when data are available from multiple water bodies or sampling locations appear similar, our review identified a few examples of differences in tissue relationships across sampling locations for a given species. For example, Yellowstone cutthroat trout from a spawning station on Henry's Lake have much greater egg-to-WB Se ratios than wild-caught trout naturally exposed to a range of Se concentrations in lotic systems and other trout reared from hatchery-sourced eggs that were provided dietary organic Se in a 2.5-year study. The greater egg-to-WB Se ratios for the Henry's Lake hatchery samples were driven by WB Se concentrations that were much less than in the wild lotic population and in the feeding study, while egg Se concentrations were not proportionally lower. Based on this observation, data for the Henry's Lake population with divergent tissue Se ratios were excluded from our analysis (see online supplementary material S4 for further discussion).

This phenomenon of tissue concentration relationships being variable among subpopulations was also observed for Arctic grayling and redside shiner. For Arctic grayling, Brix et al. (2021) hypothesized that fish from an isolated lentic population had different tissue Se relationships than those from lotic populations due to differences in Se speciation and bioaccumulation between lentic and lotic site types, which may affect Se homeostasis among tissues. However, for redside shiner, de Bruyn et al. (2023) observed that the relationship between egg and muscle Se in fish from some but not all lentic sites was similar to the relationship in fish collected from lotic sites, (i.e., there was not a clear pattern between lentic and lotic sites for redside shiner). Regardless of the mechanisms causing this variability in fish tissue Se relationships that is sometimes observed in fish of the same species collected from different water bodies, consideration should be given to all of the available data to make an informed decision on the most relevant data for the site of interest.

Variability among fish species

When comparing relationships between egg|ripe ovary Se concentrations and corresponding muscle or WB Se concentrations, there is no clear pattern between species in the same order and family. Some similarities were observed among model coefficients between species in some of the salmonid genera (see *Results* and Table 4). Although there are a limited number of examples for which there are models for more than one species in a genus, we recommend that a model for an intra-genus surrogate be used to represent a species of interest if a species-specific model is not available. This recommendation is in part based on consistency in the egg|ripe ovary versus muscle Se models for species within the genera *Oncorhynchus* and *Salvelinus*, which are based on robust datasets spanning a wide range of muscle Se concentrations (Table 4). This recommendation is also consistent with observations that, for a wide range of toxicants, species within the same genus have similar sensitivities (USEPA, 1985). However, if the regression for a species of interest is not reported and there is not a surrogate model for another species in the same genus, the bluegill model is recommended instead based on its robust dataset and its median slope relative to the other species.

Summary and conclusions

We paired Se concentrations in eggs|ripe ovaries versus muscle and WB tissue for 21 and 7 species, respectively. Significant relationships between Se concentrations in eggs|ripe ovaries and muscle were observed for 12 of the 21 fish species, and for all 7 fish species with paired egg|ripe ovary and WB Se concentration data. In addition, paired WB and muscle Se concentrations were identified for 22 fish species, 17 of which had significant relationships. The latter represents an increase to the muscle-to-WB Se ratios derived in (USEPA, 2016), which include eight fish species in the families Catostomidae, Centrarchidae, and Cyprinidae. The additional muscle-to-WB Se relationships in the present study included species in Ictaluridae, Lotidae, Salmonidae, and Percidae. Although there is variability in egg|ripe ovary Se versus muscle or WB Se relationships among species (e.g., some species are not retained in the pooled models, or some species that are retained in the pooled model have species-specific intercept or slope adjustments), there is high agreement in the behavior of the tissue Se relationships overall. Intrafamily and -order relationships are not strong enough for making assumptions among species based on taxonomic relatedness, but we suggest that this is a feature of the precision of the species-specific models and that, were the error margins wider, there would be even fewer statistical differences between the modeled species. In the absence of a species-specific model reported herein, we recommend using the model for a species in the same genus if available, or otherwise the bluegill model.

The most important observations from this study are further confirmation that oocyte development stage influences ovary Se concentrations (Brix et al., 2025), such that ovary Se concentrations tend to decrease with increasing oocyte development and, when developing muscle-to-egg|ovary Se relationships and WB-to-egg|ovary Se relationships based only on data for eggs or ripe ovaries, much of the variability among several species is reduced. This indicates that some of the variability originally assumed to be due to species-specific differences in between-tissue Se relationships is actually due to variability in oocyte development. The USEPA's ambient water quality criteria document for Se (USEPA, 2016, 2021) includes muscle-to-egg|ovary Se conversion factors and WB-to-egg|ovary Se conversion factors that were used to support development of certain criterion elements, and these conversion factors are often used as a resource for other Se assessments. Importantly, however, the data used to develop those conversion factors are not always based on eggs or ripe ovaries. Given our findings, we suggest the conversion factors developed in our analysis using additional data and constrained to fish with ripe ovaries or eggs are more appropriate for developing tissue Se relationships in support of bioaccumulation model development and other Se assessments.

Although, for a given Se exposure, consideration of only egg or ripe ovary Se concentrations is generally shown to reduce variability when compared to Se concentrations in ovaries of variable ripeness, there can still be relatively high variability in muscle-to-egg|ovary Se conversion factors and WB-to-egg|ovary Se conversion factors because these factors may be concentration-dependent. In other words, the conversion factors are generally not constant over a range of Se exposure concentrations. For this reason, when adequate data are available for a species, more reliable conversion factors may be derived using regression equations rather than from applying a conversion factor derived from data for one set of Se exposure conditions and then applied to another. We note that this is a general recommendation, but it may not always be

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possible to derive statistically significant relationships between Se concentrations in eggs or ripe ovaries versus either muscle or WB tissue (e.g., mountain whitefish).

In summary, the meta-analysis conducted in the present study emphasizes the importance of analyzing Se concentrations in eggs or ripe ovaries and using those concentrations to develop relationships between eggs|ovaries and muscle or WB tissue. Although the terms "eggs" and "ovaries" are often used interchangeably in the peer-reviewed literature, unpublished reports, and technical presentations, it is critical that these terms be applied accurately. We suggest that "eggs" and "ripe ovaries" are acceptable terms to describe samples from actively spawning fish, while the term "ovary" should be reserved for samples collected from pre-spawning fish or fish of unknown spawning status. This has important implications for determining compliance with Se criteria or guidelines based on eggs or ripe ovaries and for the development of between-tissue Se concentration relationships that may be used in the development of site-specific Se models and assessments.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Data, associated metadata, and calculation tools are available in the online supplementary material.

Author contributions

Claire Detering (Conceptualization, Formal analysis, Methodology, Visualization), Kevin Brix (Conceptualization, Methodology), Marko Adzic (Conceptualization, Data curation, Funding acquisition), Barry Fulton (Conceptualization, Methodology), and David DeForest (Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Visualization)

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Conflicts of interest

None declared.

Ethics statement

None.

Disclaimer

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