Appendix A

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Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
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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.

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.

Elizabeth Southerland
Director
Office of Science and Technology

ACKNOWLEDGEMENTS

Technical Analysis Leads

Joseph Beaman, U.S. EPA, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC
beaman.joe@epa.gov (primary contact person)

Gary Russo, U.S. EPA, Office of Water, Office of Science and Technology, Standards and Health Protection Division, Washington, DC
russo.gary@epa.gov

Charles Delos (Retired) U.S. EPA, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC

U.S. EPA Office of Water Reviewers

Elizabeth Behl
Kathryn Gallagher
Lisa Huff
Michael Elias

Intra-Agency Panel Peer Reviewers (2014-2016)

Russell Erickson, Dale Hoff and Charles Stephan, U.S. EPA, Office of Research and Development, Mid-Continent Ecology Division, Duluth, MN

Cindy Roberts, U.S. EPA, Office of Research and Development, Office of Science Policy, Washington, DC

Jim Lazorchak, U.S. EPA, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH

Jeff Gallagher, U.S. EPA, Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics, Washington, DC

Catherine Aubee and Geoff Sinclair, Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Arlington, VA

David Hair, Laura Phillips, and Scott Wilson, U.S. EPA, Office of Water, Office of Wastewater Management, Washington, DC

Rosaura Conde, Ruth Chemerys, and Eric Monschein, U.S. EPA, Office of Water, Office of Wetlands, Oceans, and Watersheds, Washington, DC

Jim Keating. Julianne McLaughlin, and Lars Wilcut and, U.S. EPA, Office of Water, Office of Science and Technology, Washington, DC

Cheryl Atkinson and Frank Borsuk, U.S. EPA Region 3, Philadelphia, PA, and Wheeling, WV

Joel Hansel, U.S. EPA Region 4, Atlanta, GA

Dean (Robie) Anson, Candice Bauer, Katharine Marko, and Angela Vincent, U.S. EPA Region 5, Chicago, IL

Jason Gildea and Lareina Guenzel, U.S. EPA Region 8, Denver, CO

Diane Fleck, Eugenia McNaughton, and Daniel Oros, U.S. EPA Region 9, San Francisco, CA

Lisa Macchio, and Burt Shepard, U.S. EPA Region 10, Seattle, WA

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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).

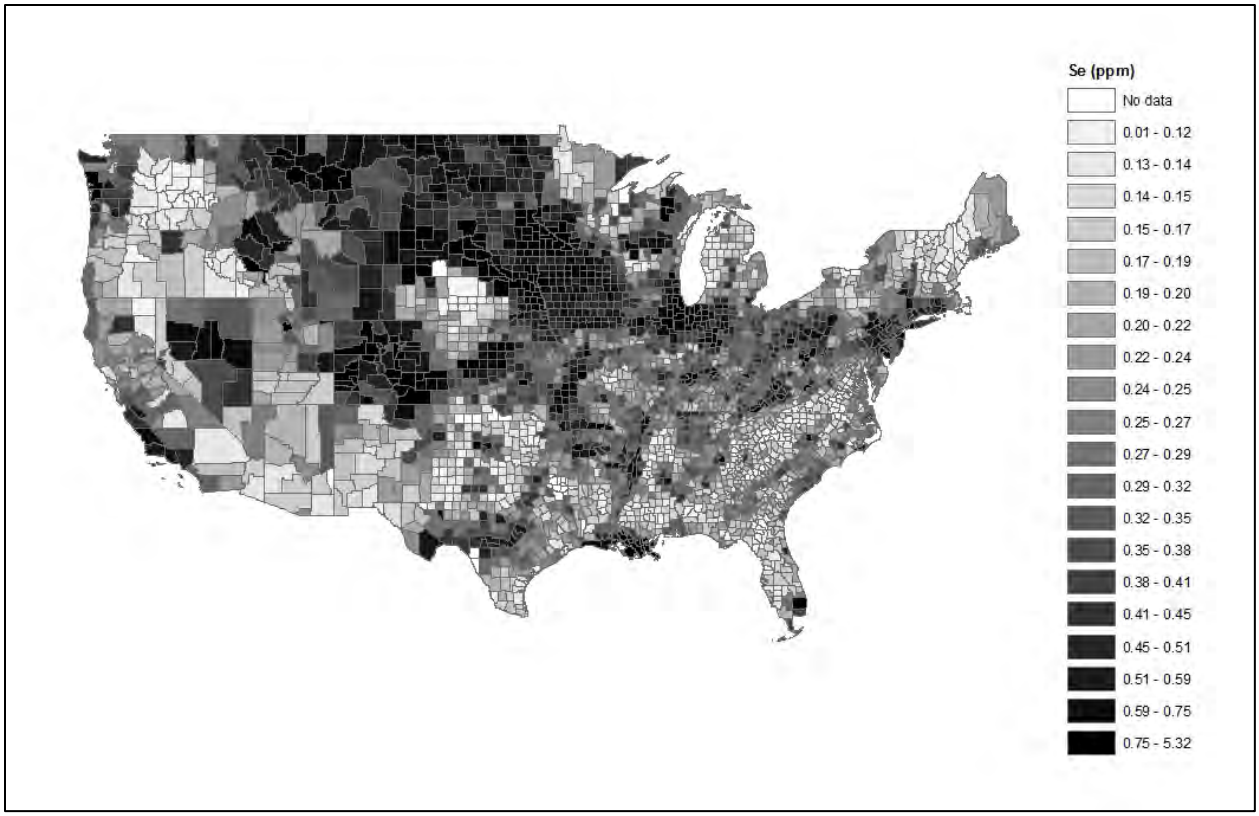


Figure 2.1. Selenium in Surficial Soils and Aquatic Sediments in counties of the Conterminous United States.
U.S. Geological Survey Open-File Report 2004-1001. URL: http://mrdata.usgs.gov/geochem/doc/averages/countydata.htm. Data are available from: http://mrdata.usgs.gov/geochem/doc/groups-cats.htm.

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

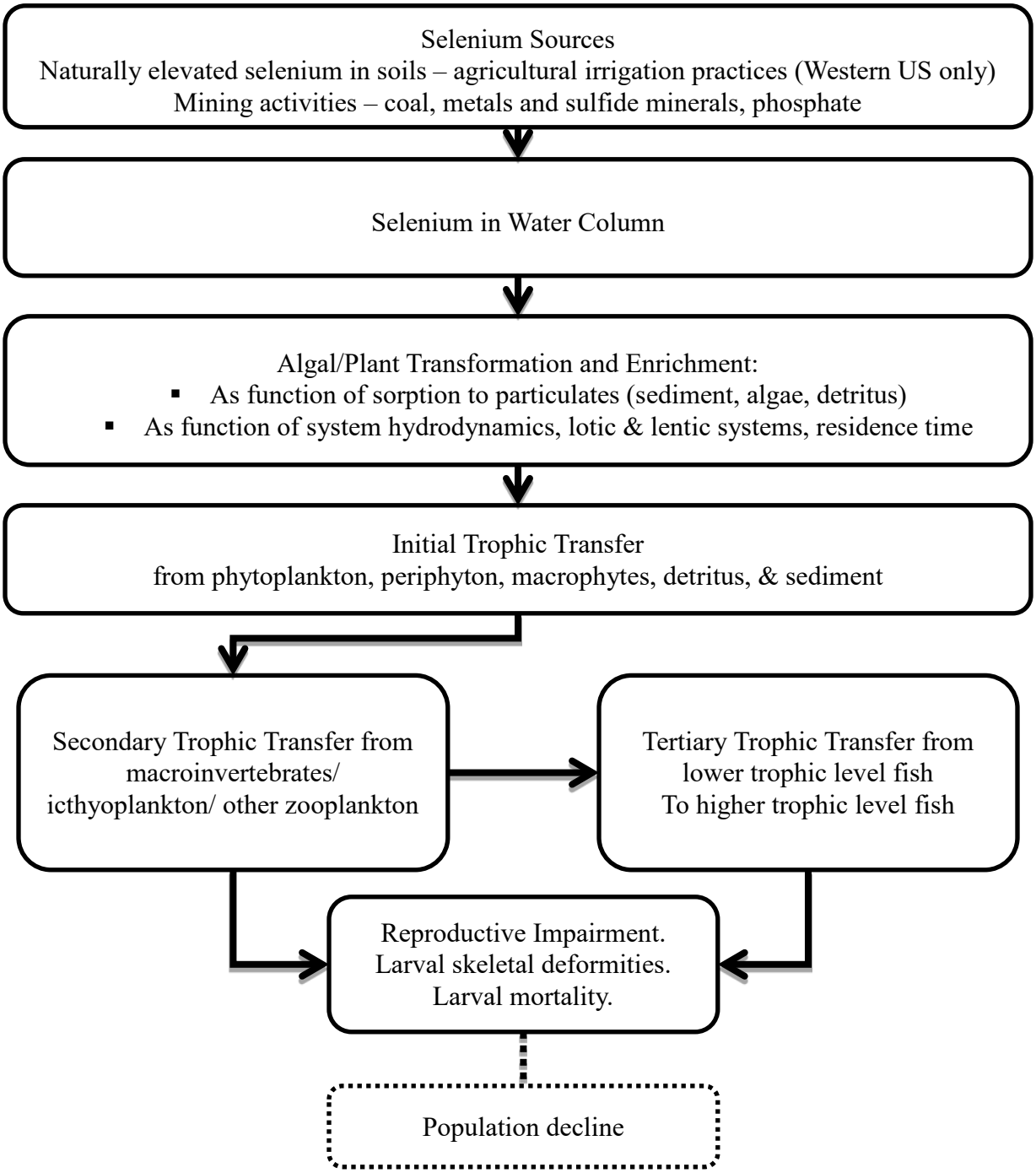


Figure 2.2. Diagram of Selenium Partitioning, Bioaccumulation, and Effects in the Aquatic Environment.

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.

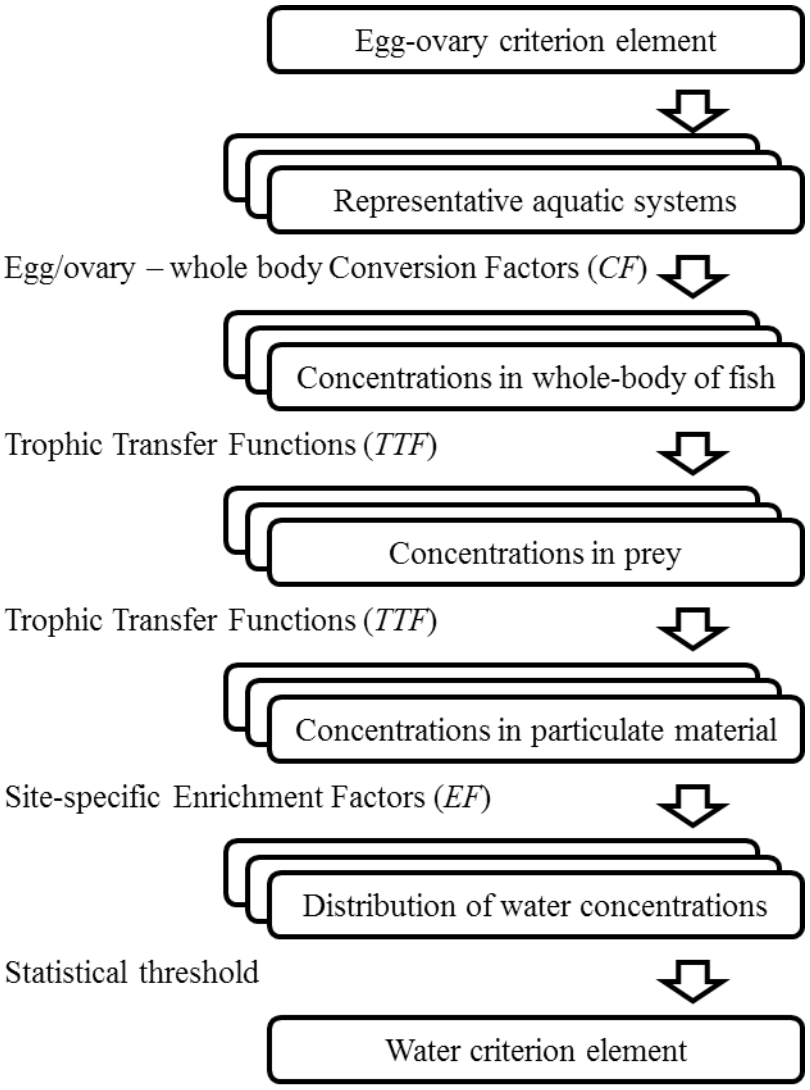


Figure 2.3. Conceptual Model for Translating the Selenium Egg-Ovary Concentration to a Water Column Concentration.

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**.

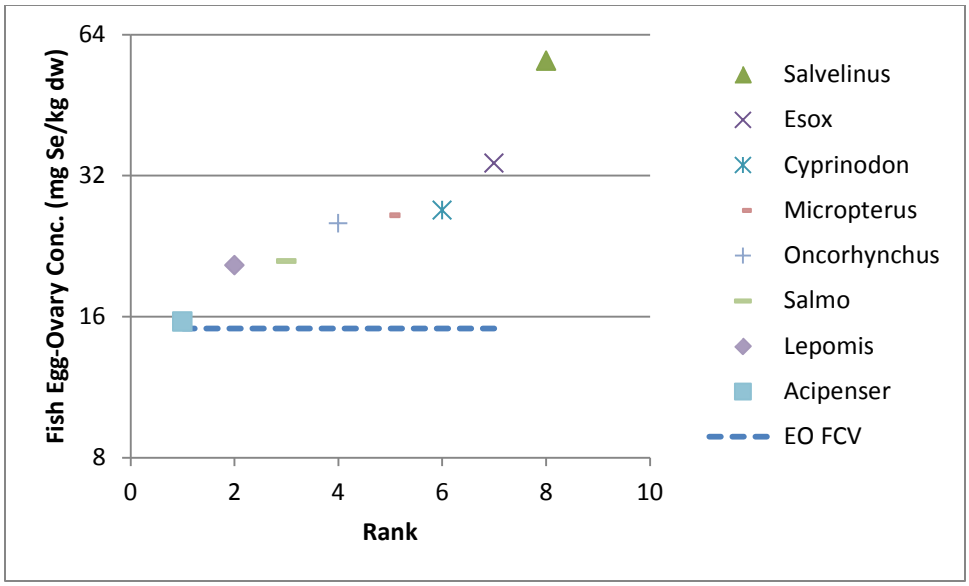


Figure 3.1. Distribution of Reproductive-Effect GMCVs for Fish Measured as Egg-Ovary Concentrations.

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**.

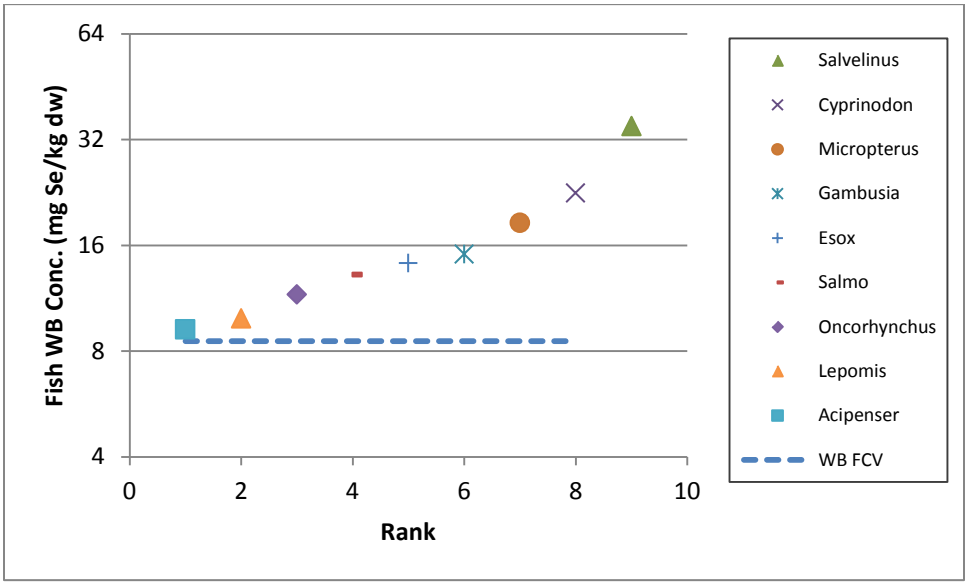


Figure 3.2. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Whole-Body Concentrations in the Original Tests, or Measured as Egg-Ovary Concentrations but Converted to Whole-Body.
(As shown in **Table 3.4**).

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**.

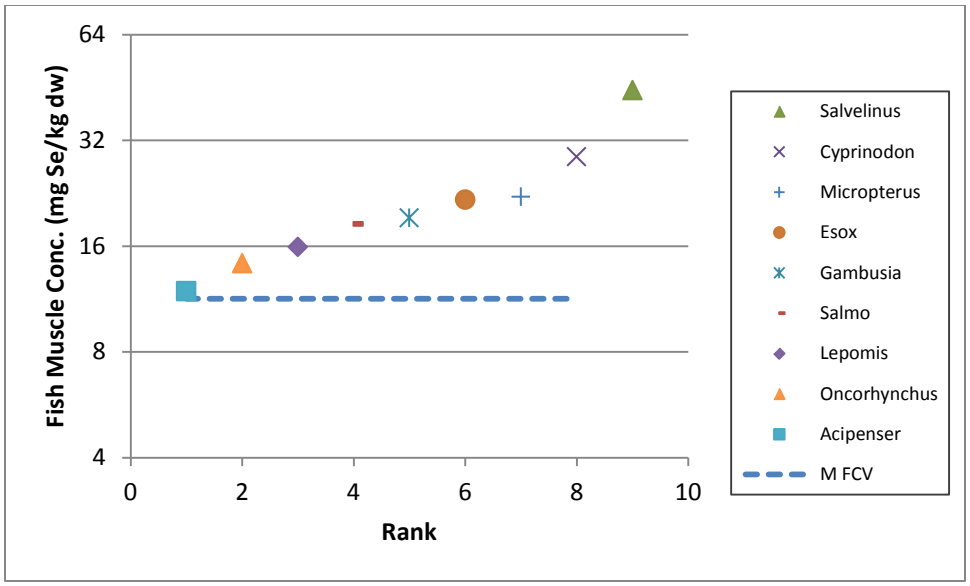


Figure 3.3. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Muscle in the Original tests, or Measured as Egg-Ovary Concentrations but Converted to Muscle Concentrations.
(As shown in **Table 3.6**). (Live-bearer *Gambusia* was converted from WB to muscle).

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.

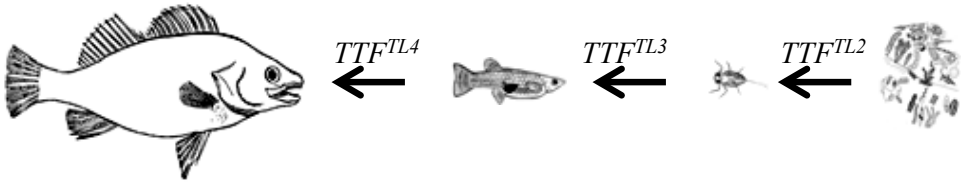
The parameter $TTF^{composite}$ quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system, w_i, the proportion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

A) Three trophic levels (simple):

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

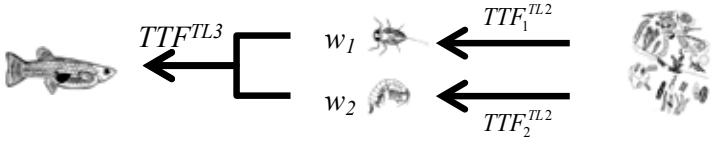
B) Four trophic levels (simple):

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



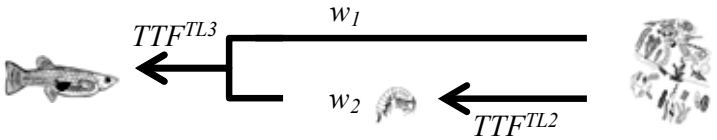
C) Three trophic levels (mix within trophic levels):

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



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):

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

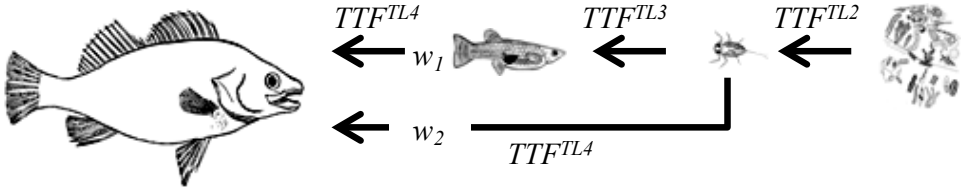


Figure 3.4. Example Aquatic System Scenarios and the Derivation of the Equation Parameter $TTF^{composite}$.

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 (%)

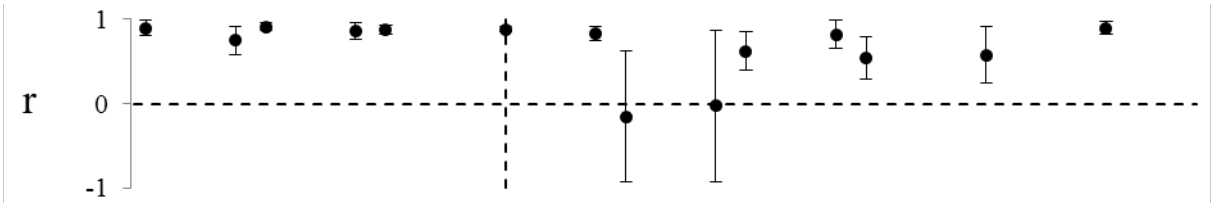
IR = Ingestion rate (g/g-d)

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

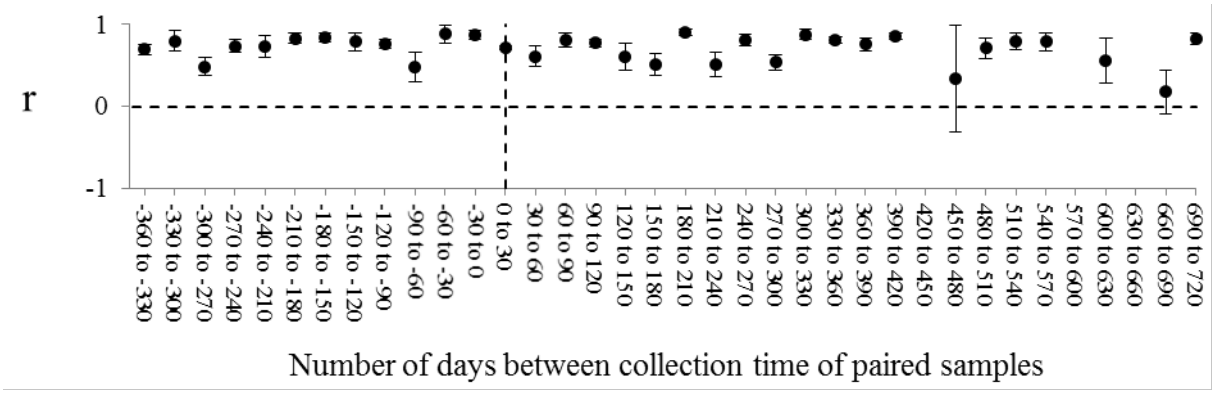


Figure 3.5. Effect of Relative Sample Collection Time on Correlation Coefficients of Selenium Measurements in Particulate Material, and Invertebrate and Fish Tissue. Error bars indicate the 95% confidence interval of r calculated using Fisher's r to z transformation. Horizontal dashed line indicates 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**.

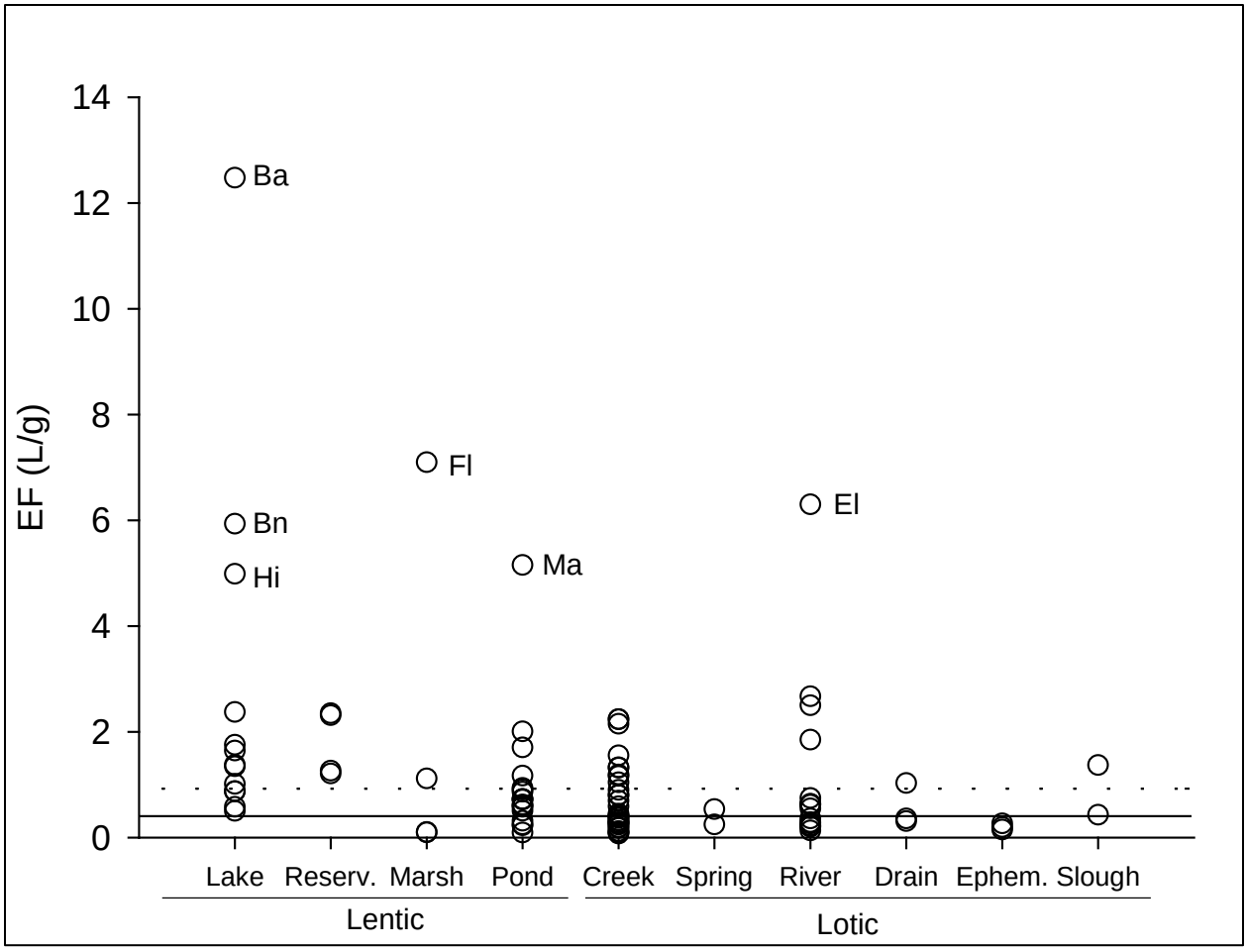


Figure 3.6. Enrichment Factors (*EF*) for 96 Aquatic Sites Derived from Published Studies and Organized by Waterbody Type.
The dashed line represents the median *EF* for the 39 lentic sites (0.9 L/g), and the solid line represents the median *EF* for the 57 lotic sites (0.4 L/g). See text for information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

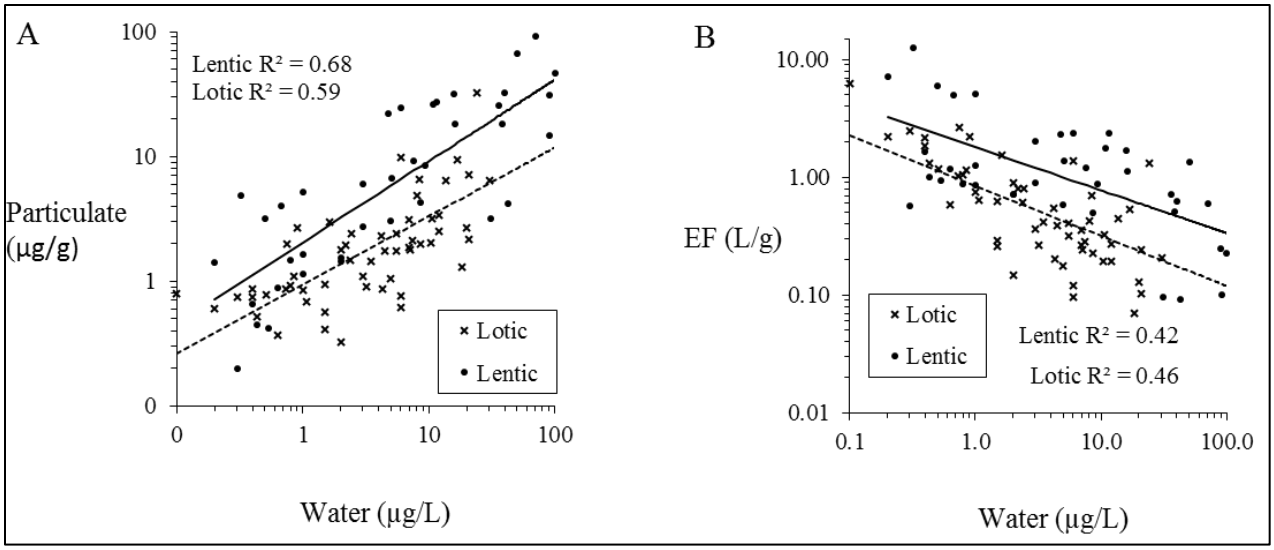


Figure 3.7. The Relationship between C_{water} and $C_{particulate}$, and C_{water} and *EF* for the 39 Lentic and 57 Lotic Aquatic Systems.
A: Relationship between C_{water} and $C_{particulate}$ by site category.
B: Relationship between C_{water} and *EF* by site category.
Solid line, ordinary least 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.

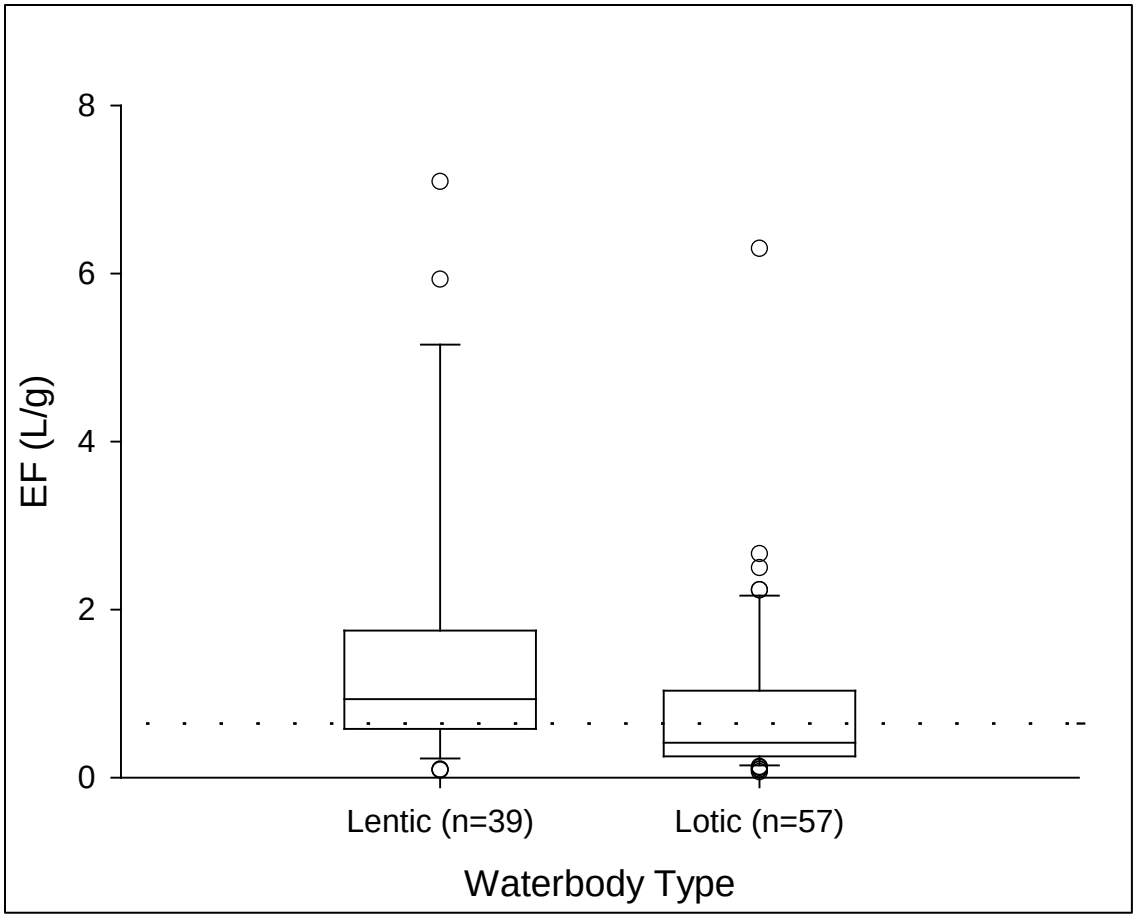


Figure 3.8. Distribution of *EF* Values for the Same 96 Aquatic Systems.
(As shown in **Figure 3.6** and **Figure 3.7** grouped by lentic and lotic aquatic system categories). Boxes show the 25th centile, median, and 75th centile *EF* values; whiskers show the 10th and 90th centiles. Circles represent *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.

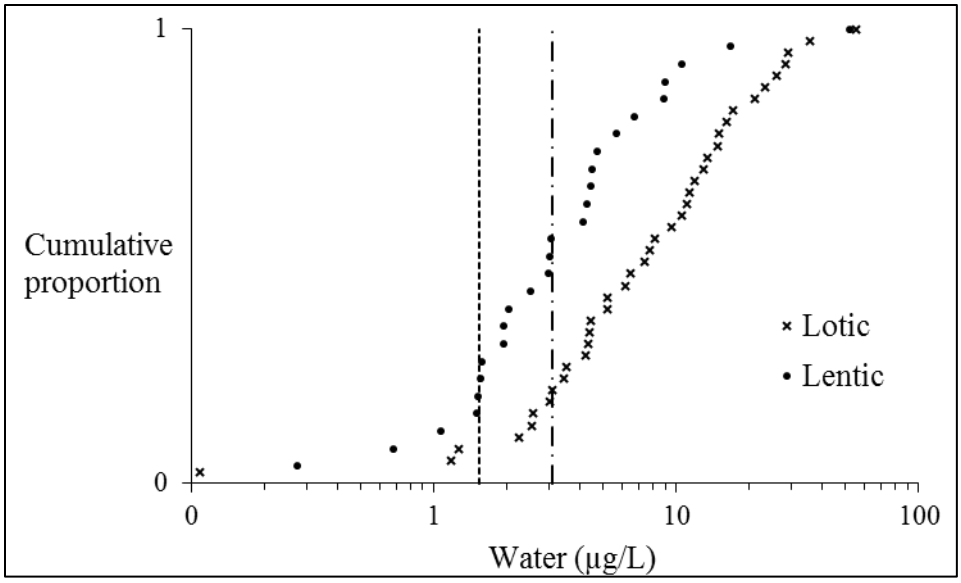


Figure 3.9. Probability Distribution of the Water Column Concentrations Translated from the Egg-Ovary Criterion Element at 26 Lentic and 39 Lotic Aquatic Sites.
Dashed and dash-dot lines show the 20th percentiles of the lentic and lotic distributions, respectively.

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.

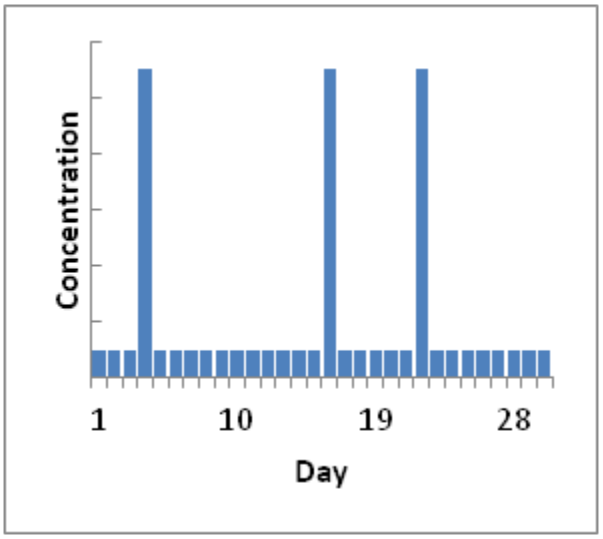


Figure 3.10. Illustration of Intermittent Spike Exposure Occurring for a Certain Percentage of Time (e.g., 10%) Over a 30-Day Period, and Background Exposure Occurring for the Remaining Percentage of Time (e.g., 90%).

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.

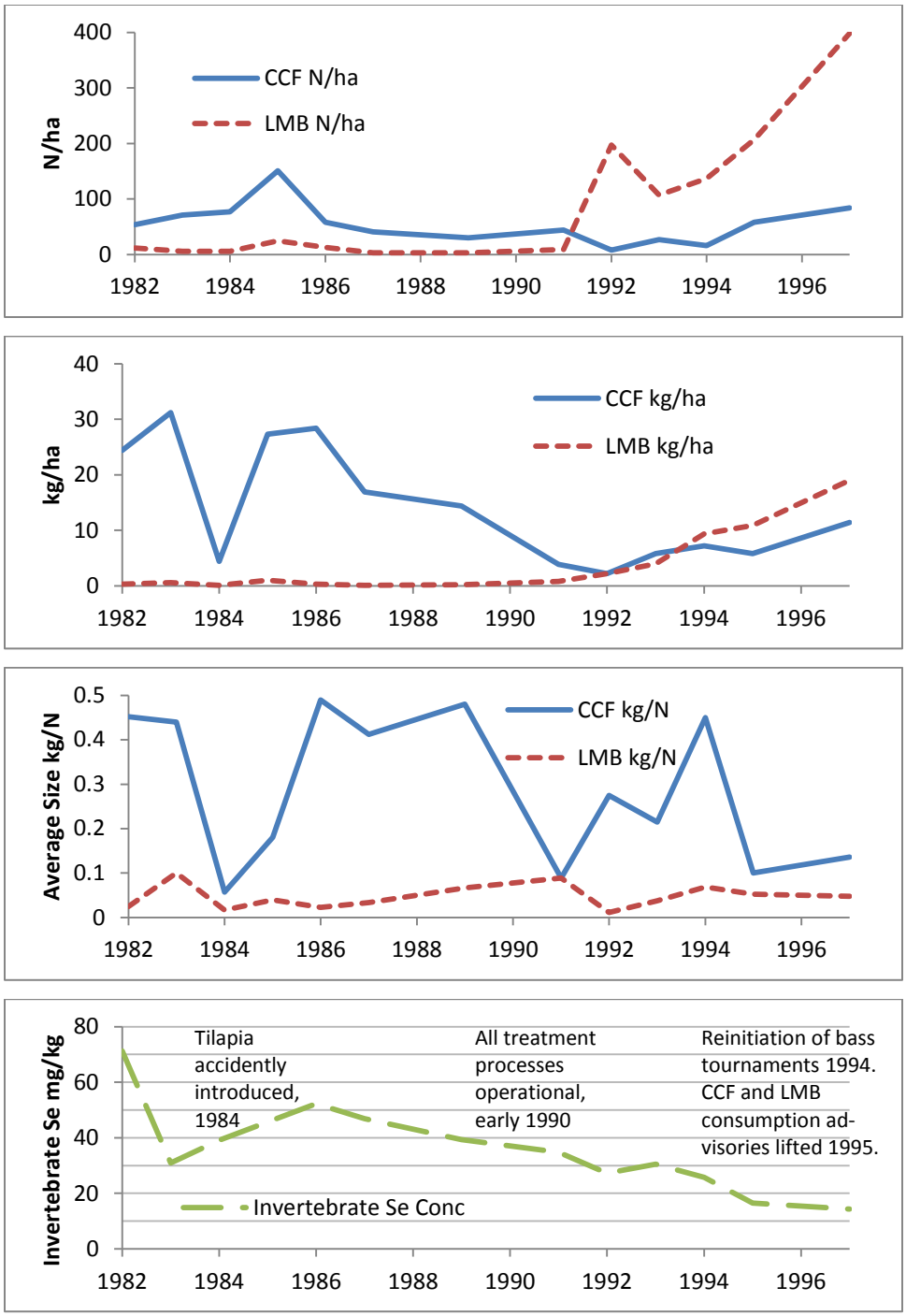


Figure 6.1. Crutchfield (2000) Observations of Channel Catfish (CCF) and Largemouth Bass (LMB) in Hyco Reservoir Beginning a Few Years after Populations of Largemouth Bass had been Reduced by Se Contamination.
(A) number of individuals/ha, (B) mass/ha, (C) mass/ha divided by number/ha, yielding average 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**.

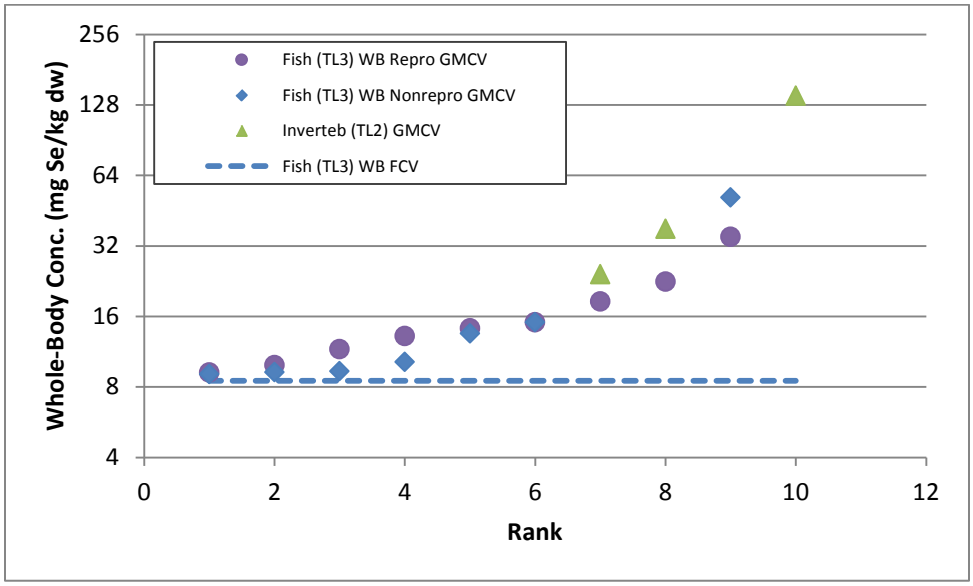


Figure 6.2. Distribution of Fish Reproductive Effect GMCVs from Figure 3.2 and Distribution of Fish Nonreproductive Effect GMCVs and Invertebrate GMCVs.

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.

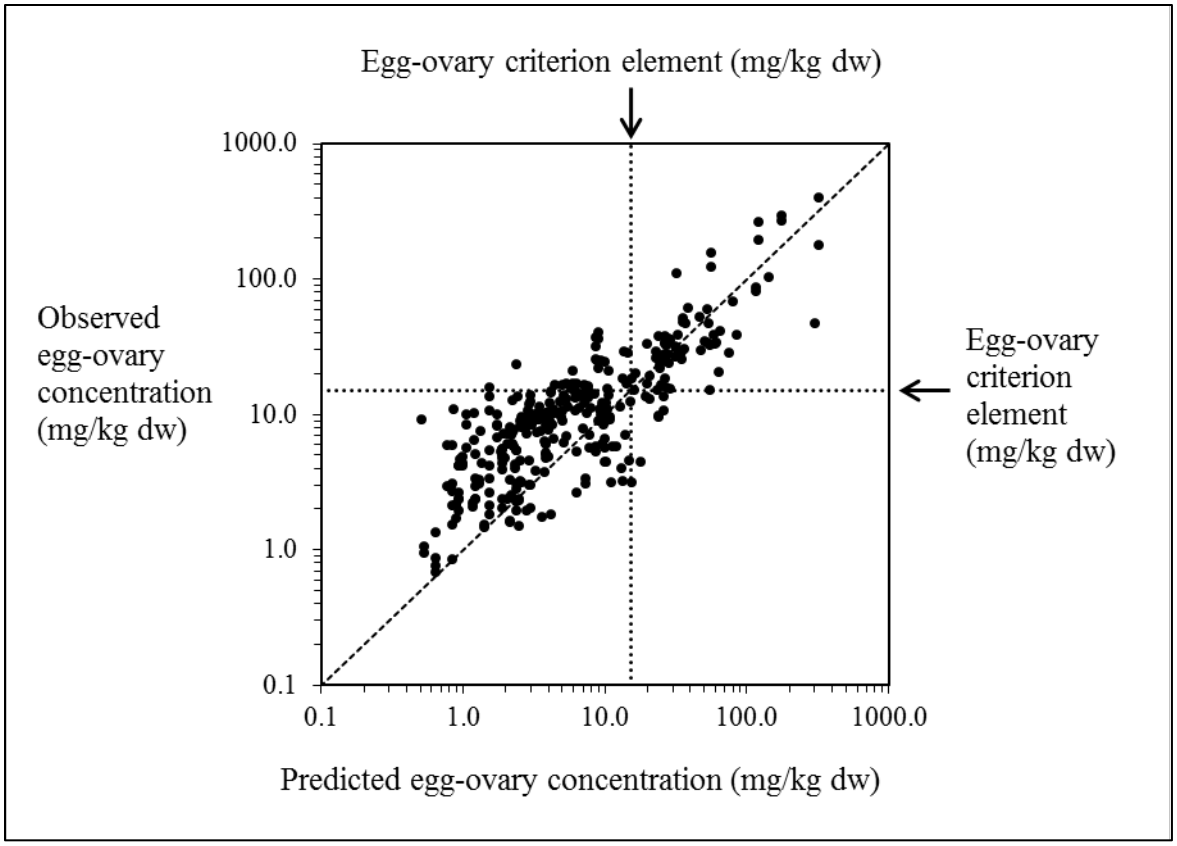


Figure 6.3. Scatter Plot of Predicted Versus Measured Concentrations of Selenium in Fish. Solid line shows unity y = x line; dashed lines show the egg-ovary criterion element value.

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.

LIST OF APPENDICES

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.

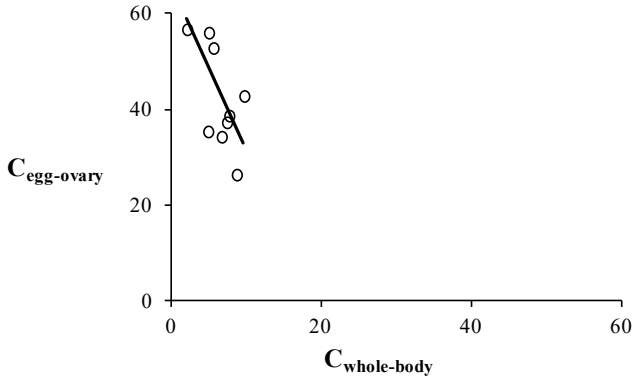
2.2 CF values calculated directly from whole-body and egg-ovary selenium measurements

$C_{whole\text{-}body}$ = 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



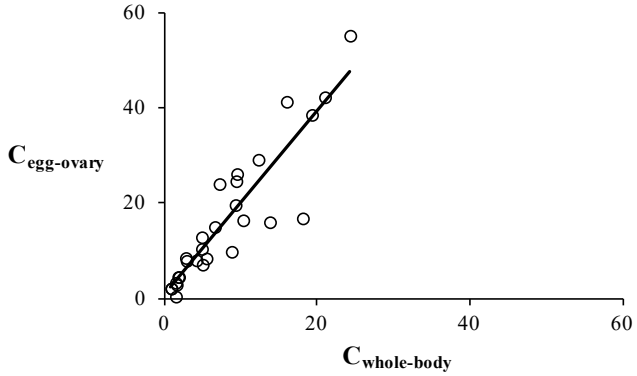
Median ratio: 6.29

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

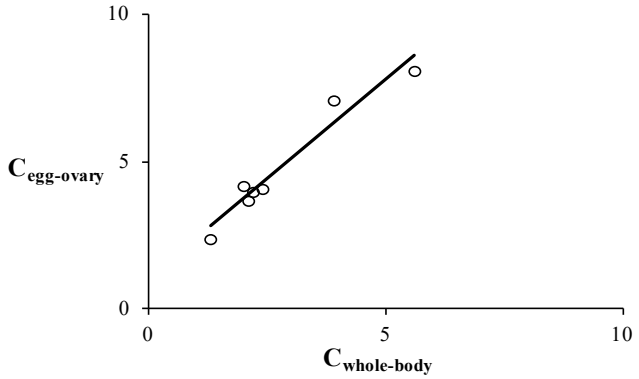


Median ratio: 2.13

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



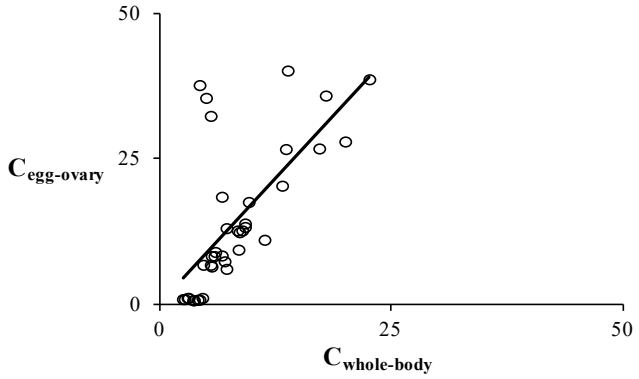
Median ratio: 1.82

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

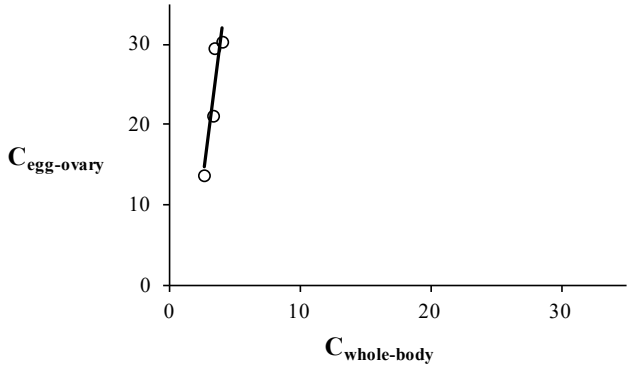


Median ratio: 1.45

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



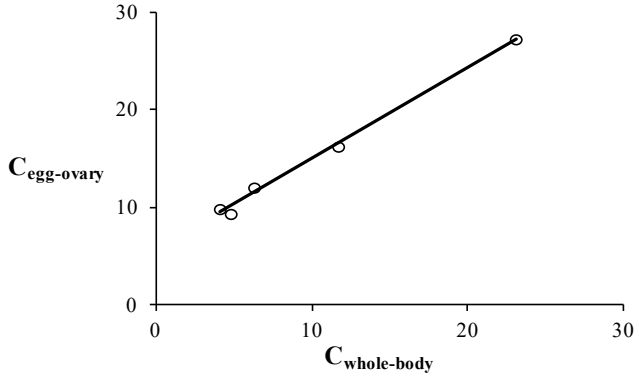
Median ratio: 6.98

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

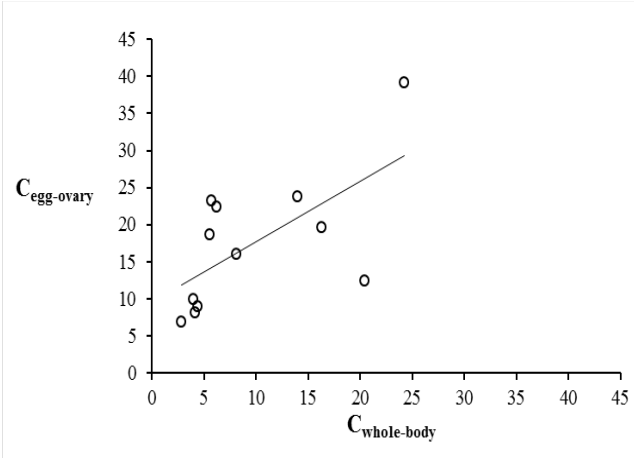


Median ratio: 1.92

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



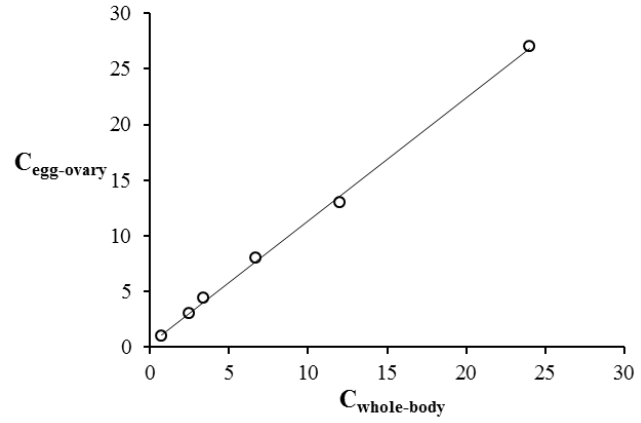
Median ratio: 1.99

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*)



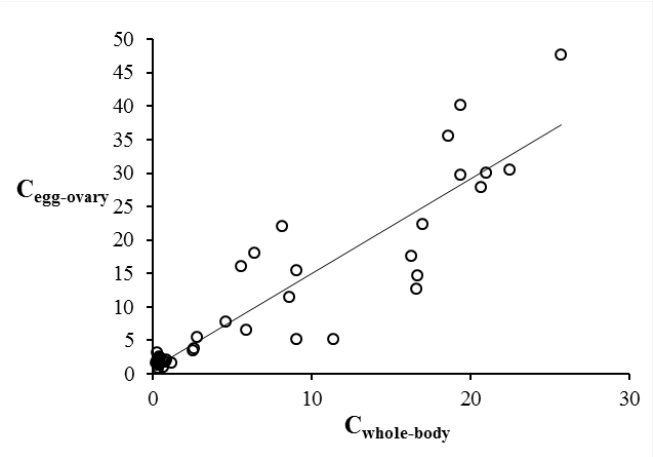
Median ratio: 1.20

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



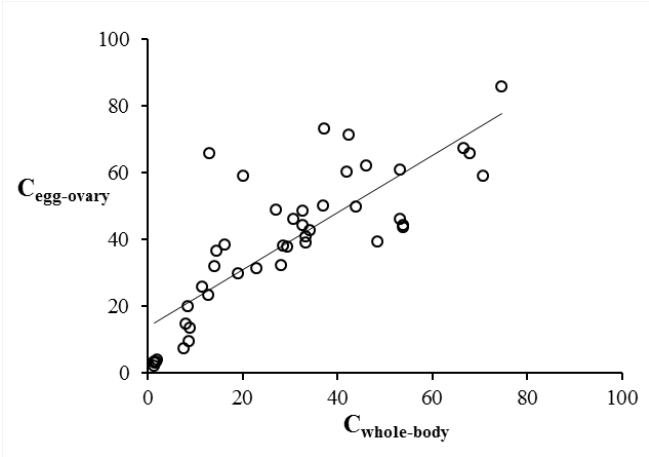
Median ratio: 1.96

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*)

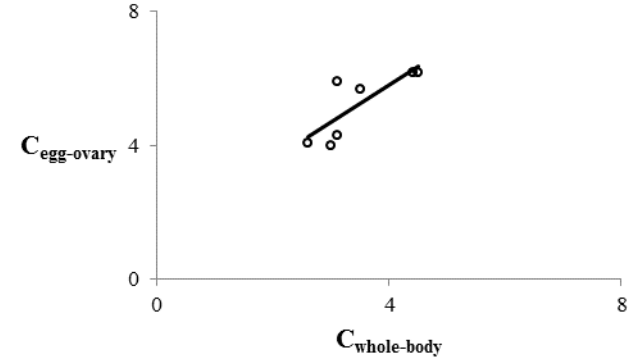


Median ratio: 1.40

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



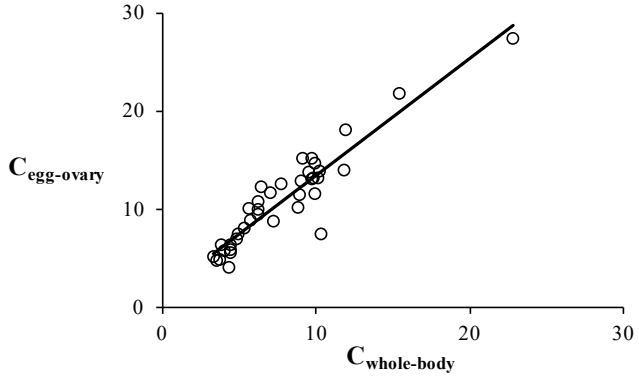
Median ratio: 1.41

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*)

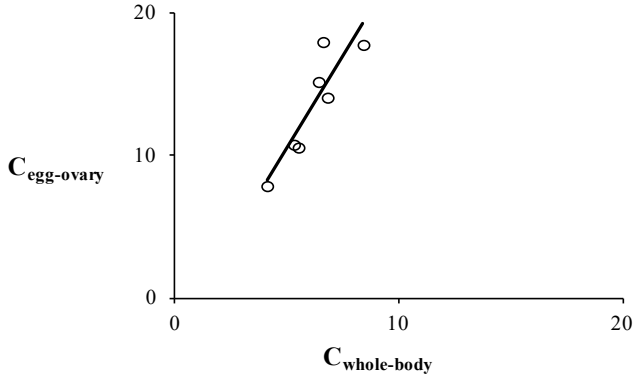


Median ratio: 1.45

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

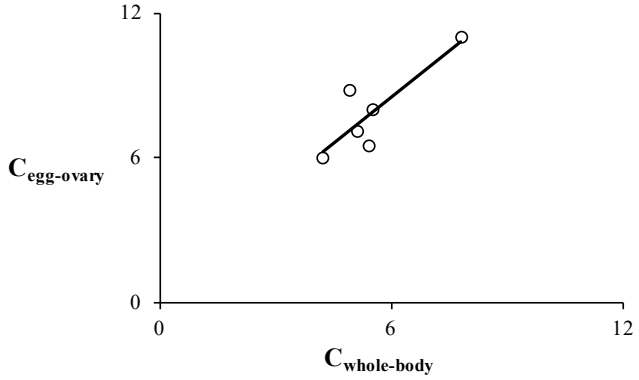


Median ratio: 2.07

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



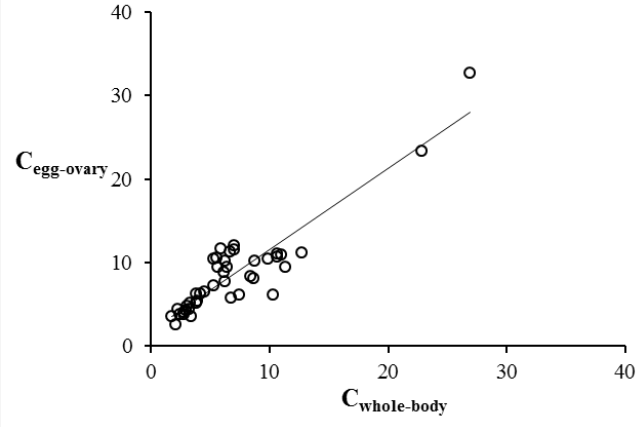
Median ratio: 1.42

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

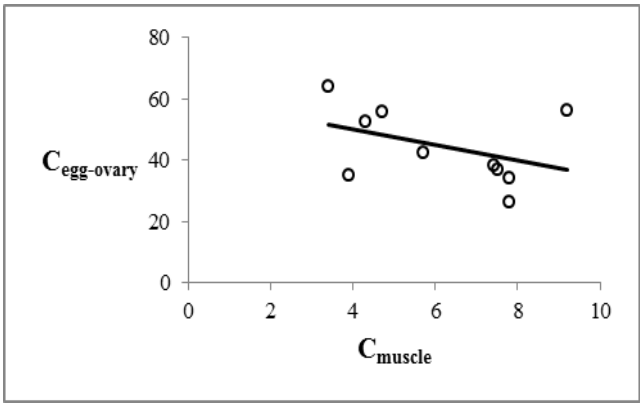
2.3 Muscle to egg-ovary conversion factors

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



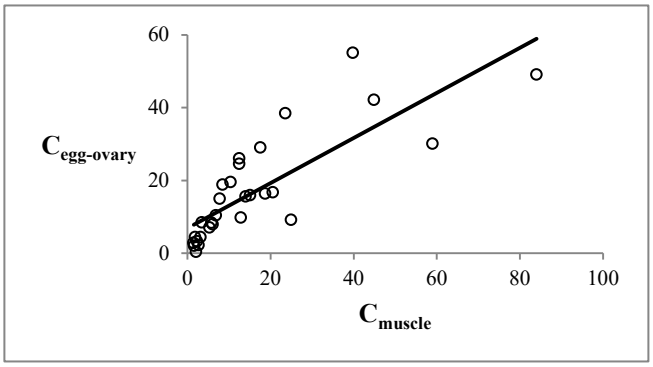
Median ratio: 6.84

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

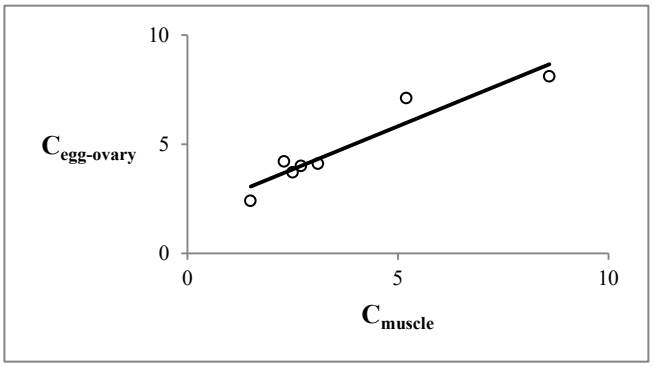


Median ratio: 1.38

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



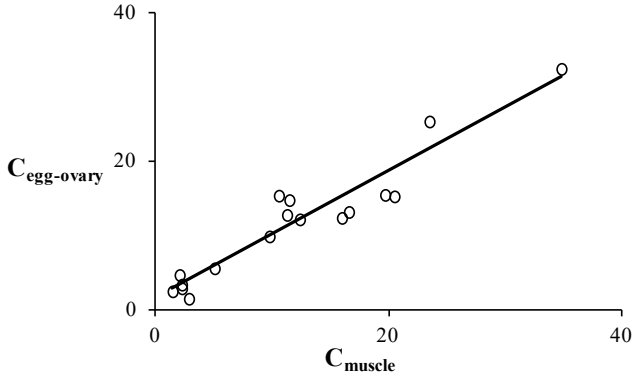
Median ratio: 1.48

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*)

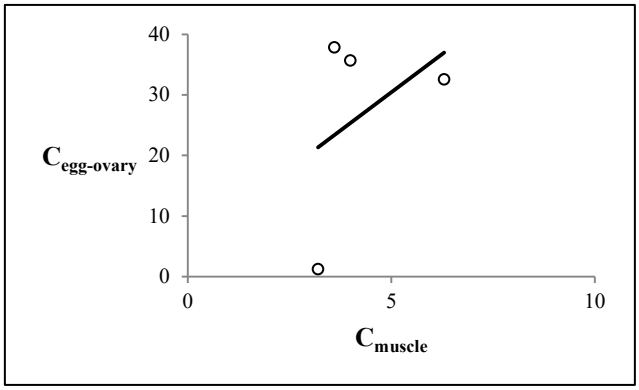


Median ratio: 1.09

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



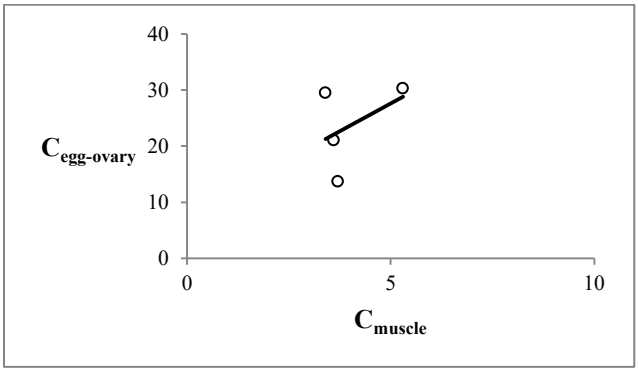
Median ratio: 7.03

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



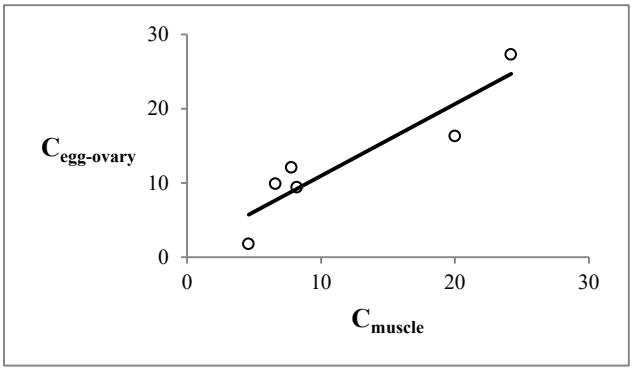
Median ratio: 5.79

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

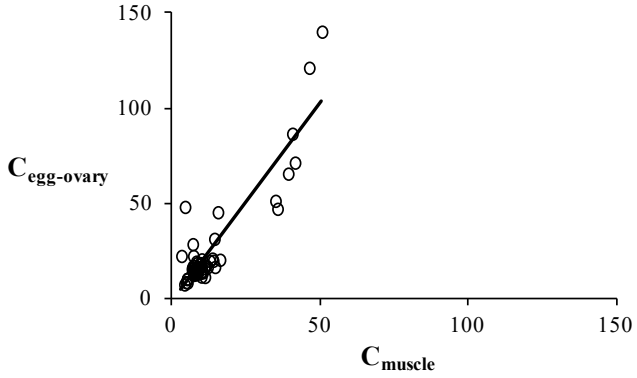
R^2: 0.84
F: 21.7
df: 4
P: 0.007

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

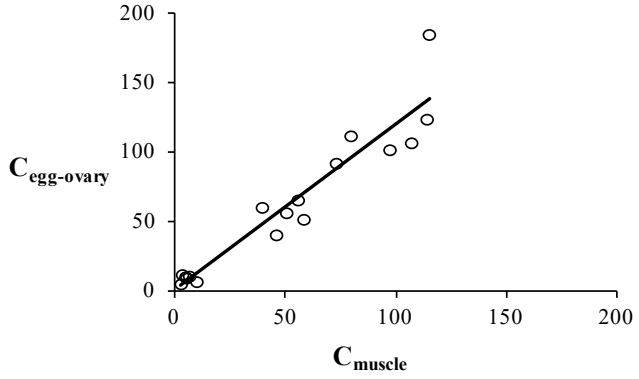


Median ratio: 1.81

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

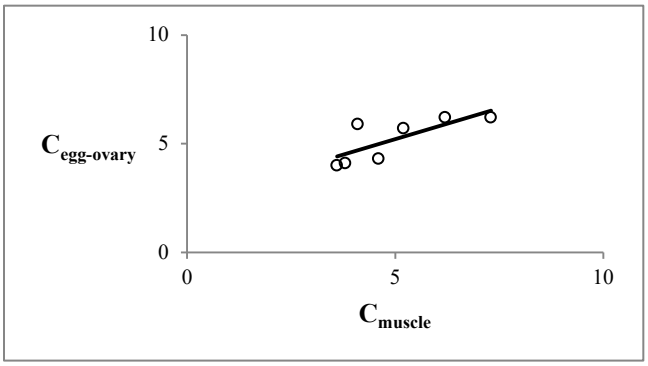


Median ratio: 1.26

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

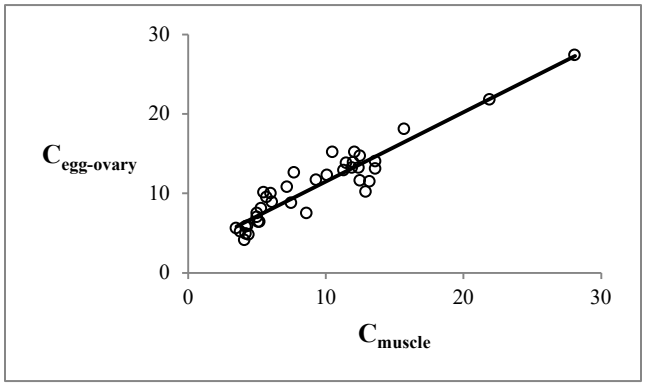
R^2: 0.58
F: 6.92
df: 5
P: 0.036

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

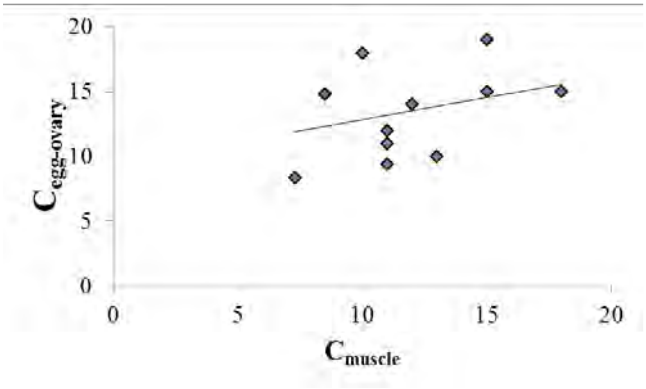


Median ratio: 1.21

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

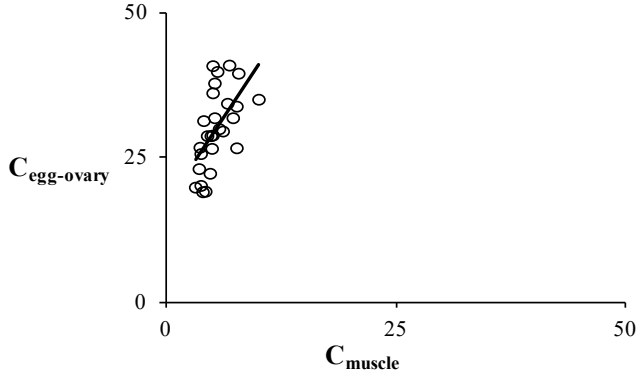


Median ratio: 1.09

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

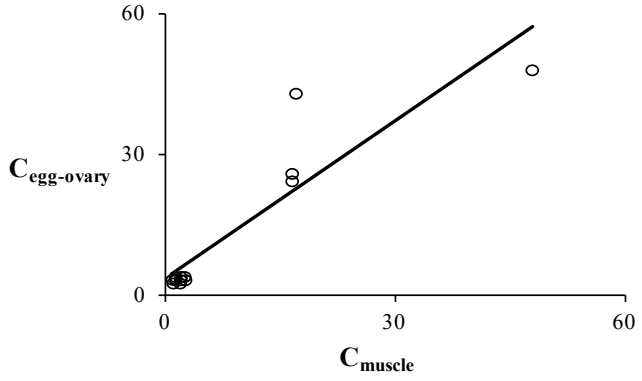


Median ratio: 5.80

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*)

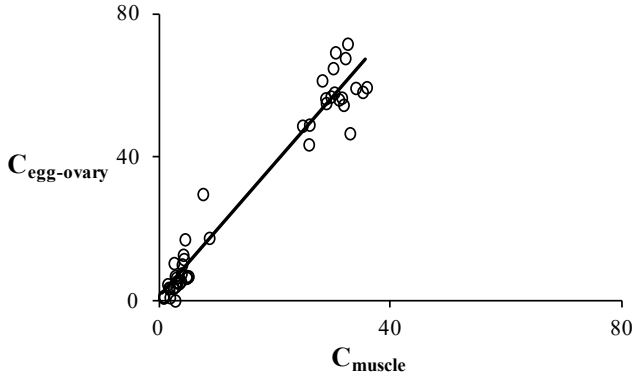
Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.10	11.60	-	11.60	2.83
Casey and Siwik 2000	3.80	10.10	-	10.10	2.66
Casey and Siwik 2000	2.60	0.10	-	0.10	0.04
Casey and Siwik 2000	3.30	4.90	-	4.90	1.48
Casey and Siwik 2000	2.30	3.60	-	3.60	1.57
Casey and Siwik 2000	2.80	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

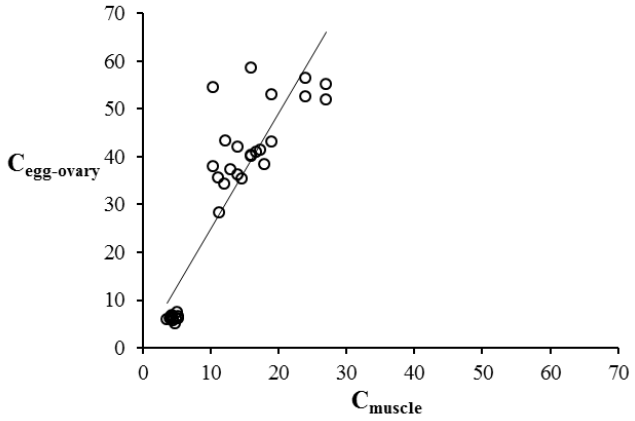
R^2: 0.96
F: 990.0
df: 45
P: <0. 001

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

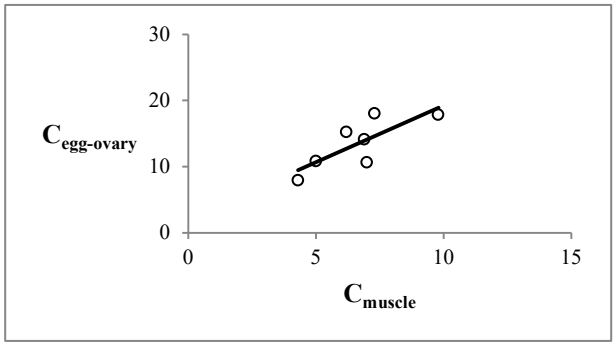
R^2: 0.80
F: 125.6
df: 32
P: <0.001

[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*)

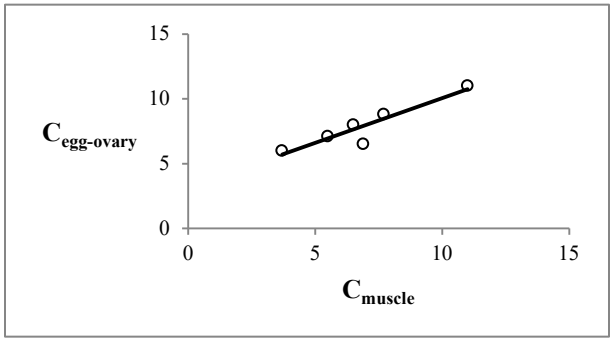


Median ratio: 2.04

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

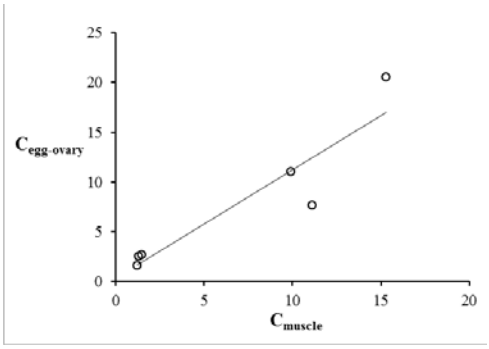


Median ratio: 1.19

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

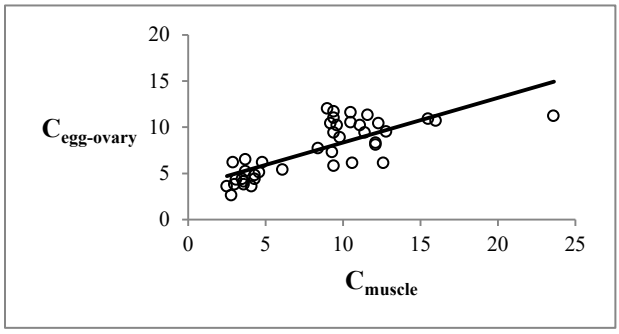
R^2: 0.86
F: 24.96
df: 4
P: 0.006

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

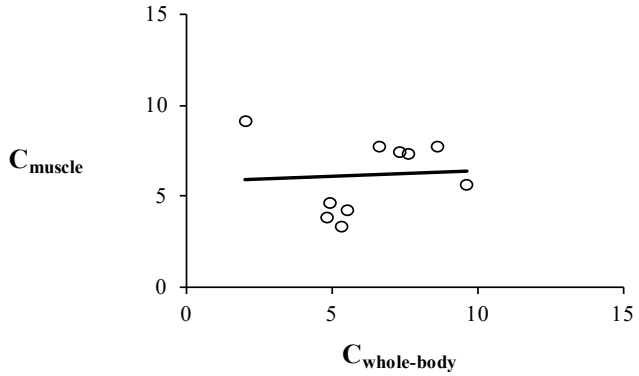
2.4 Muscle to whole-body conversion factors

$C_{whole\text{-}body}$ = 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

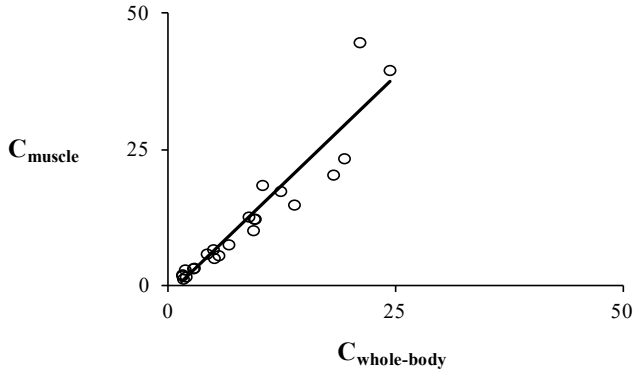
Not used because P > 0.05.

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

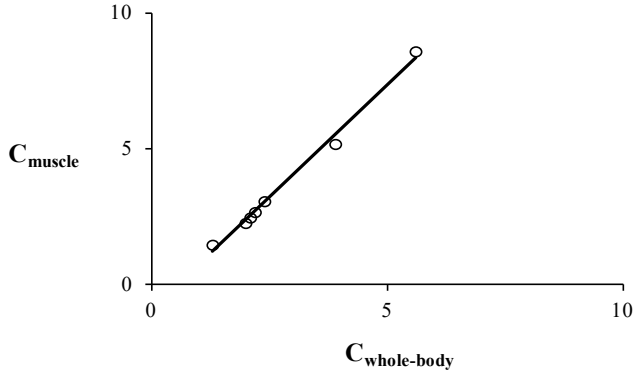


Median ratio: 1.32

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

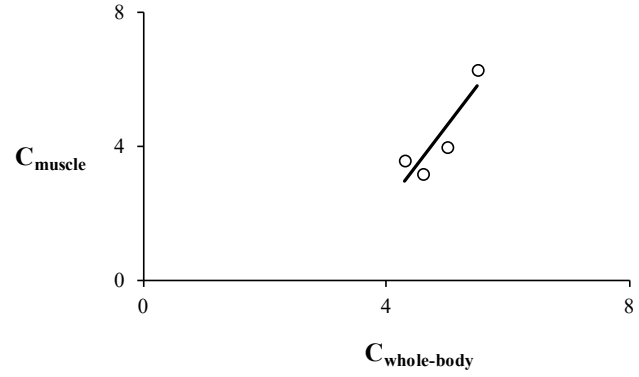


Median ratio: 1.23

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



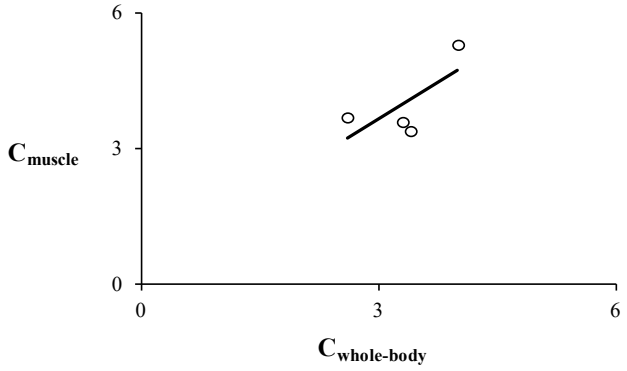
Median ratio: 0.82

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



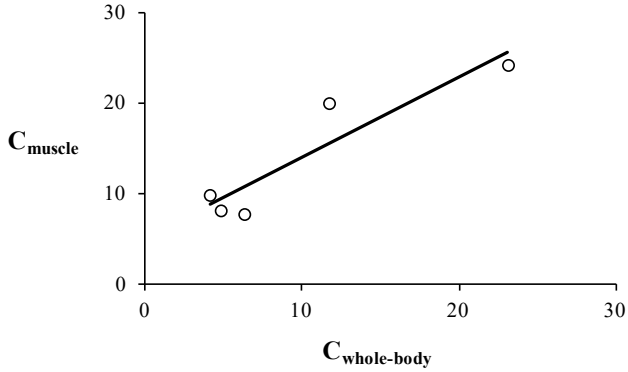
Median ratio: 1.21

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

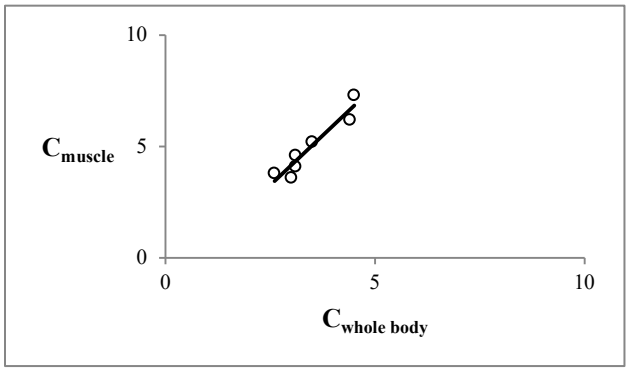


Median ratio: 1.61

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*)

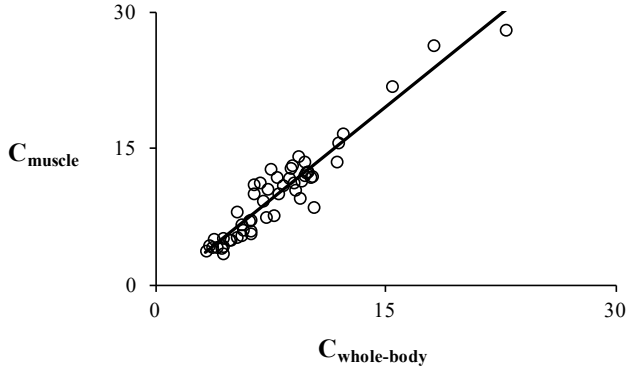
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	22.80	28.10	1.23
Osmundson et al. 2007	8.80	12.90	1.47
Osmundson et al. 2007	15.40	21.90	1.42
Osmundson et al. 2007	4.80	5.00	1.04
Osmundson et al. 2007	5.70	6.10	1.07
Osmundson et al. 2007	4.40	5.20	1.18
Osmundson et al. 2007	3.80	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

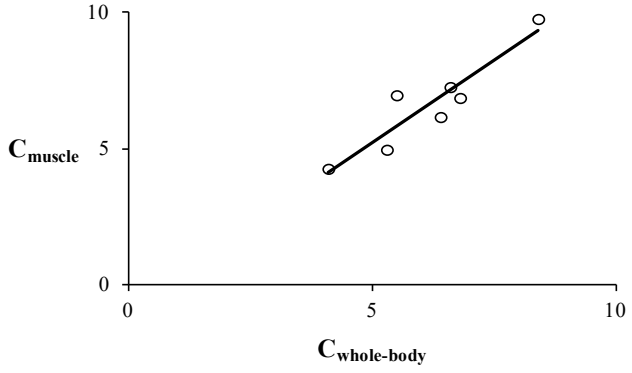


Median ratio: 1.23

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

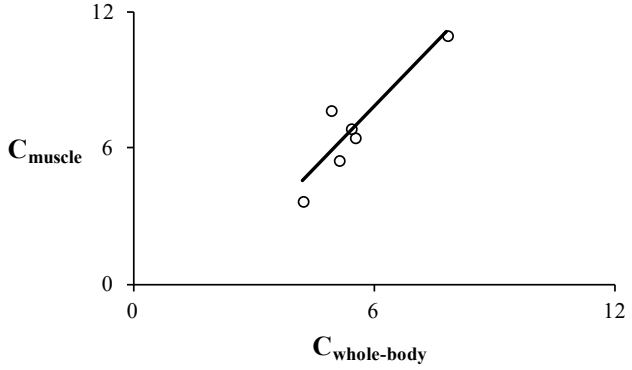


Median ratio: 1.05

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*)

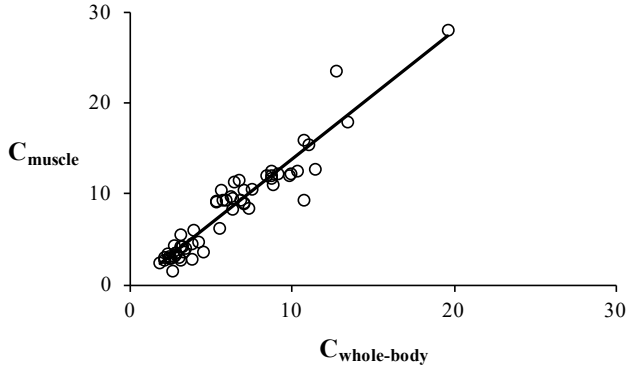
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.80	2.90	0.76
Osmundson et al. 2007	4.20	4.80	1.14
Osmundson et al. 2007	3.30	3.70	1.12
Osmundson et al. 2007	4.50	3.70	0.82
Osmundson et al. 2007	6.30	8.40	1.33
Osmundson et al. 2007	6.80	9.40	1.38
Osmundson et al. 2007	11.00	15.50	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

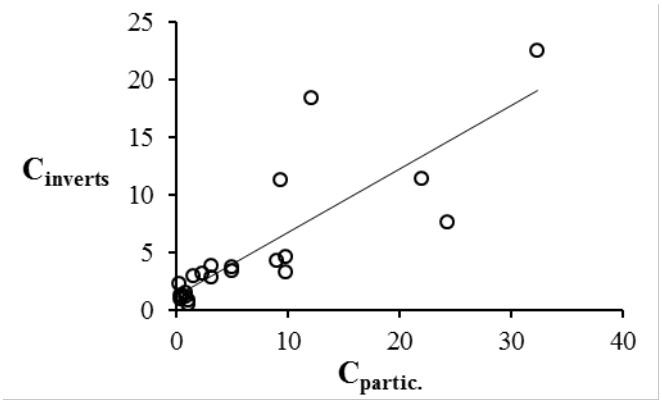
3.3.1 Invertebrates

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)

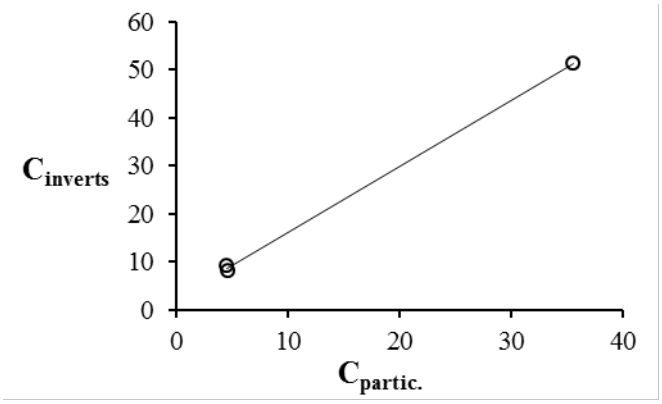


Median ratio: 1.22

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



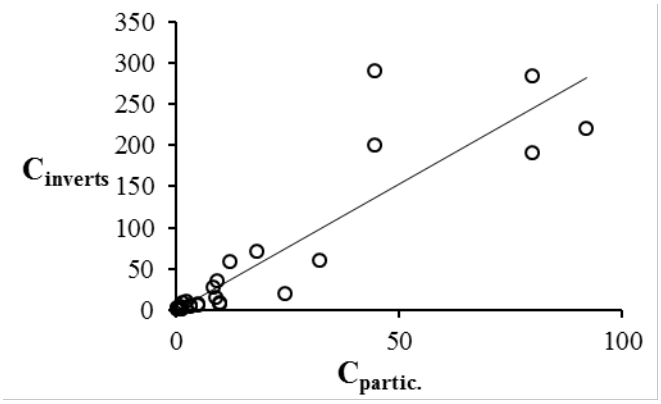
Median ratio: 1.78

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)

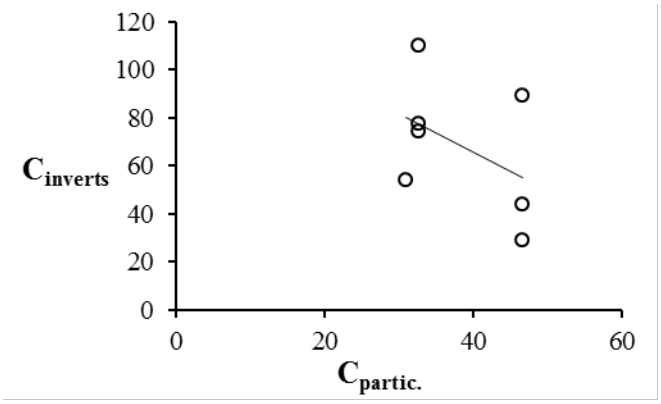


Median ratio: 1.90

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

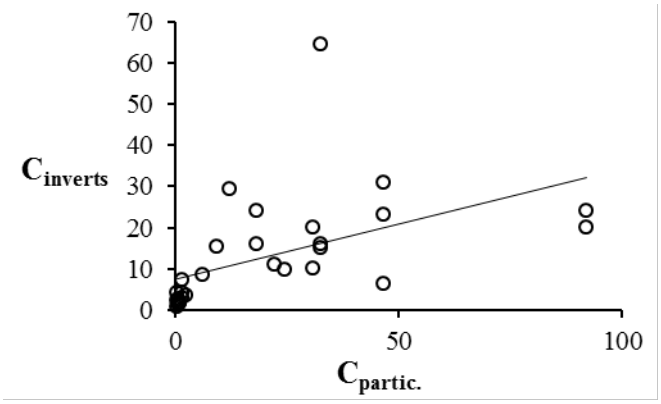
R^2: 0.20
F: 1.24
df: 5
P: 0.36
Not used because P > 0.05 and negative slope.

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

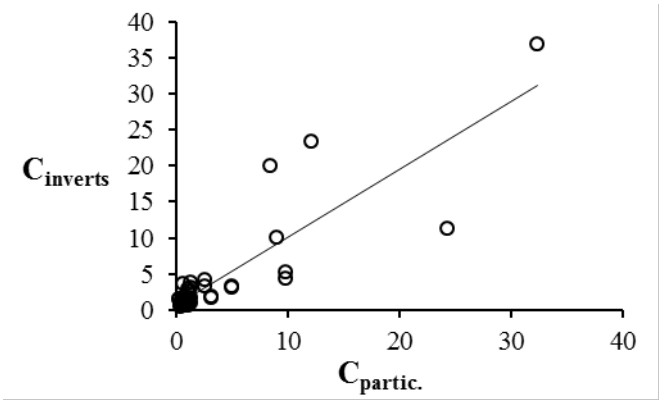
R^2: 0.25
F: 9.17
df: 27
P: < 0.001

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

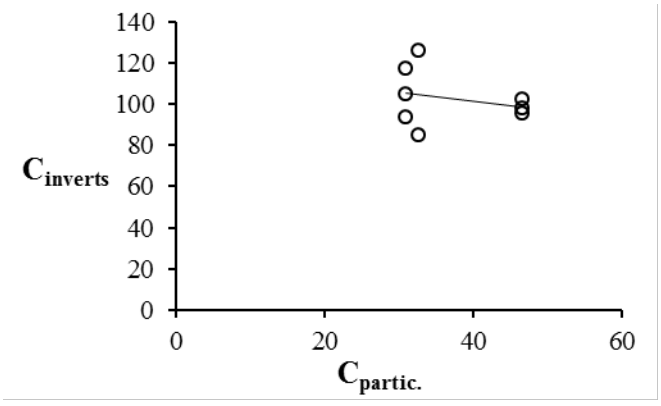


Median ratio: 1.46

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

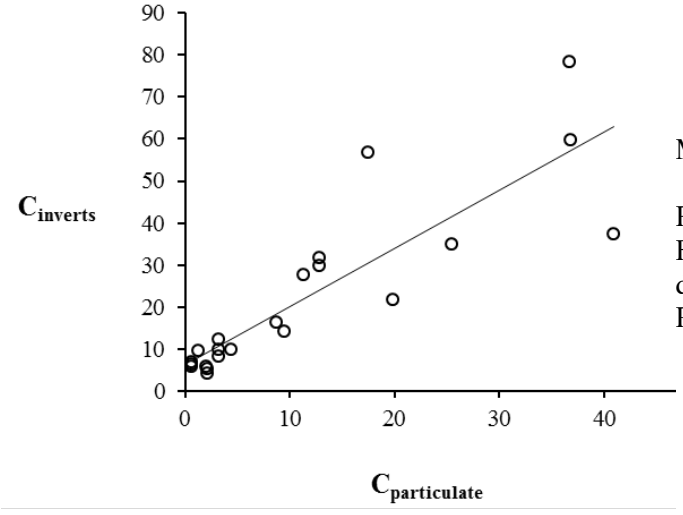


Median ratio: 2.81

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

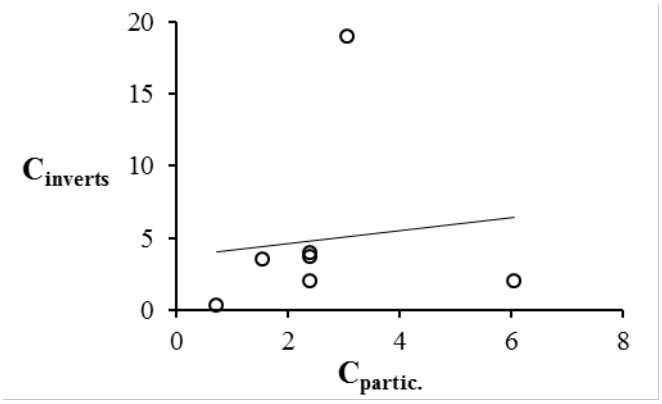


Median ratio: 2.38

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

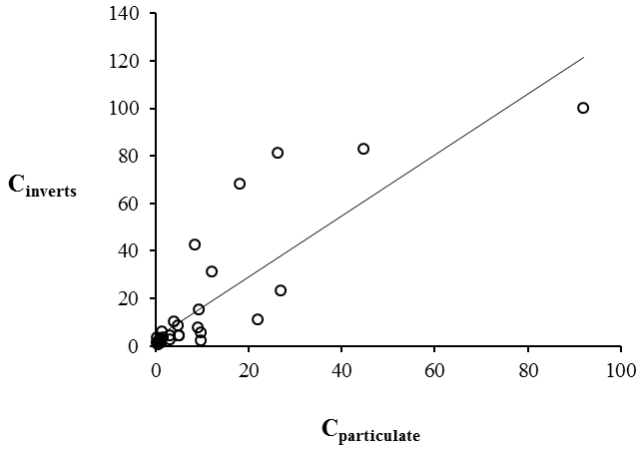
R^2: 0.01
F: 0.07
df: 5
P: 0.93
Not used because P > 0.05.

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)

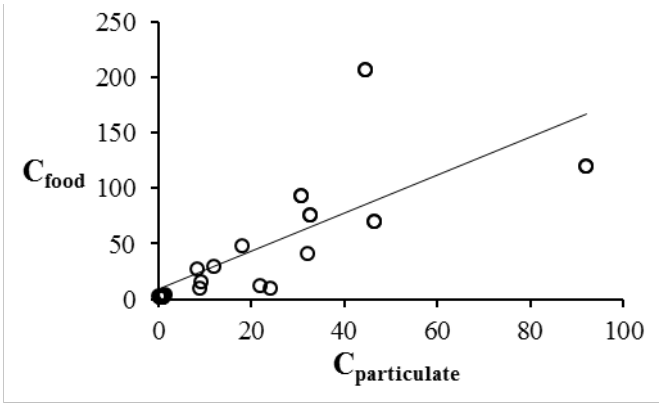
Ratio = $\frac{C_{food}}{C_{part}}$, $\frac{C_{damsel}}{C_{food}}$, or $\frac{C_{dragon}}{C_{food}}$

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

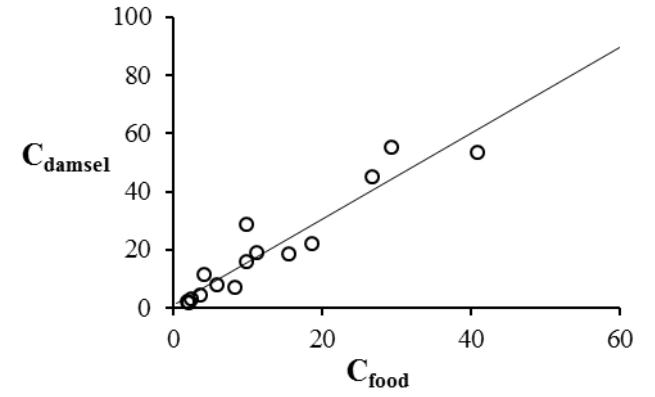
R^2: 0.54
F: 28.7
df: 24
P: < 0.001

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



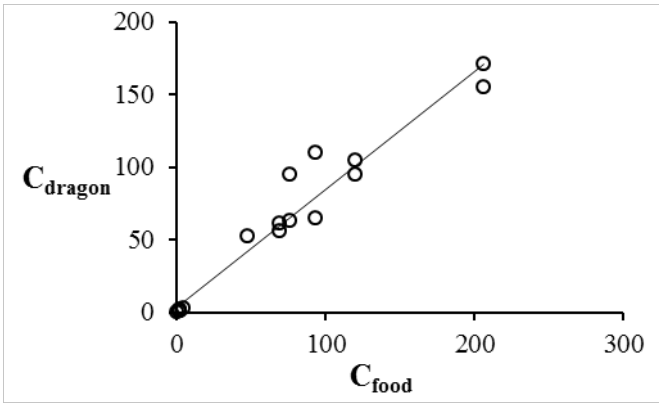
Median ratio: 1.30 x 2.21
(damselfly food to particulate) = 2.88

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

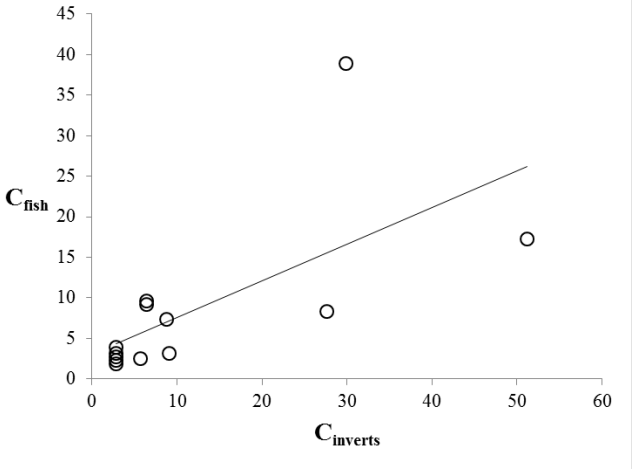
C_{invert} = Selenium concentration in invertebrate tissue (µg/g)
C_{fish} = Average selenium concentration in the whole-body of fish (µg/g)

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*)



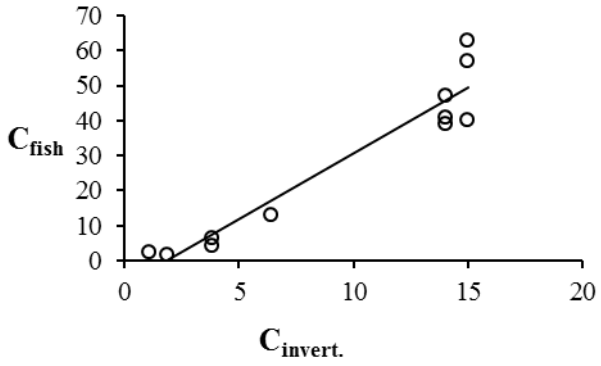
Median ratio: 0.85

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

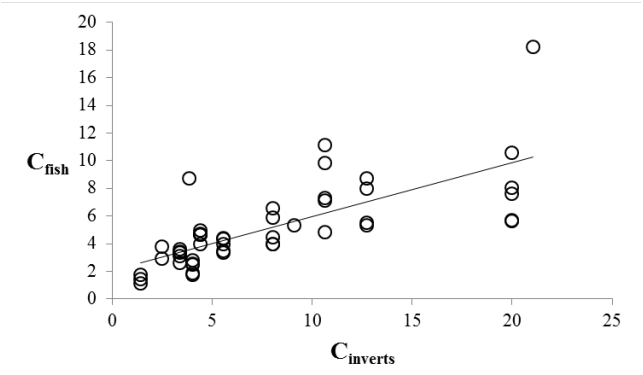
R^2: 0.92
F: 97.9
df: 9
P: < 0.001

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

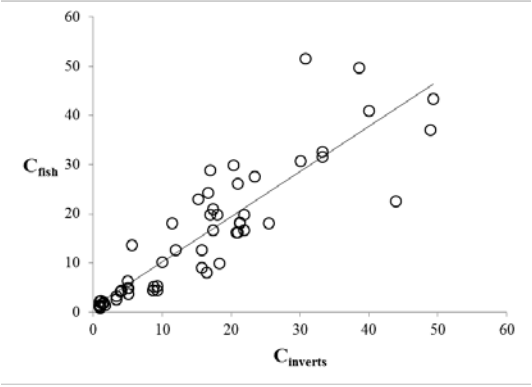
R^2: 0.52
F: 48.97
df: 45
P: < 0.001

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*)

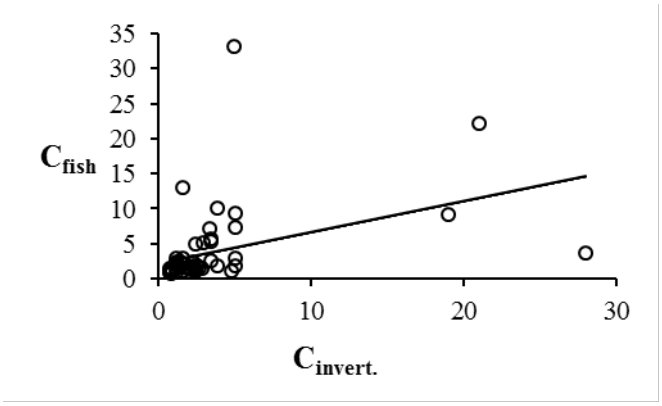
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	AK	0.78	0.94	1.21
Butler et al. 1995	HD1	0.83	0.83	1.01
Butler et al. 1995	HD1	0.83	0.86	1.04
Butler et al. 1995	HD1	0.83	1.20	1.45
Butler et al. 1995	HD1	0.83	1.40	1.70
Butler et al. 1995	DD	0.86	0.64	0.74
Butler et al. 1995	DD	0.86	0.88	1.02
Butler 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

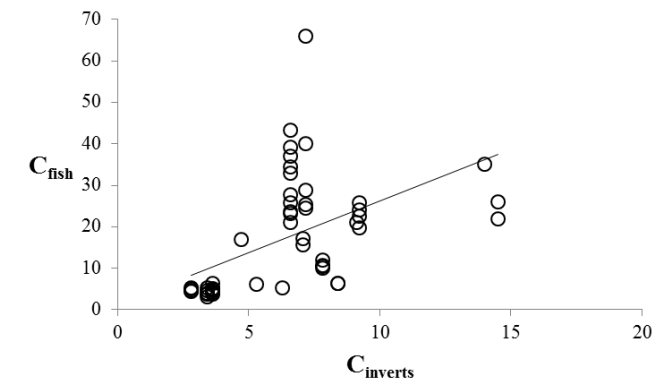
R^2: 0.16
F: 9.6
df: 51
P: < 0.001

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

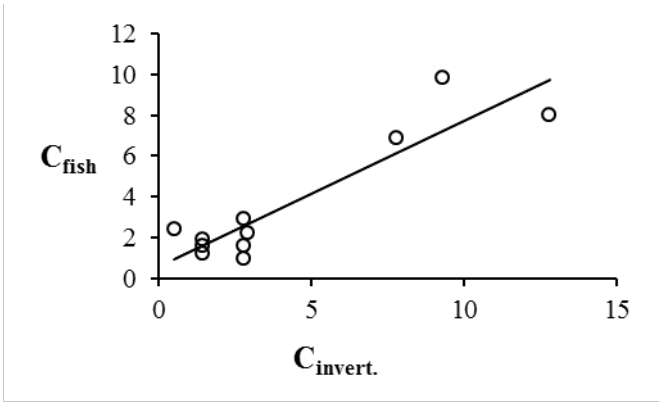


Median ratio: 1.79

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

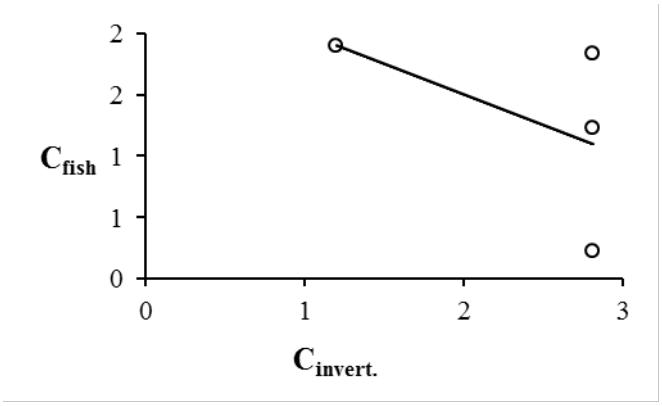


Median ratio: 0.88

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*)

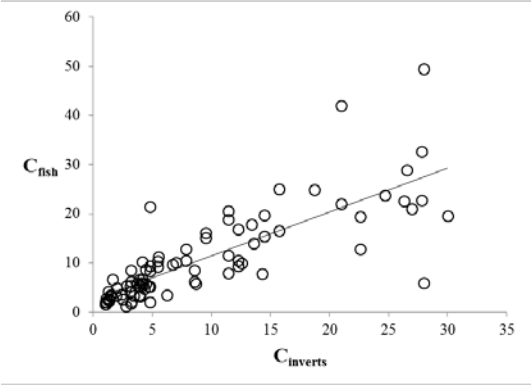
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.00	0.42
Butler et al. 1991	12	2.80	5.40	1.93
Butler et al. 1991	4	3.90	3.30	0.85
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1991	3	6.20	3.50	0.56
Butler et al. 1993	SP2	3.40	3.40	1.00
Butler et al. 1993	SP2	2.75	1.20	0.44
Butler 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

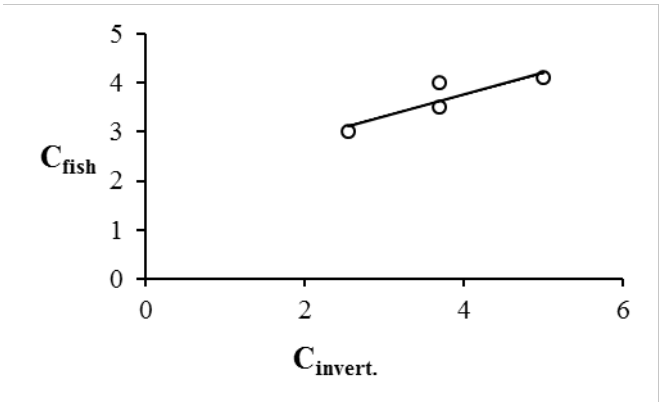


Median ratio: 1.38

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

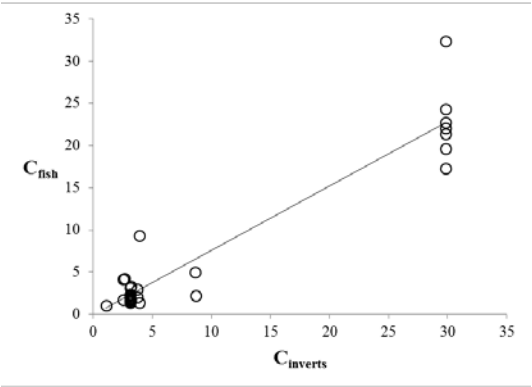
R^2: 0.77
F: 6.58
df: 2
P: 0.13
Not used because P > 0.05

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*)

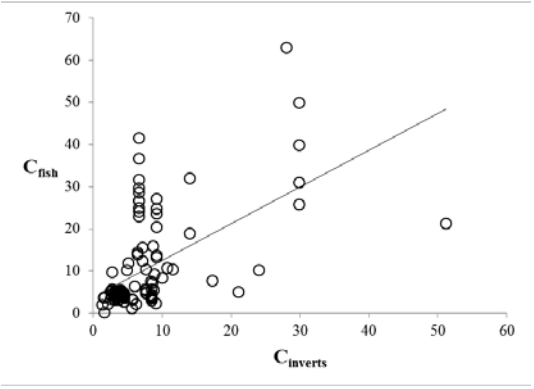
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	BSW1	5.00	12.00	2.40
Butler et al. 1994	RB1	21.00	5.10	0.24
Butler et al. 1995	ME4	1.55	3.90	2.52
Butler et al. 1995	ME4	1.55	3.70	2.39
Butler et al. 1995	ME4	1.55	3.80	2.45
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	ME3	2.55	5.20	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*)

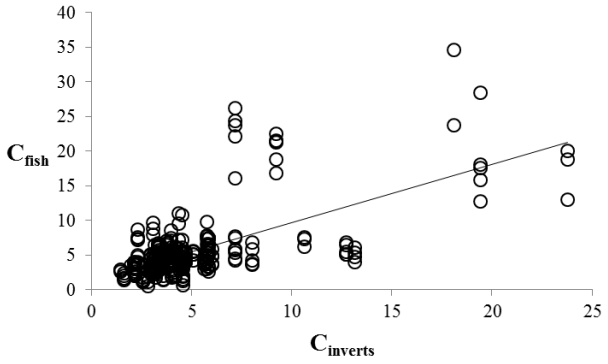
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-4	5.10	5.46	1.07
GEI 2014	Grassy Creek, GC-4	5.10	5.63	1.10
GEI 2014	Grassy Creek, GC-4	5.10	5.15	1.01
GEI 2014	Grassy Creek, GC-4	5.76	7.72	1.34
GEI 2014	Grassy Creek, GC-4	5.76	6.04	1.05
GEI 2014	Grassy Creek, GC-4	5.76	7.88	1.37
GEI 2014	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*)

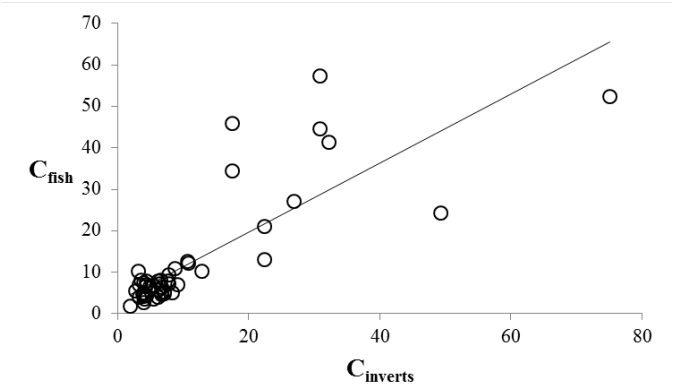
Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ShpC	1.90	1.80	0.95
McDonald and Strosher 1998	ER 745	2.74	5.40	1.97
Hamilton and Buhl 2005	SC	4.10	3.50	0.85
McDonald and Strosher 1998	ER 747	4.29	6.57	1.53
Hamilton and Buhl 2005	UAC	5.00	6.60	1.32
Hamilton and Buhl 2004	ACM	6.70	6.30	0.94
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*)

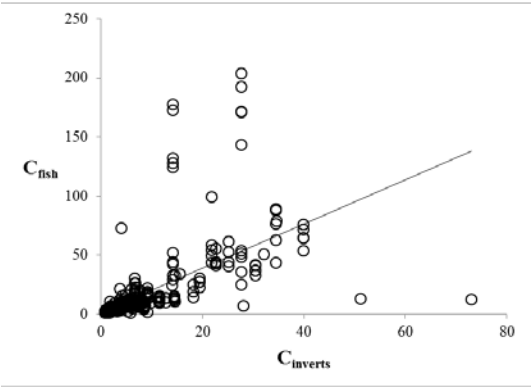
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	DC-4	19.42	27.88	1.44
GEI 2014	DC-4	19.42	23.05	1.19
GEI 2014	DC-4	19.42	30.61	1.58
GEI 2014	DC-4	18.10	19.32	1.07
GEI 2014	DC-4	18.10	14.48	0.80
GEI 2014	DC-4	18.10	25.42	1.40
GEI 2014	FOC-2	2.18	4.13	1.90
GEI 2014	FOC-2	2.18	4.10	1.89
GEI 2014	FOC-2	2.18	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*)

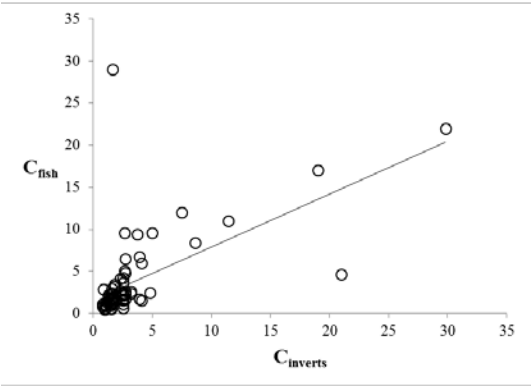
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.50	0.52
Butler et al. 1991	7	29.80	22.00	0.74
Butler et al. 1991	4	3.90	1.70	0.44
Butler et al. 1991	9	4.10	1.50	0.37
Butler et al. 1991	9	4.10	6.00	1.46
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	P1	1.50	2.40	1.60
Butler 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

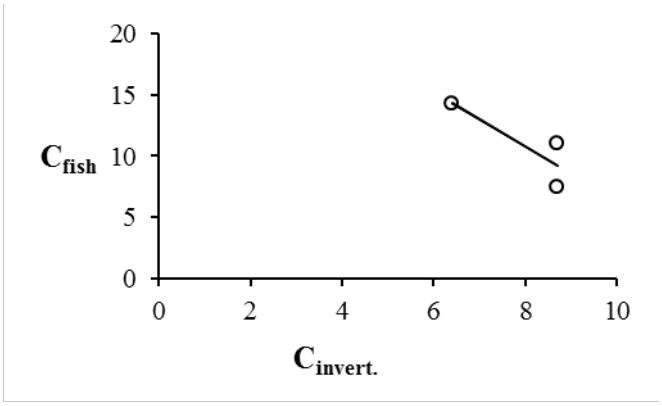


Median ratio: 0.98

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

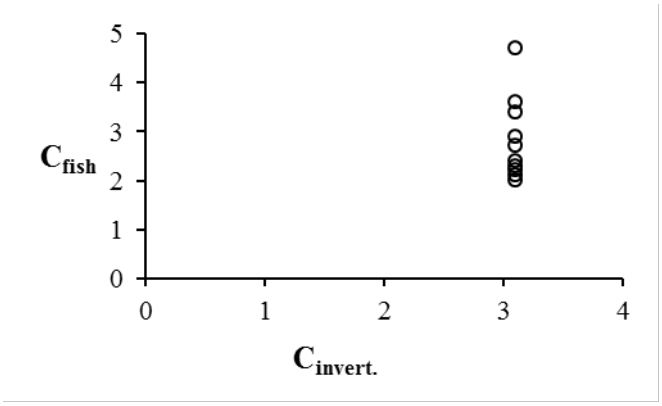
R^2: 0.74
F: 2.78
df: 1
P: 0.39
Not used because P > 0.05 and negative slope.

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*)

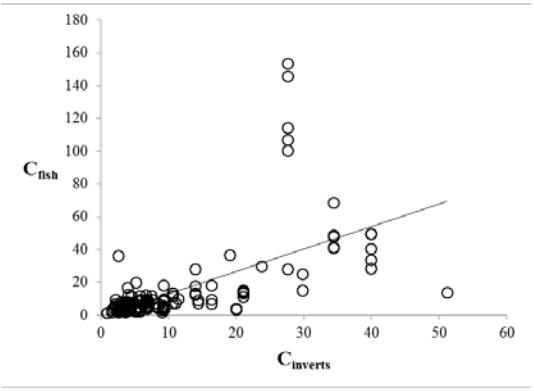
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	C-CLF2	6.85	5.11	0.75
GEI 2014	C-CLF2	6.85	4.10	0.60
GEI 2014	C-LFWOC1	9.32	10.10	1.08
GEI 2014	C-TF1	20.00	4.15	0.21
GEI 2014	C-TF1	20.00	3.47	0.17
GEI 2014	C-TF1	20.00	4.14	0.21
GEI 2014	C-TF1	20.00	4.11	0.21
GEI 2014	C-TF1	20.00	3.41	0.17
GEI 2014	C-WOC1	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



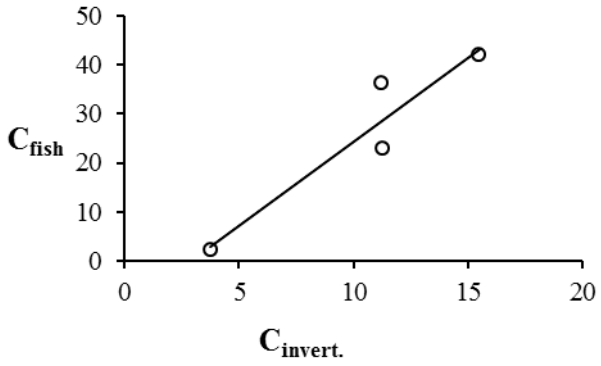
Median ratio: 1.12

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*)

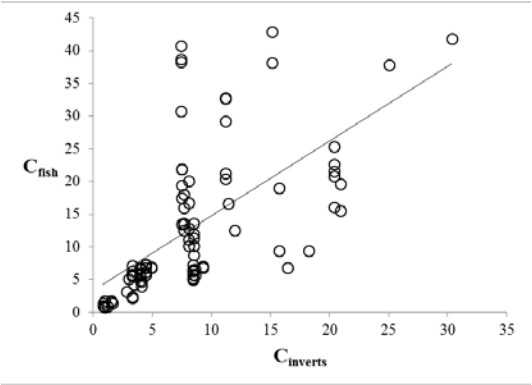
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	MP	1.60	1.40	0.88
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	5.10	1.70
GEI 2013	SWA1	2.81	3.17	1.13
GEI 2013	SW11	8.41	5.02	0.60
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	5.19	0.62
GEI 2013	SW11	8.41	6.26	0.74
GEI 2013	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

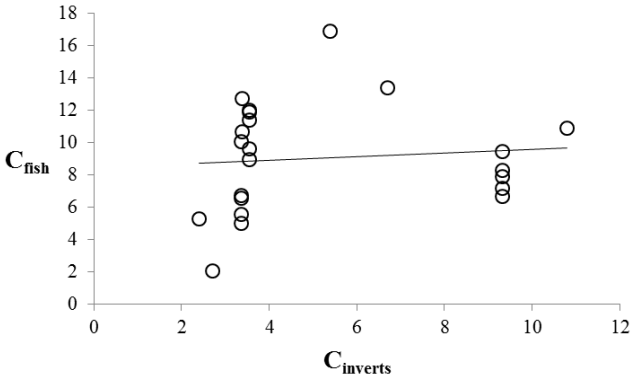


Median ratio: 1.39

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

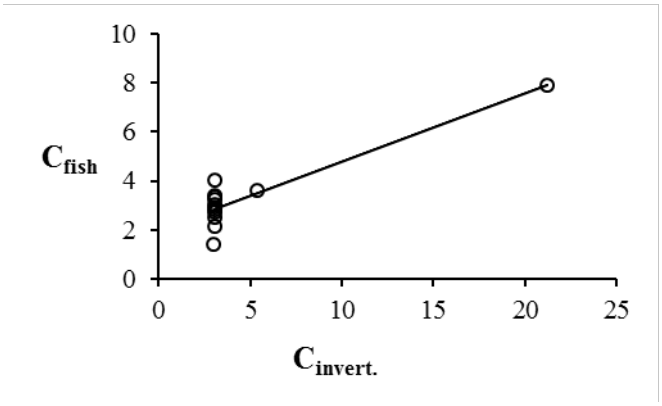


Median ratio: 2.00

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

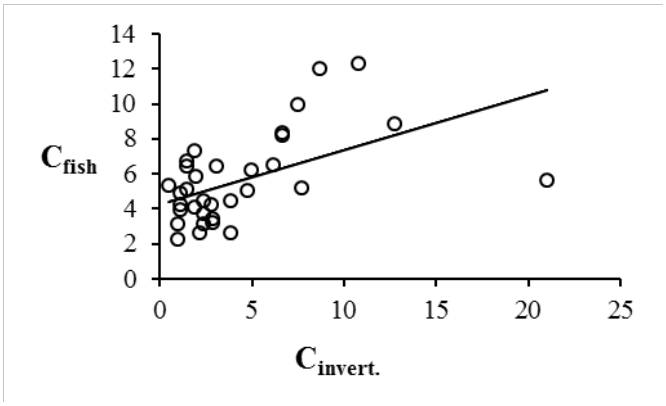
R^2: 0.83
F: 54.66
df: 11
P: <0.001

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

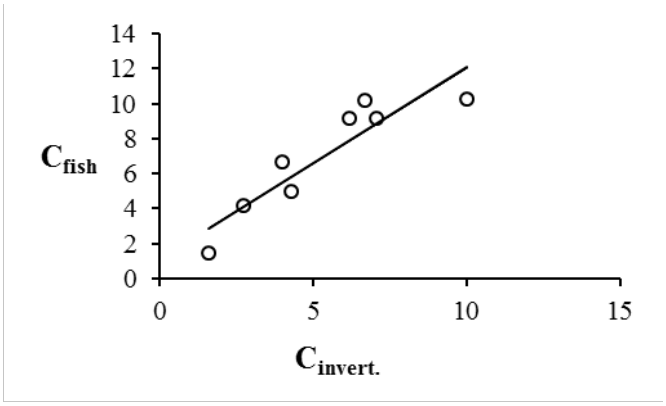
R^2: 0.27
F: 11.62
df: 31
P: < 0.001

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



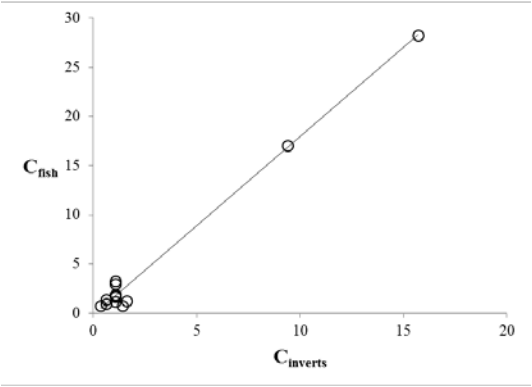
Median ratio: 1.38

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*)

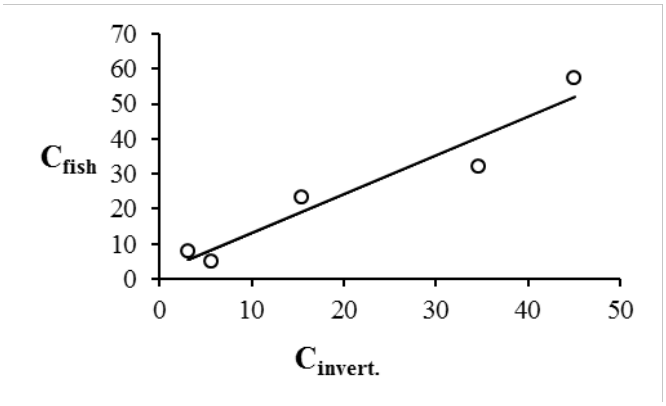


Median ratio: 1.78

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



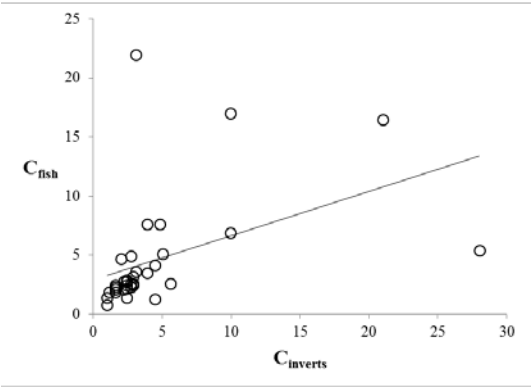
Median ratio: 1.27

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*)

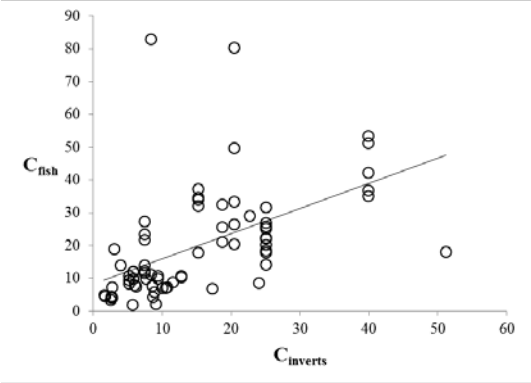
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	3	6.20	7.70	1.24
Butler et al. 1994	IW	8.35	83.00	9.94
Butler et al. 1994	AD	2.70	7.30	2.70
Butler et al. 1994	LW	3.00	19.00	6.33
Butler et al. 1994	LSW1	3.90	14.00	3.59
Butler et al. 1995	ME4	1.55	5.10	3.29
Butler et al. 1995	ME3	2.55	4.60	1.80
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



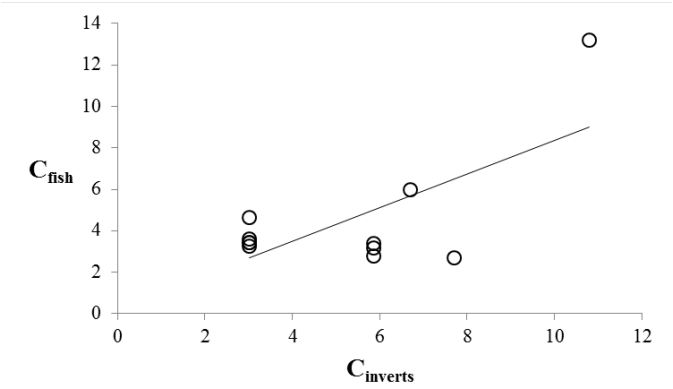
Median ratio: 1.31

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*)



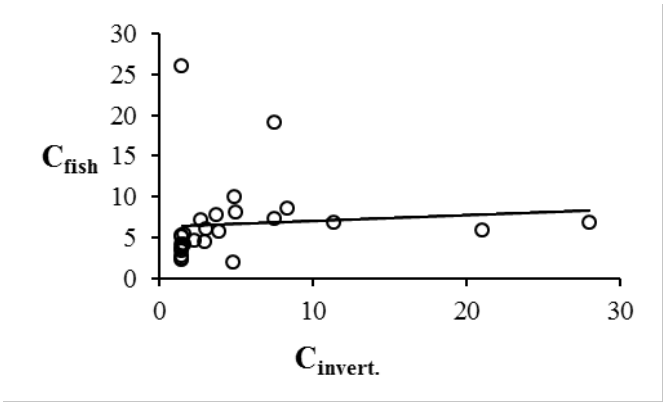
Median ratio: 1.08

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*)

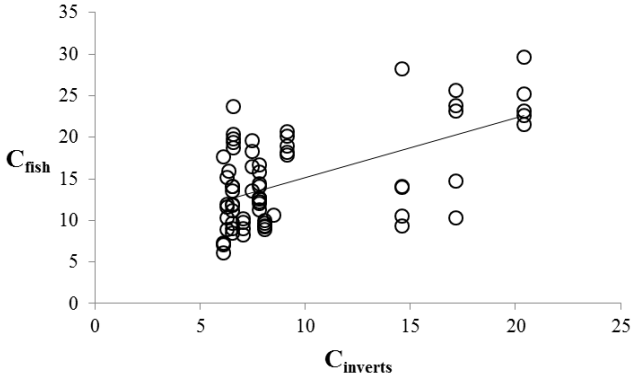
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	6.54	8.43	1.29
GEI 2013	SW1	6.54	9.02	1.38
GEI 2013	SW1	6.54	9.66	1.48
GEI 2013	SW1	6.54	11.21	1.71
GEI 2013	SW1	6.54	11.85	1.81
GEI 2013	SW1	6.54	11.94	1.83
GEI 2013	SW1	6.54	13.50	2.06
GEI 2013	SW1	6.54	14.05	2.15
GEI 2013	SW1	6.54	14.14	2.16
GEI 2013	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

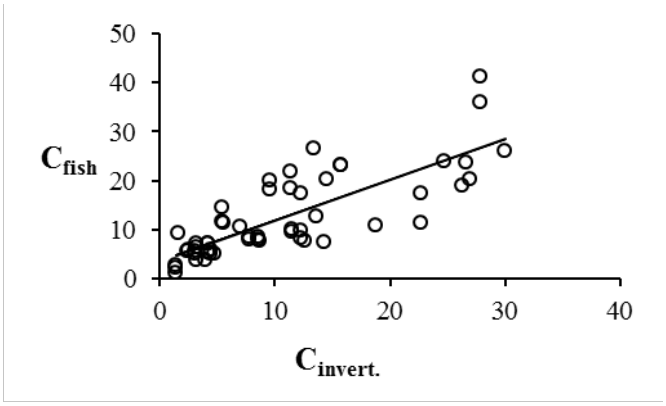
R^2: 0.32
F: 32.15
df: 67
P: <0.001

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*)

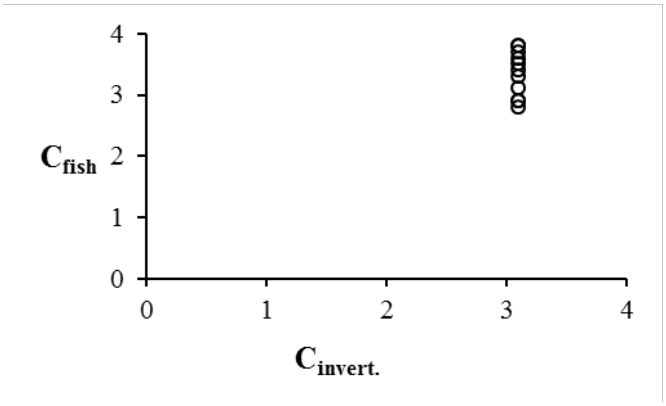
Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	8.53	7.87	0.92
Formation 2012	DC-600	8.53	8.50	1.00
Formation 2012	DC-600	8.65	7.63	0.88
Formation 2012	LSV-4	9.54	18.28	1.92
Formation 2012	LSV-4	9.54	20.01	2.10
Formation 2012	HS-3	11.40	18.57	1.63
Formation 2012	HS-3	11.40	21.85	1.92
Formation 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

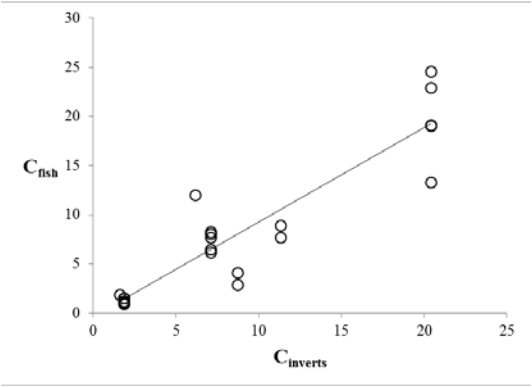


Median ratio: 1.10

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

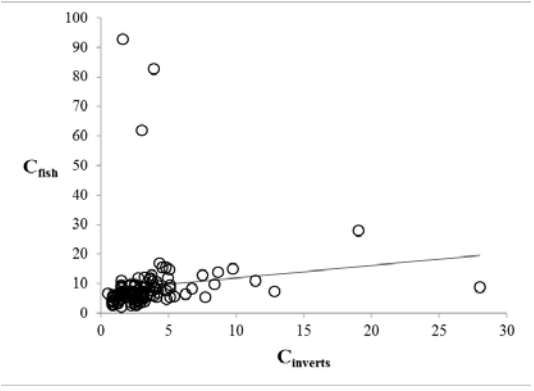
R^2: 0.84
F: 107.1
df: 20
P: <0.001

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

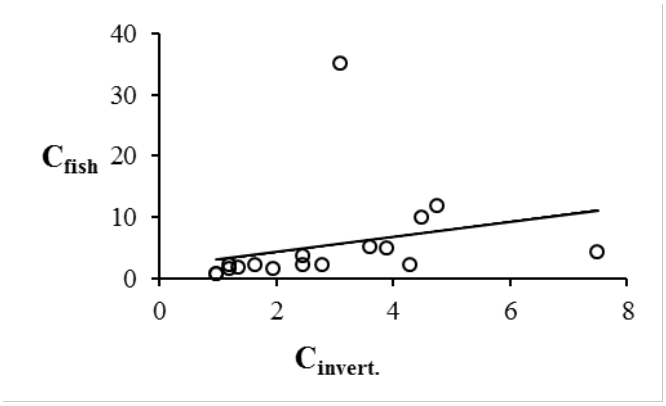


Median ratio: 2.76

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

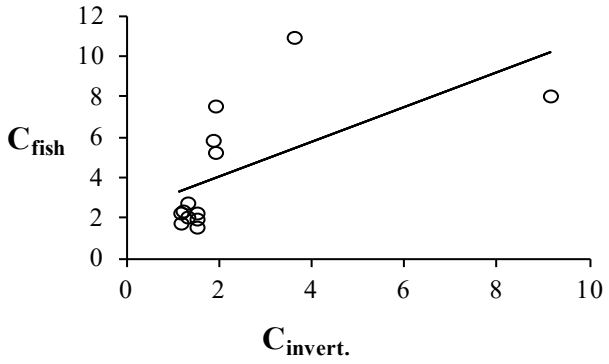


Median ratio: 1.33

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

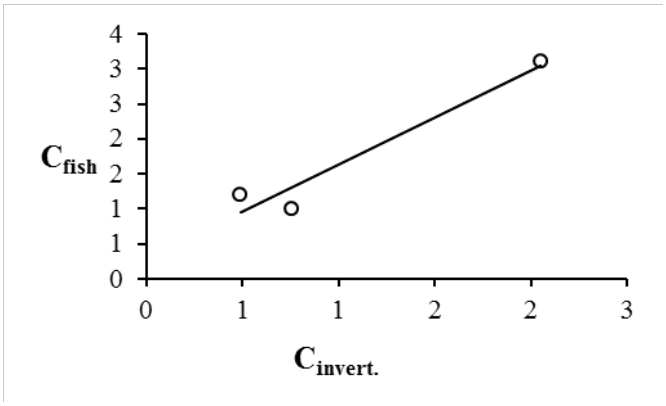
Sunfish (Centrarchidae)				
GEI 2013	SW2-1	9.14	8.10	0.89



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

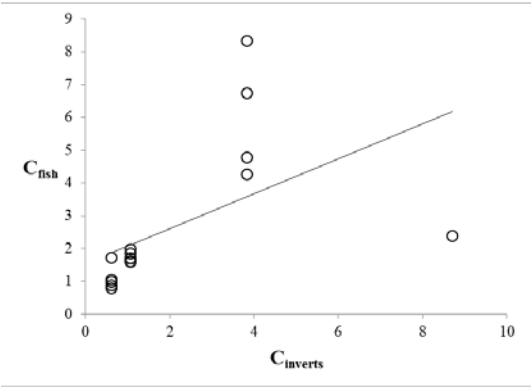


Median ratio: 1.51

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



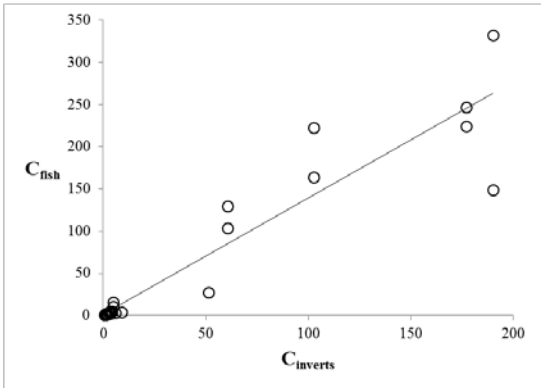
Median ratio: 1.60

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

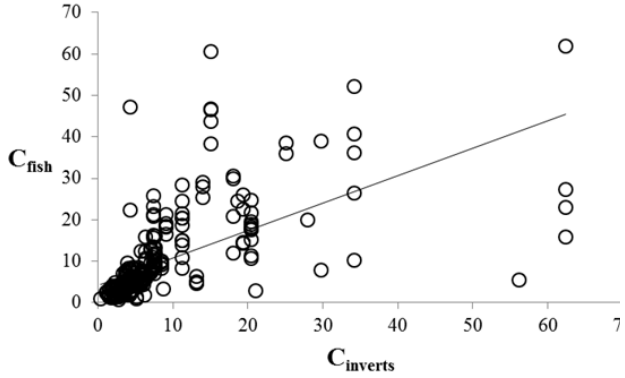
White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Grasso et al. 1995	17	1.91	4.00	2.09
Grasso et al. 1995	17	1.91	4.01	2.10
Butler et al. 1994	NFK3	2.00	3.90	1.95
Butler et al. 1993	ST1	2.25	4.90	2.18
Lambing et al. 1994	S33	2.40	3.50	1.46
Mueller et al. 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



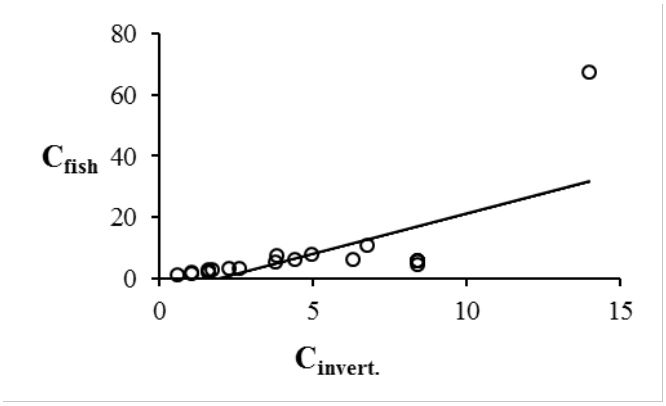
Median ratio: 1.11

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 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				
			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	
Ephemeroptera	Atenella margarita	CN	CG	3												2																3				5
Ephemeroptera	Baetis spp.	SW	CG	5		3	5	56	14	85	89	27	90	68	38	61	253	76	67	76	9	2	56	249	7	316	27	53	46	57	32	62	56		61	2041
Ephemeroptera	Centroptilum conturbatum	SW	CG	2																											1					1
Ephemeroptera	Cinygmula spp.	CN	SC	4													2	14																		16
Ephemeroptera	Diphetor hageni	SW	CG	5													1				11						9	6	3							30
Ephemeroptera	Drunella coloradensis	CN	P	0				1				7		7			1						1		3				5	3			1			29
Ephemeroptera	Drunella grandis	CN	P	0		2	4		9	3			3		4	7								1			4							1		38
Ephemeroptera	Epeorus longimanus	CN	SC	0													4	5	3																	12
Ephemeroptera	Ephemerella dorothea infrequens	CN	CG	1		5	3							1							5		1	5	2	2							1			25
Ephemeroptera	Ephemerella aurivillii	CN	CG	1					5									7																		12
Ephemeroptera	Paraleptophlebia spp.	SW	CG	1		2	12	3	9		11	4		1	1		11				5	7				4			2			2	1			75
Ephemeroptera	Tricorythodes minutus	CN	CG	4												2																8	7	5	3	25
Plecoptera	Hesperoperla pacifica	CN	P	1		21	12	3	11								21	62	13	20	23			9	4							3			15	217
Plecoptera	Isoperla sp.	CN	P	2						7	11	5							7				1		3		2	6	1		3					46
Plecoptera	Malenka sp.	CN/SP	SH	2		10	33	5	25	16		5	14	3	2	4	14	30					2	1		4	9	3	29	1		2				212
Plecoptera	Pteronarcys sp.	CN/SP	SH	0			21																												3	24
Plecoptera	Skwala sp.	CN	P	2		3		4	14			1		1	8																2			4		37
Plecoptera	Sweltsa sp. p	CN	P	1		7					1		3				13	35		14	2					2										77
Trichoptera	Agapetus sp.	CN	SC	0																2																2
Trichoptera	Arctopsyche sp.	CN	P	1					4	18		9	35			2		14	6					13	33		34	23								191
Trichoptera	Brachycentrus sp.	CN	F	1				4	4	29		3		88	4	13			3				3	17	65	6	153	29	4	20	73	11	27	61	18	635
Trichoptera	Cheumatopsyche sp.	CN	F																											8			13			21
Trichoptera	Dicosmoecus sp.	BU	SH	1		3	2																			2										7
Trichoptera	Dolophilodes	CN	F	1														25	3																	28

Order	Genus	Habitat / Behavior	Functional Feeding Groups	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
					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 paravula	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					
					Location	SFTC1		CC75			CC150			CC350			DC600			HS			HS3			LSV2C			LSV4		CC1A		CC3A			Total
					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 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		Total
					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

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

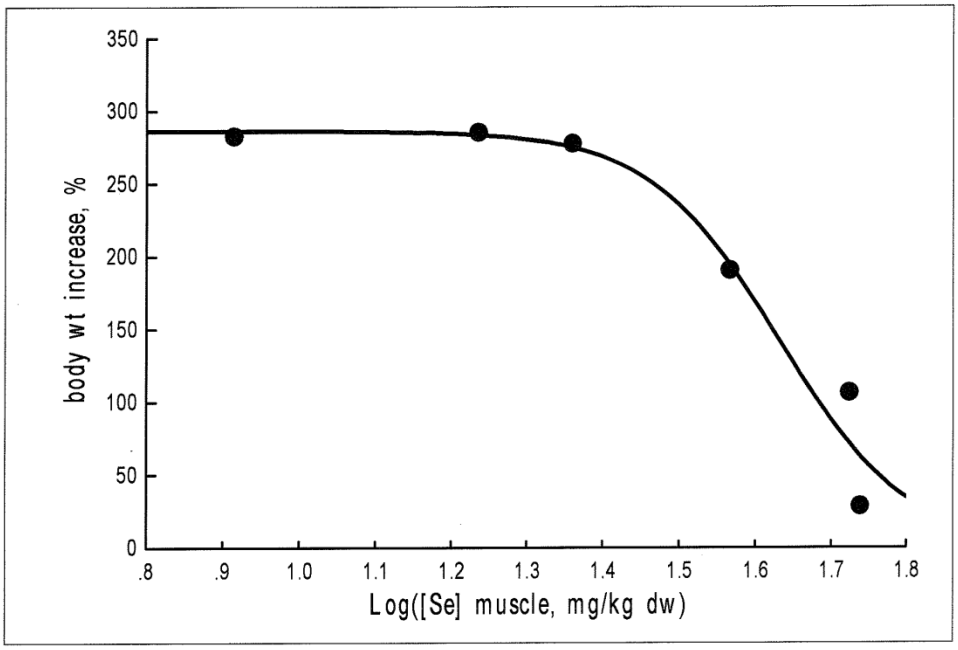
Treatment group	Moisture, % ww	Lipid, % ww	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	76.8 (0.5) b	9.5 (4) abc	8.2 (0.6) e	5.2 (0.4) c
9.6	77.0 (0.7) b	9.5 (0.9) abc	17.2 (0.7) d	11.8 (0.9) b
20.5	76.8 (0.3) b	10.1 (0.4) ab	22.9 (1.5) c	14.7 (0.8) b
41.7	77.3 (0.5) b	9.6 (0.7) abc	36.8 (1.8) b	22.5 (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)

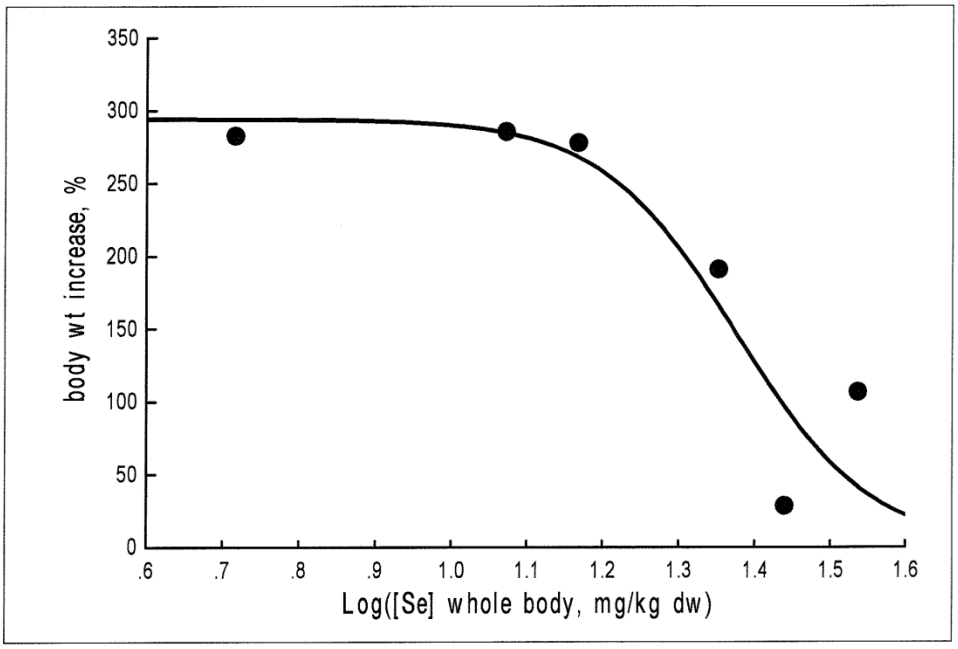
Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.6006	1.6303	0.0314	1.5304	1.7301
S	1.6574	2.938	0.925	-0.005	5.882
Y0	284.2	286.3	18.9	226.1	346.5

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.

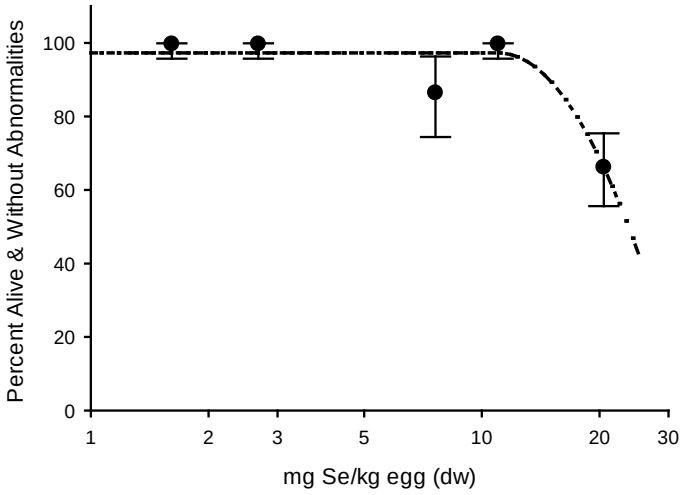


Figure 1. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in eggs. TRAP is used to interpolate between the last two points; EC10 = 15.6 mg Se/kg egg dw.

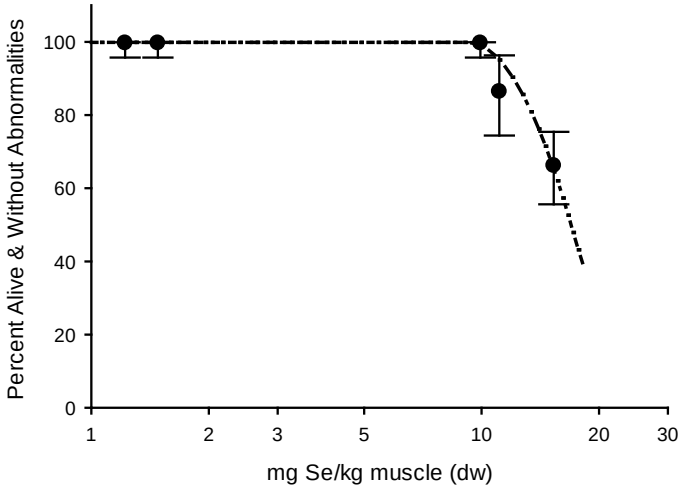
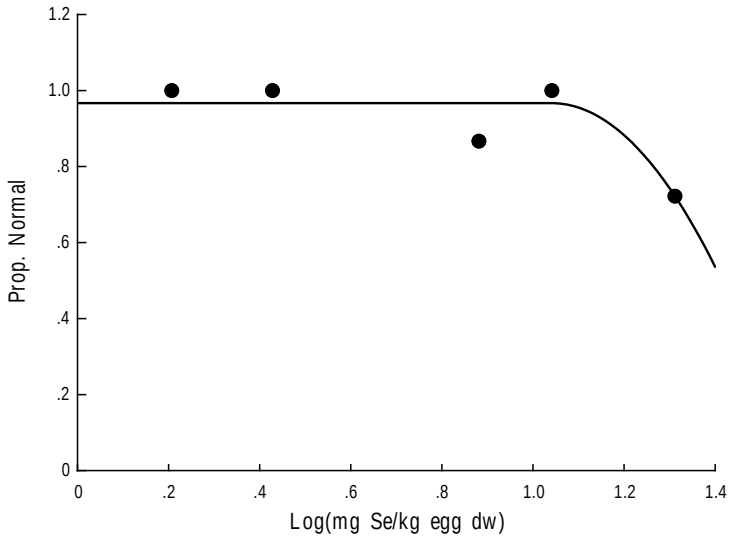


Figure 2. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in female muscle. TRAP tolerance distribution analysis with the log-triangular model; EC10 = 11.9 mg Se/kg muscle dw.



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

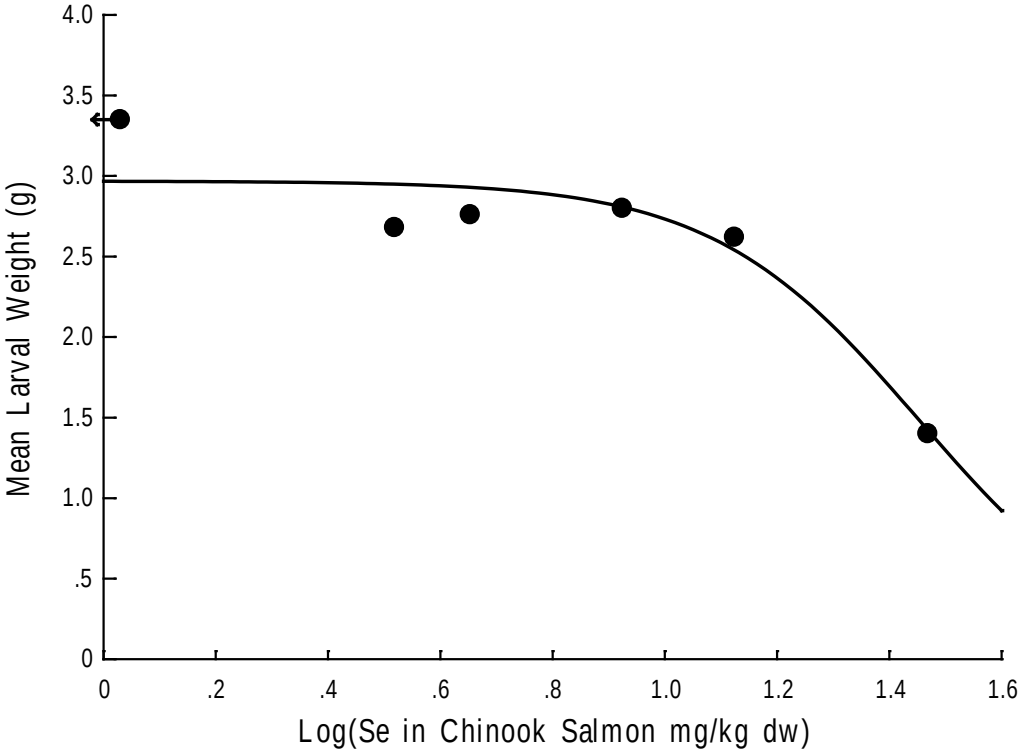
Chronic Value: Due to unacceptable control mortality of swim-up larvae in control treatments after 90 days (33.3 percent - SLD diet; 27.5 percent - SeMet diet), chronic values had to be determined from respective values reported after 60 days (tables above).

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

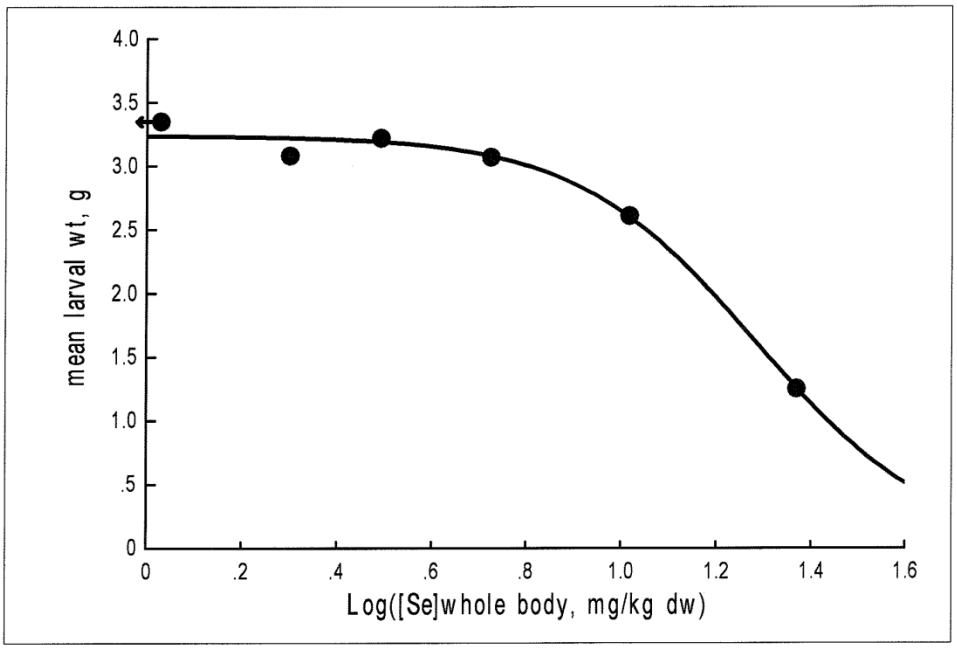


	Guess	FinalEst	SE	95%LCL	95%UCL
LogX50	1.453	1.453	7.30E-02	1.2206	1.6854
StDev	1.353	1.353	6.67E-01	-7.71E-01	3.4769
Y0	2.968	2.968	1.89E-01	2.3651	3.5709

%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		

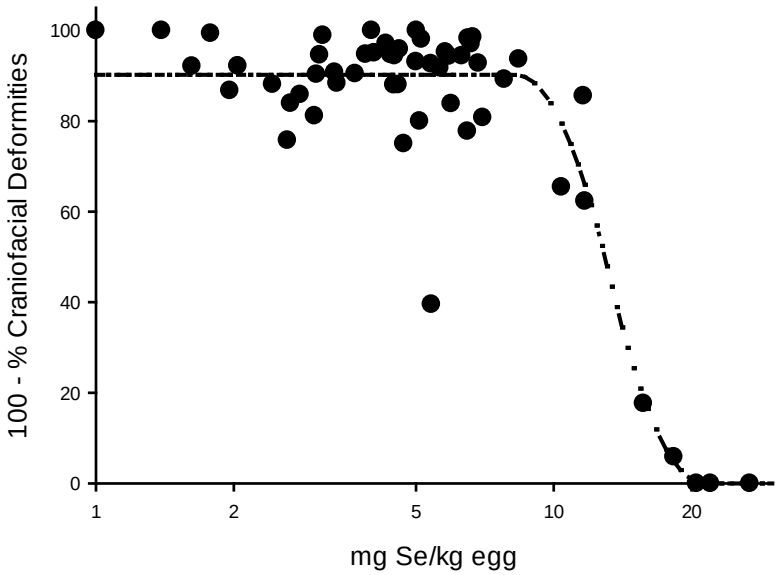


Figure 1. Rainbow trout percent normal (100 - % craniofacial deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 90.2%, the slope 4.8%, and the EC_{10} 10.2 mg Se/kg egg ww.

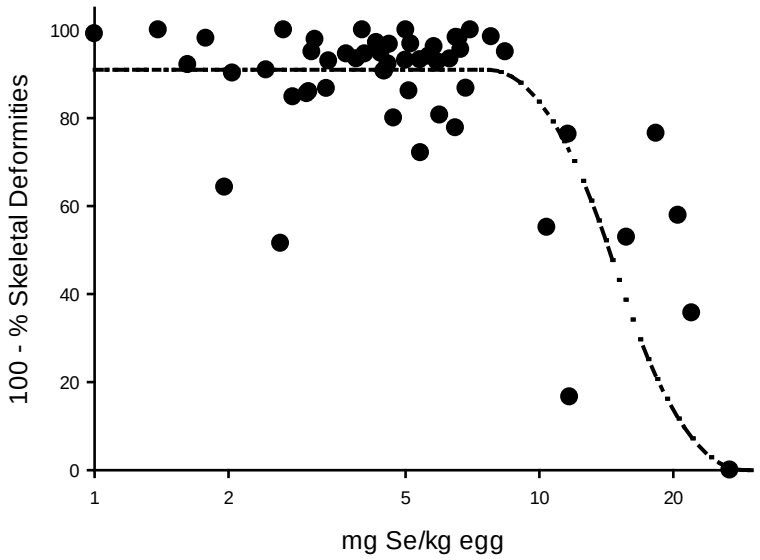


Figure 2. Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 91%, the slope3.5%, and the EC_{10} 10.3 mg Se/kg egg ww.

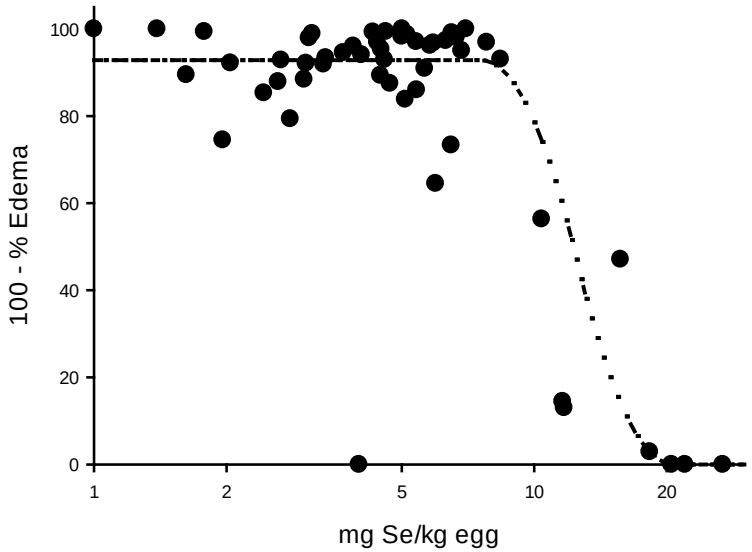


Figure 3. Rainbow trout percent normal (100 - % edema) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoidal equation excluding the one outlier with 100% edema at 4 mg/kg. The background value was 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.

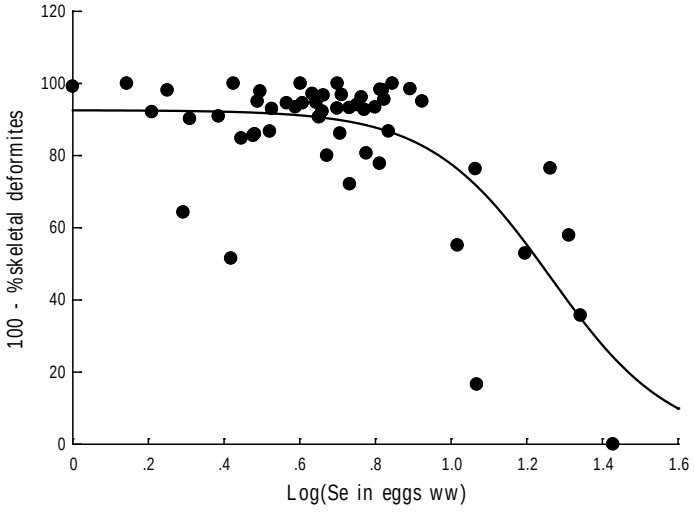
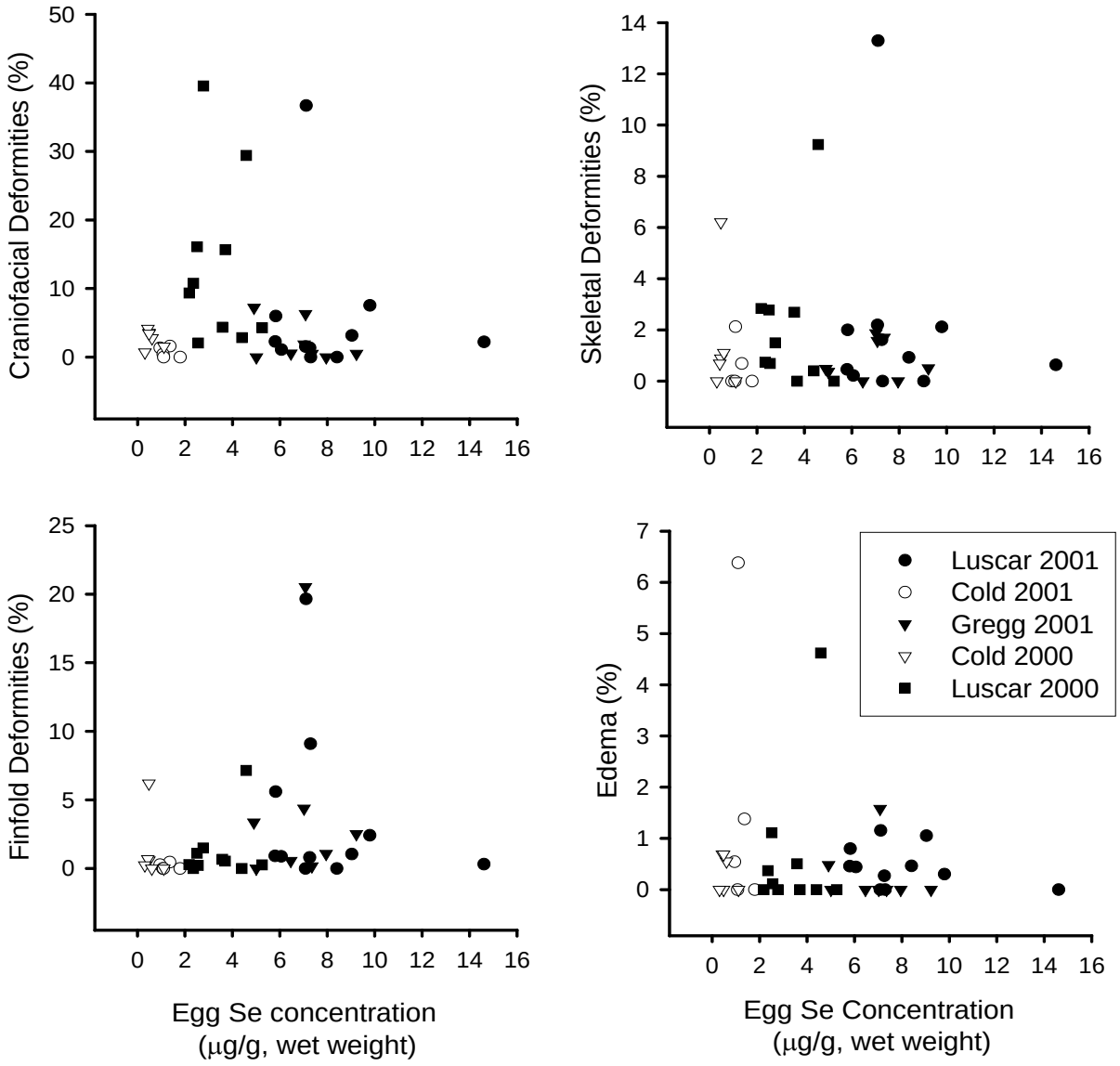


Figure 4. (From Previous Draft) Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). EC_{10} = 8.2 mg/kg ww.

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

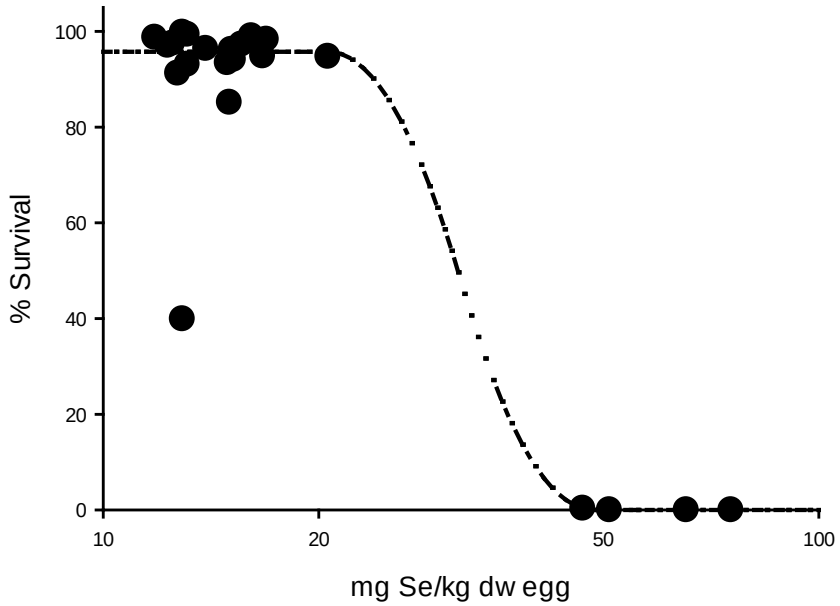


Figure 1. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in eggs.

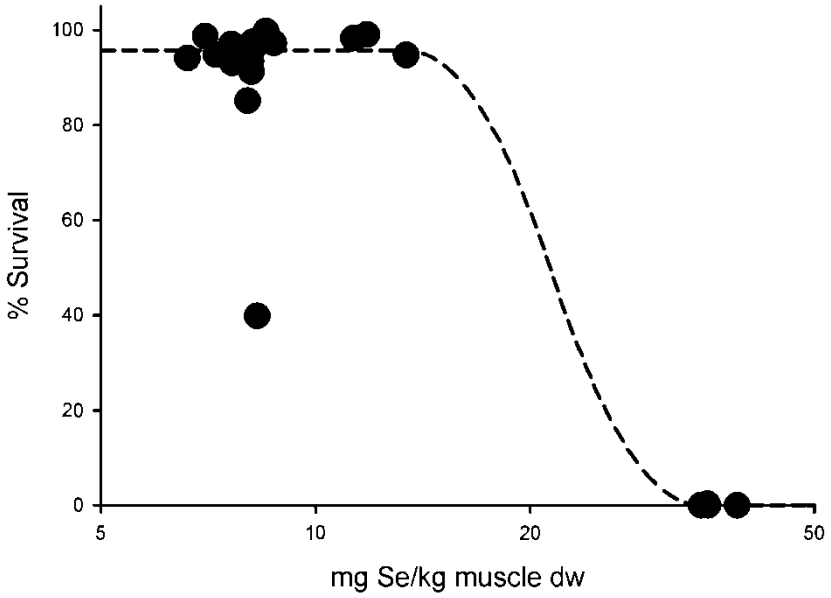


Figure 2. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in maternal muscle.

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

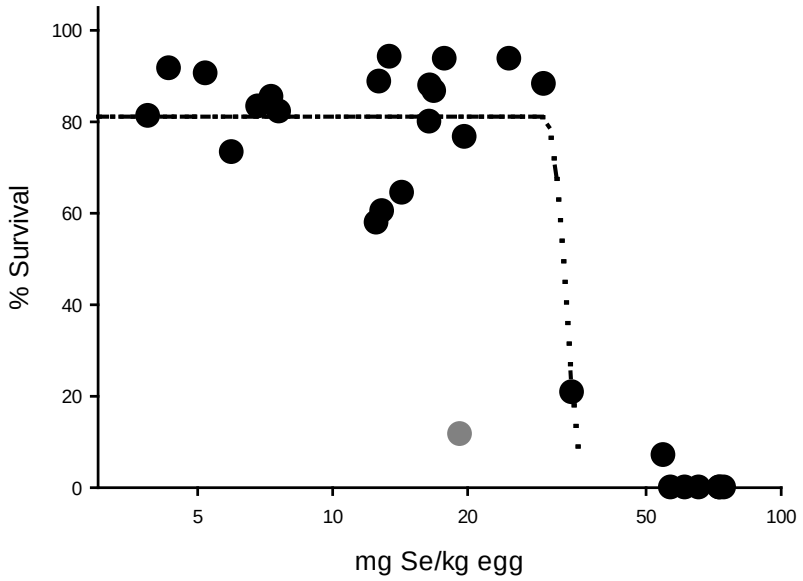


Figure 1. Labs A and B datasets. EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (29.6 mg/kg and 81.1%). The slope is 20.5 and the EC10 is 31.1 mg/kg. Note: the gray point denotes egg batch with quality problems noted by authors and was not used in the analysis.

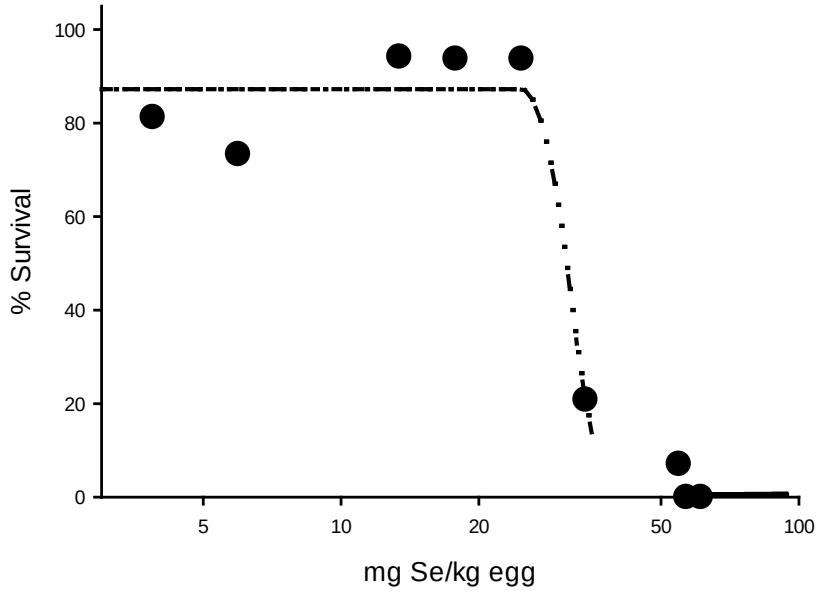


Figure 2. Lab A dataset.* EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (24.8 mg/kg and 87.25%). The slope is 9.4 and the EC10 is 27.7 mg/kg.

*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.

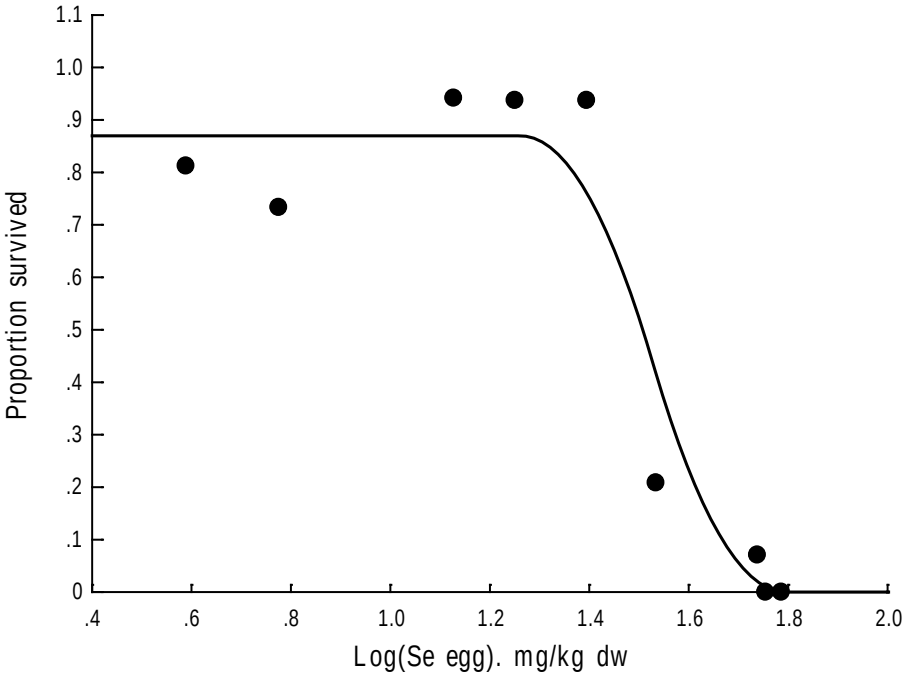


Figure 3. (From previous draft) Tolerance distribution; Model option – Triangular distribution (3 parameter). Includes Laboratory "A" dataset only TRAP EC_{10} estimate = 24.0 mg/kg.

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.

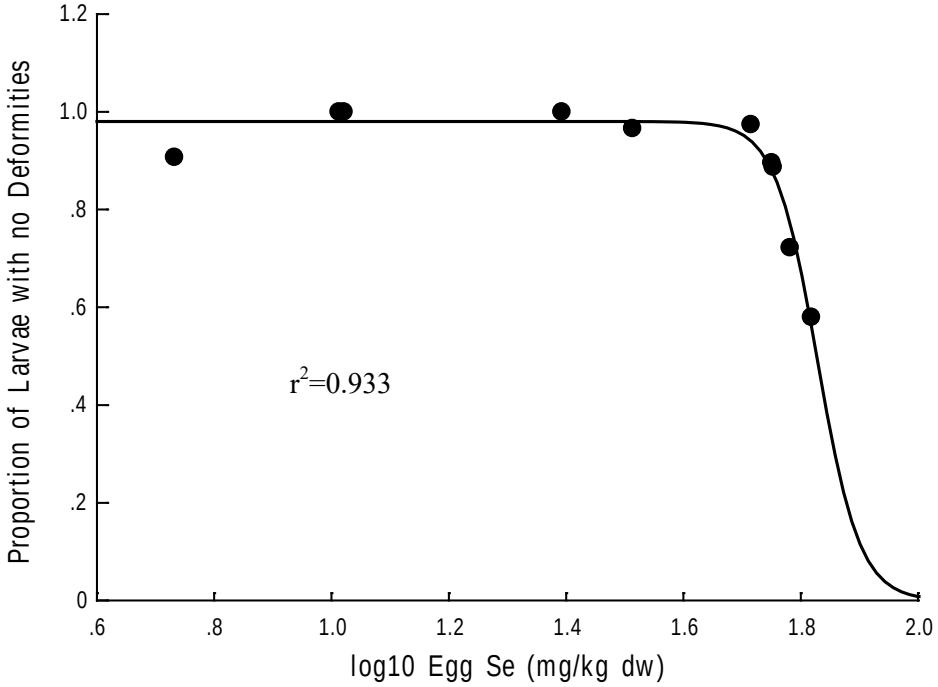
A graduated severity index (GSI) was used for deformity assessment (skeletal, craniofacial, and finfold as well as edema). The narrative criteria were the same as used by Holm et al. (2005) and Rudolph et al. (2008).

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.

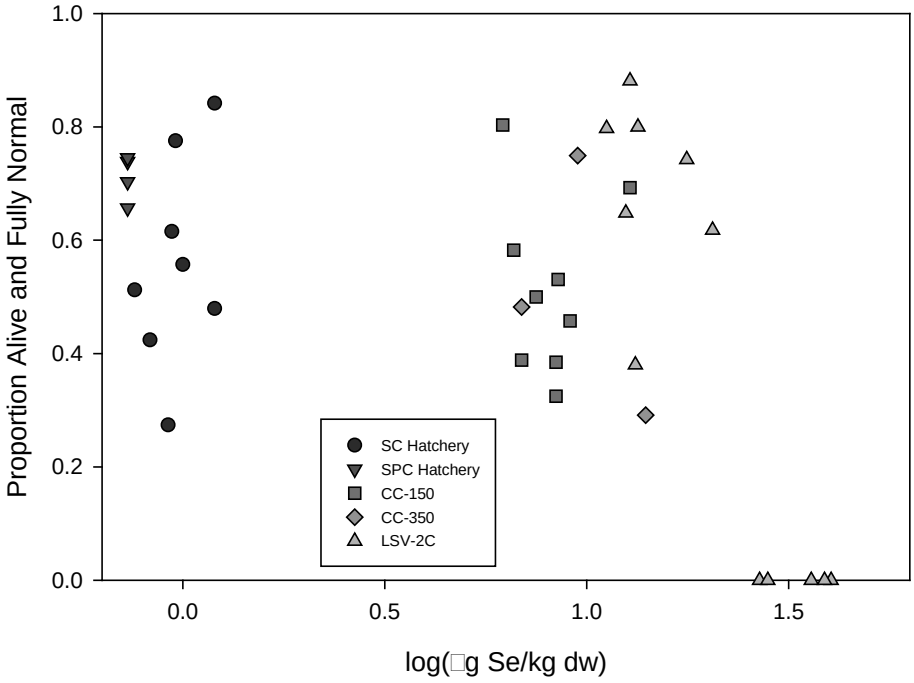


Figure 1. Proportion of alive and normal larvae plotted against Se concentrations in eggs. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

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.

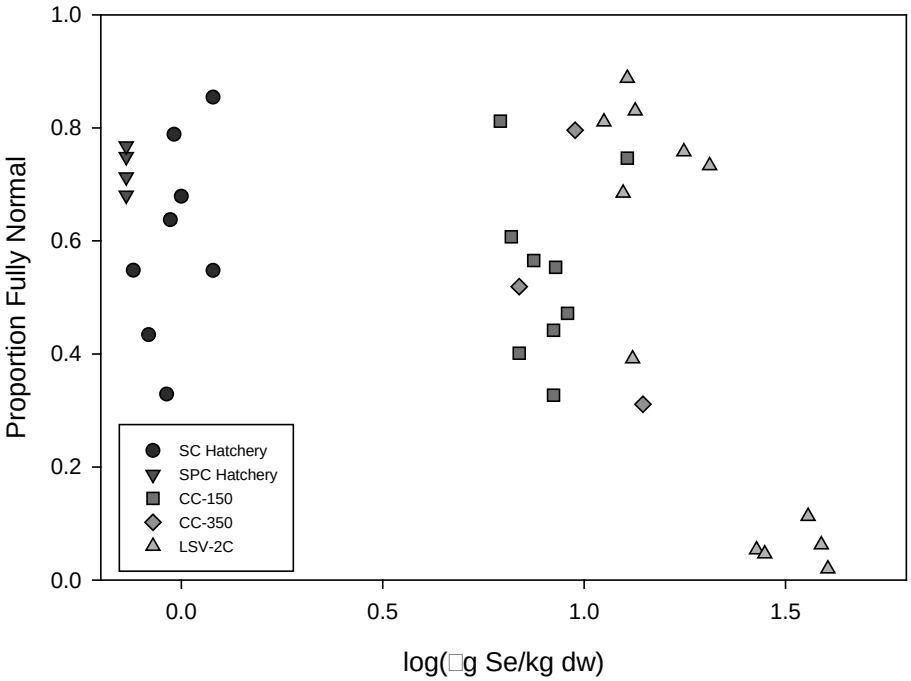


Figure 2. Proportion of normal (free from deformities) larvae plotted against Se concentrations in eggs, hatch through 15-days post swim up. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for 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.

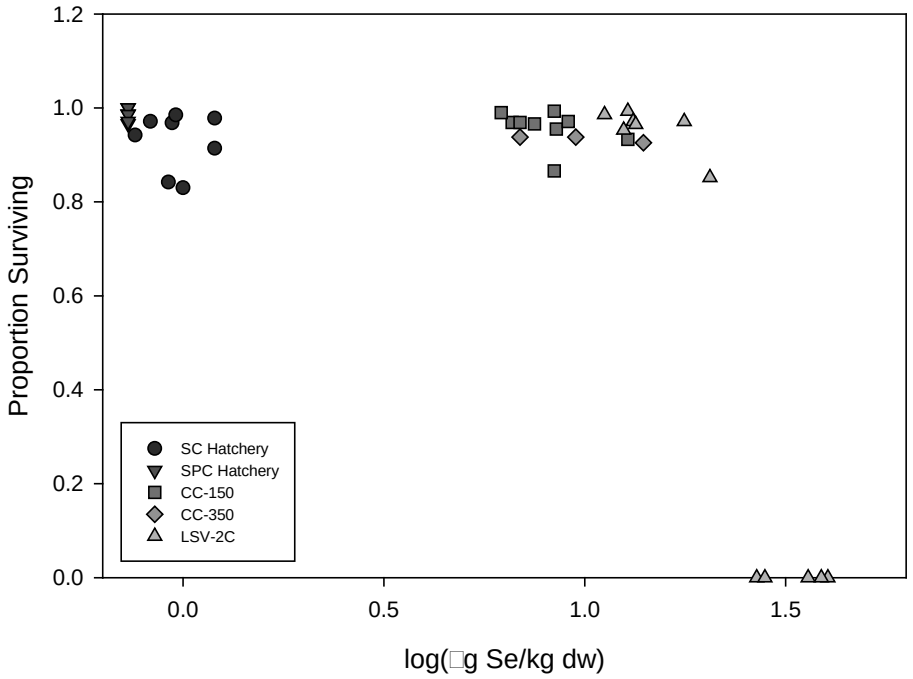


Figure 3. Proportion of larval survival plotted against log transformed Se concentrations in eggs, hatch through 15-day post swim up. Larvae from the five highest Se concentration treatments failed to reach swim up and were assumed to not have survived in the wild.

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."

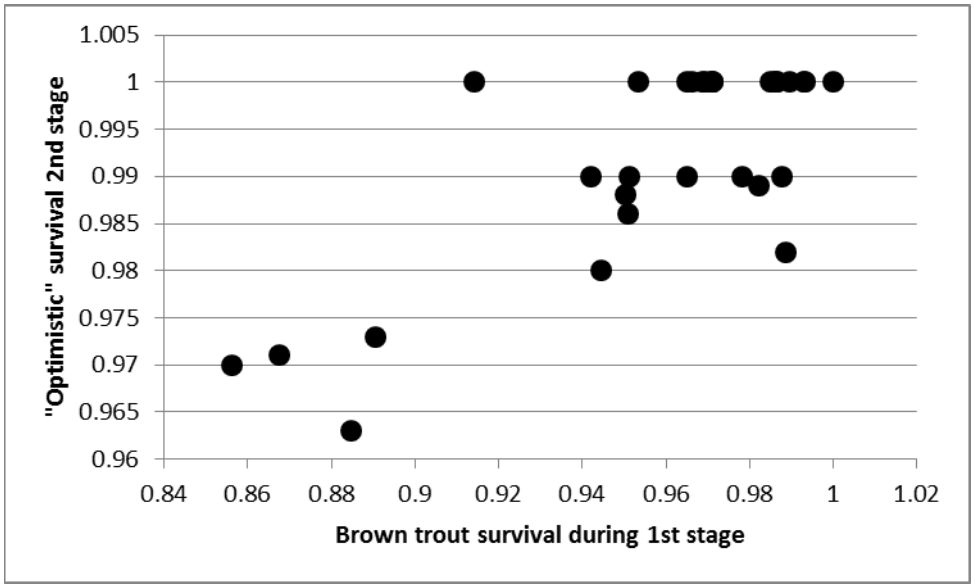


Figure 4. Relationship between survival during the first and second portions of the test. All treatments where larvae successfully reached swim up (Se concentrations of 20.5 mg/kg and lower).

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.

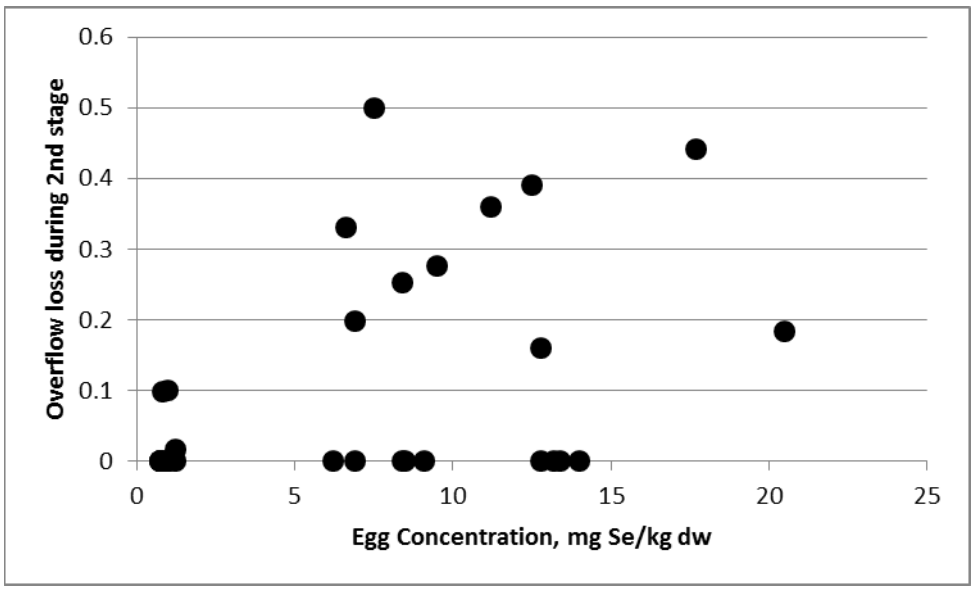


Figure 6. Relationship between egg Se concentration and overflow loss during the second stage of the test. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

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.

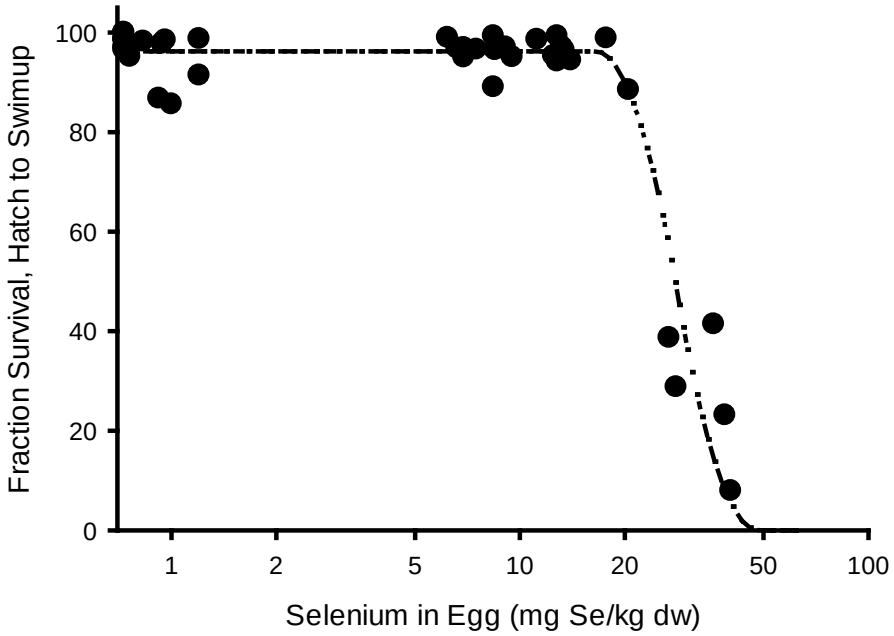


Figure 7. Brown trout survival, hatch to swim up. EC_{10} of 21.0 mg/kg calculated using a weighted nonlinear regression model.

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.

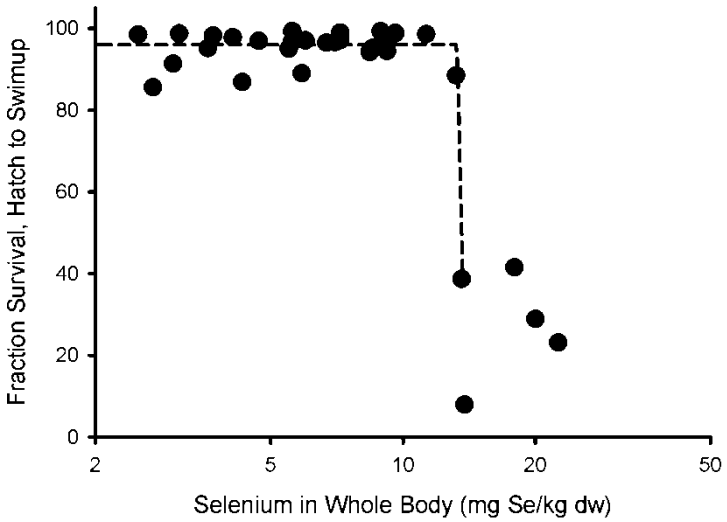


Figure 8. Fraction survival of brown trout larvae as a function of selenium in eggs.

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

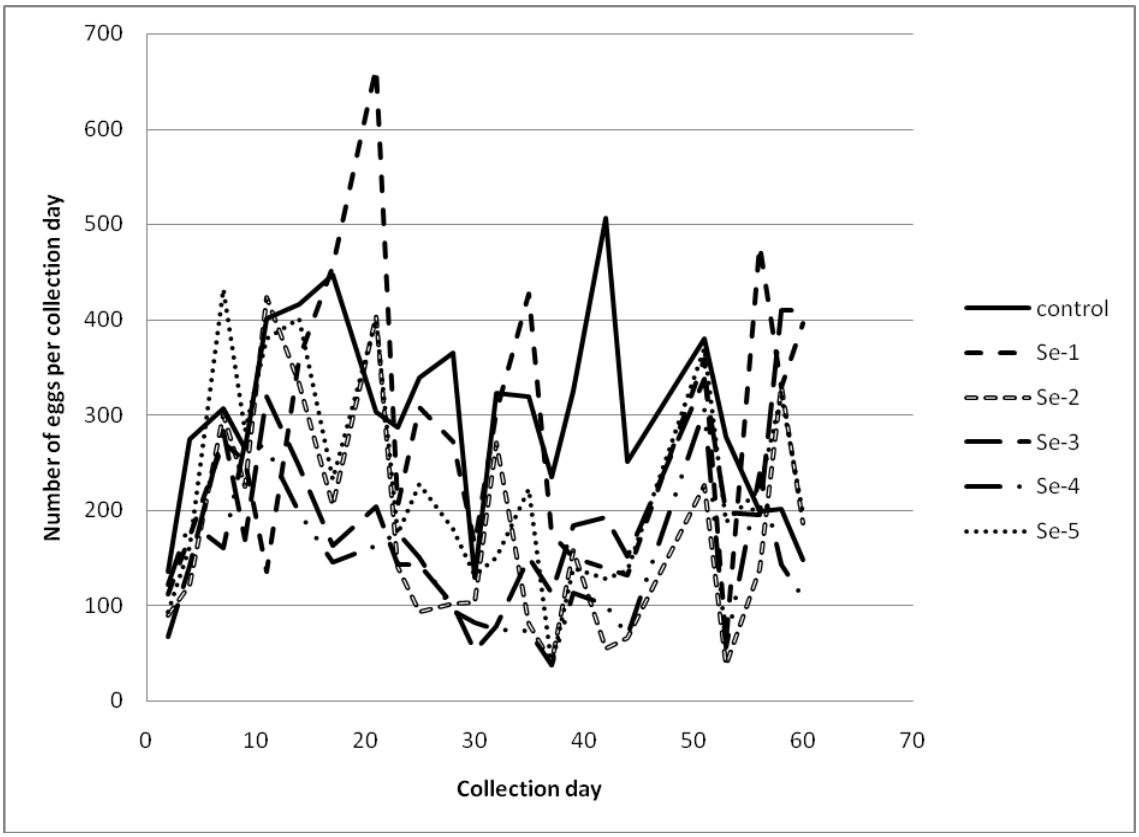


Figure 1. Pupfish egg production by sampling date

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

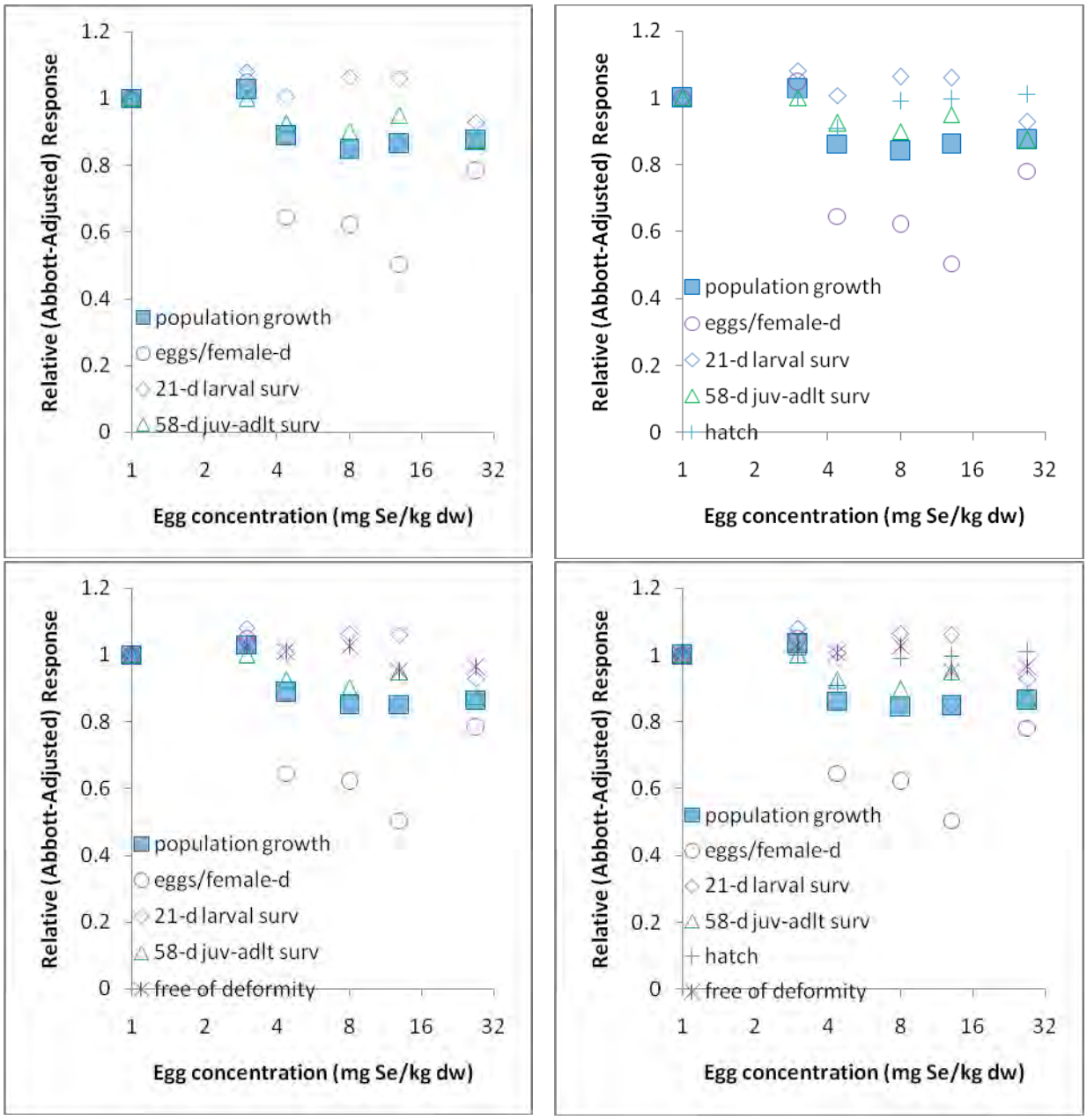


Figure 2. Abbott-adjusted pupfish response as modeled population growth rate (solid-filled symbols) and observed eggs per female per day, larval survival, and juvenile and adult survival (open symbols). Where used in the population model (to modify fecundity), hatch and deformity are shown as open symbols. 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.

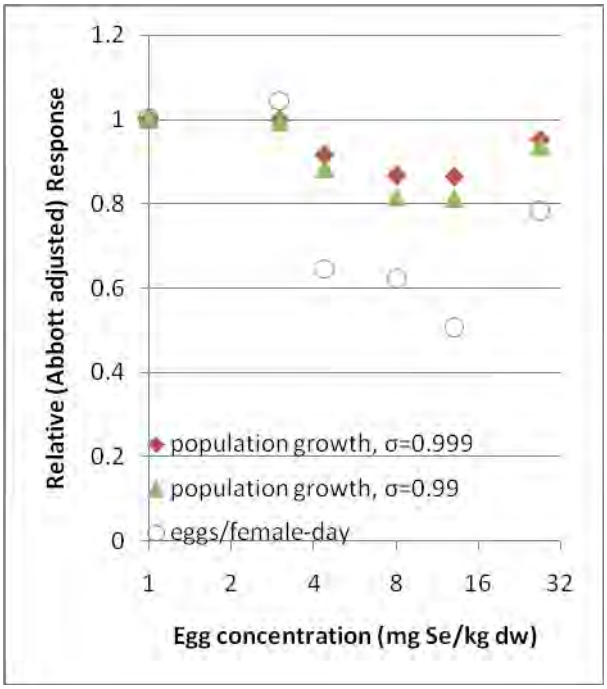


Figure 3. Predicted population growth rate calculated considering differences only in egg production and timing (having assigned uniform survival rates across treatments).

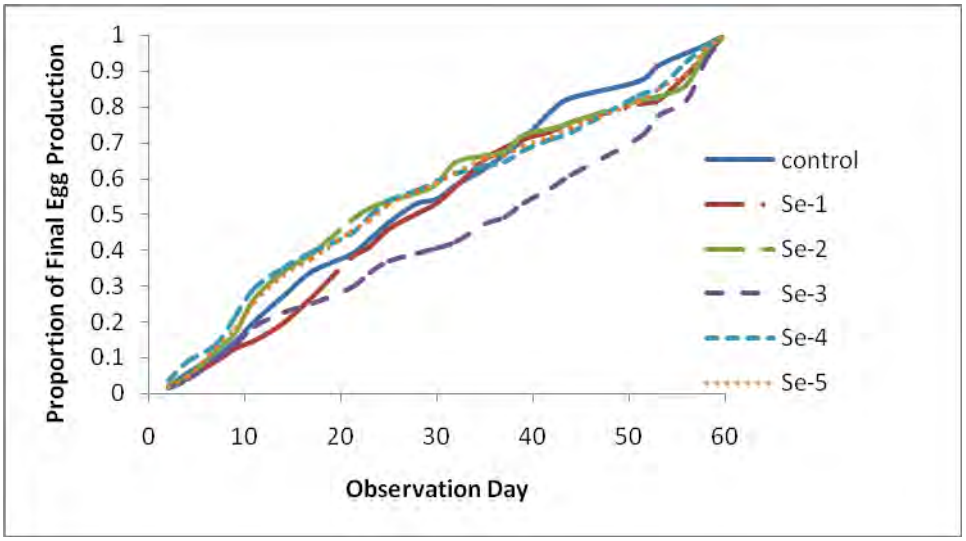


Figure 4. Cumulative pattern of egg production over time. (Control: continuous line. Se-1: dot, dot, long dash. Se-2: long dashes. Se-3: medium dashes. Se-4: short dashes. Se-5: dots.)

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

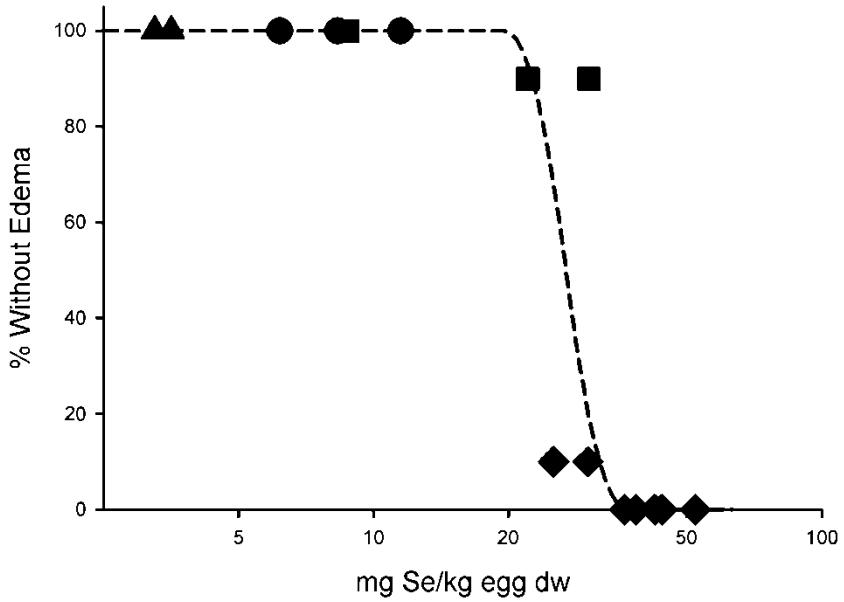


Figure 1. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

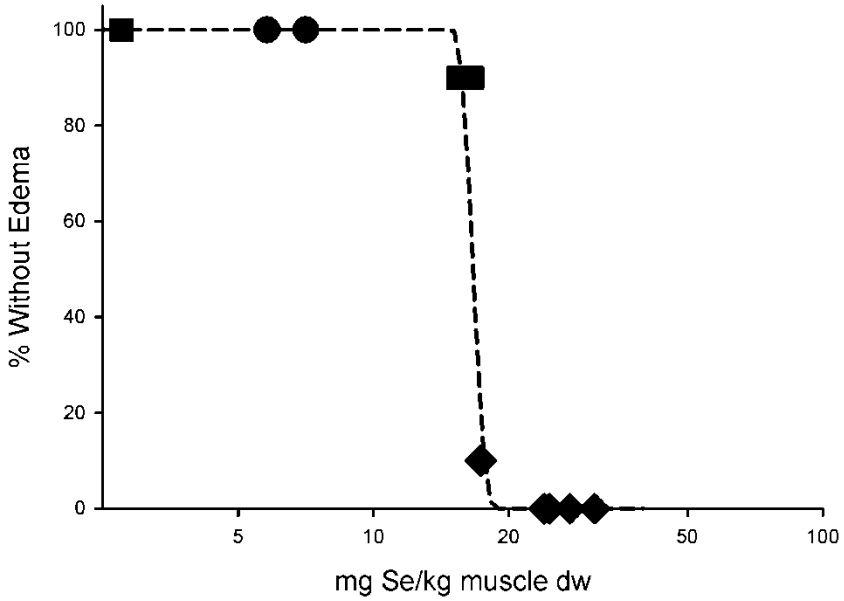


Figure 2. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in maternal muscle. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution option with the log-triangular distribution. EC_{10} for replicate data is 22.6 mg Se/kg egg dw.

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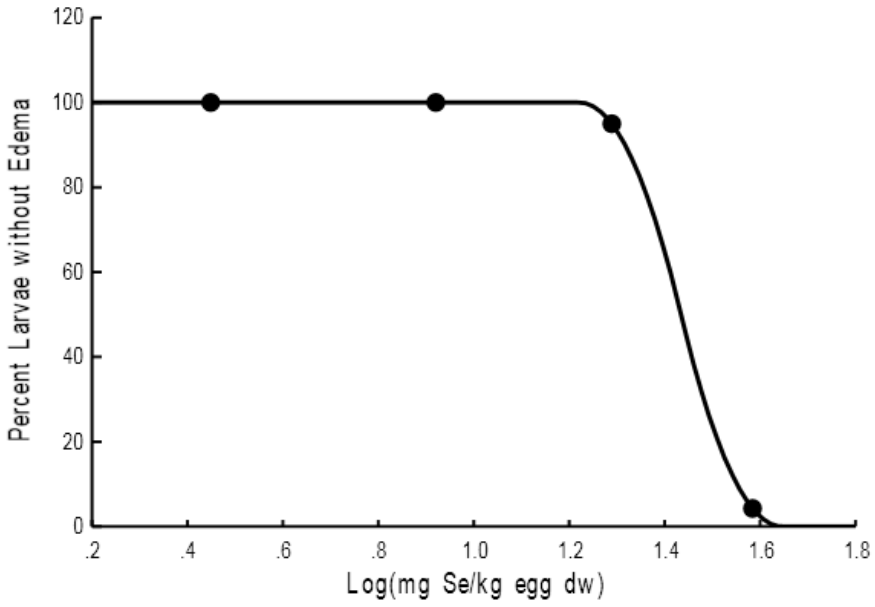

Parameter Summary (Threshold Sigmoid Regression Analysis)

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

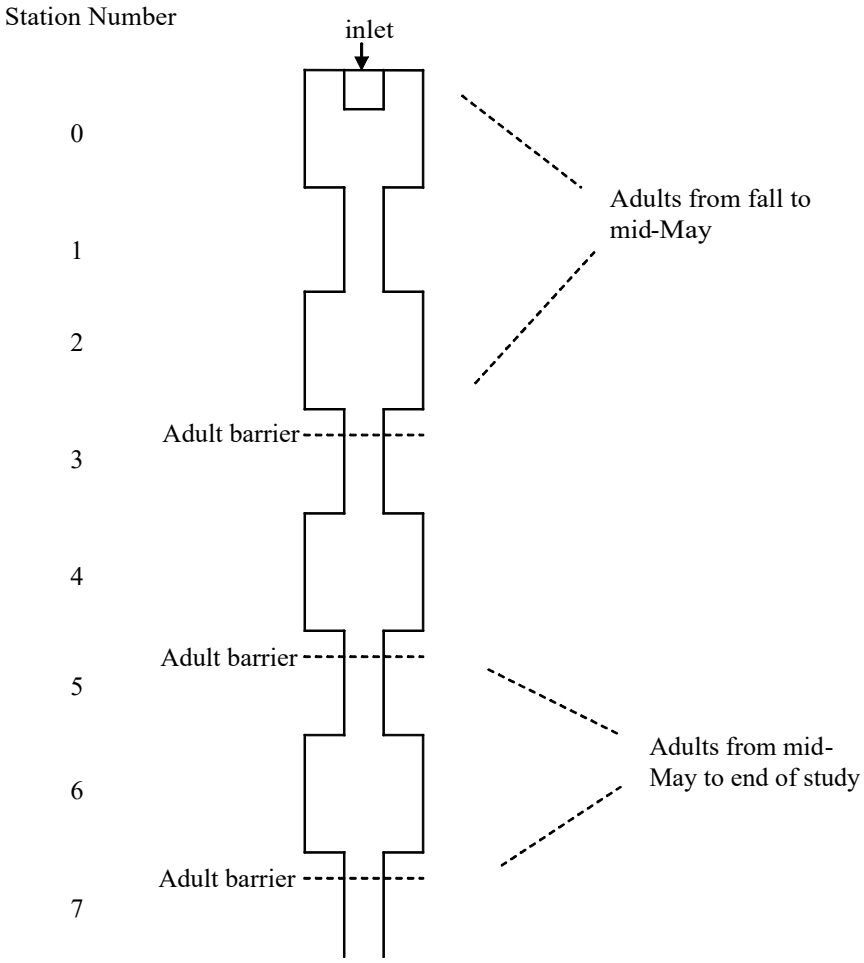


Table 2. Effects on Progeny - Study I[a]

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.

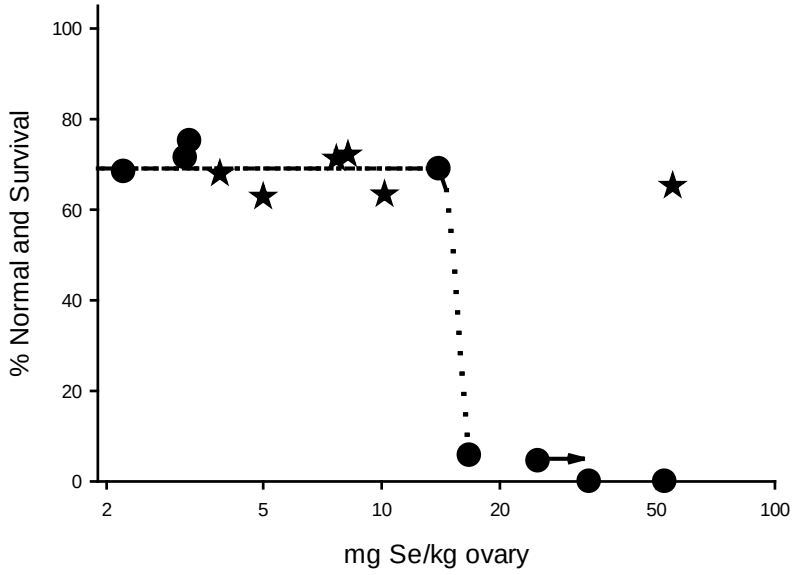


Figure 2. TRAP interpolation curve for the Table 5 ovary data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 14.0 mg/kg and the average background survival/normal 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.

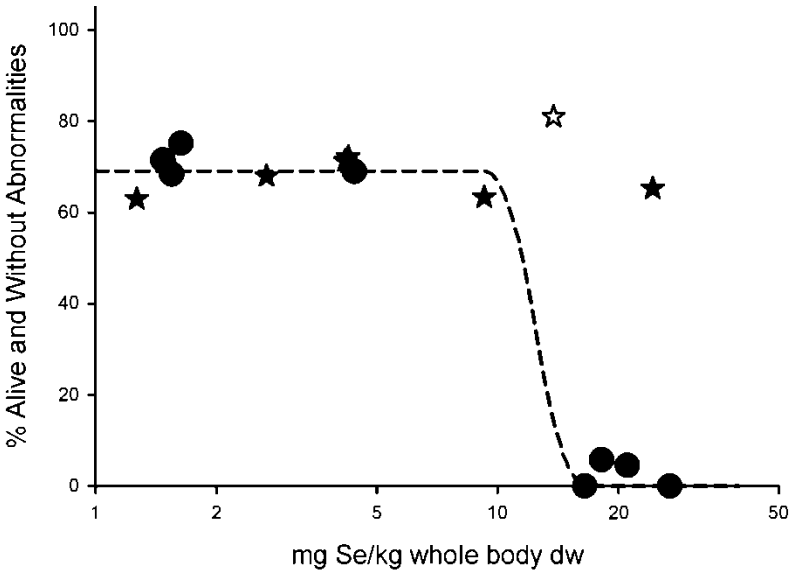


Figure 3. TRAP interpolation curve for the Table 5 whole body data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 9.3 mg/kg whole body and the average background 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

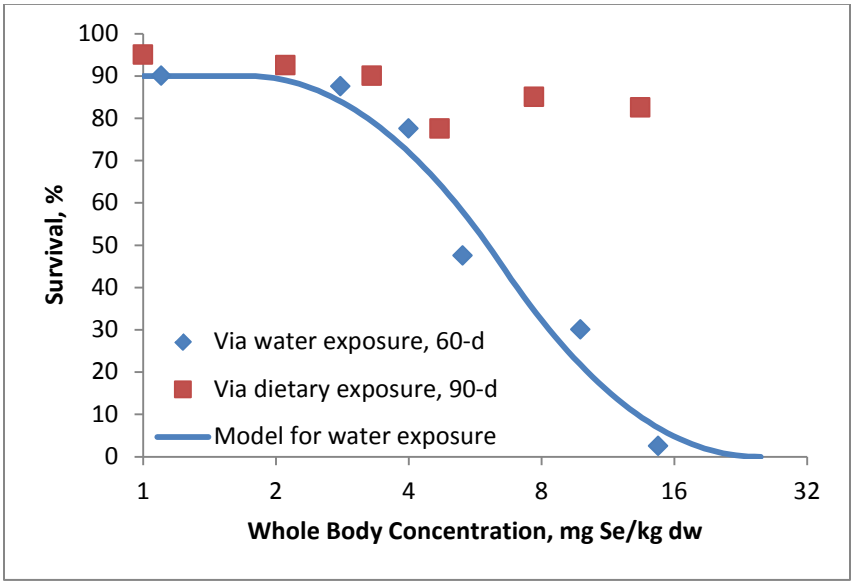
*[a] 30-d measurement because all fish were dead at 60 days in this concentration.

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).

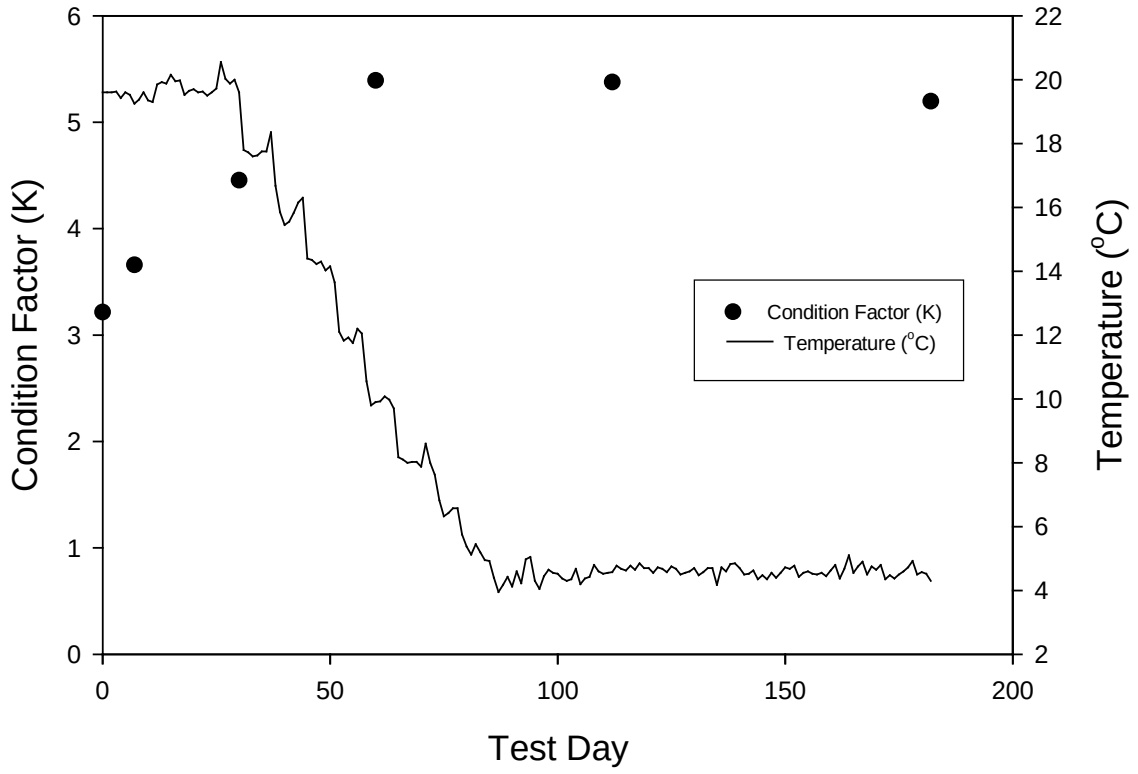
Several other studies have reported decreased feeding and activity levels for several fish species. McCollum et al. (2003) observed decreased overwinter feeding, and subsequent weight loss, of white crappie. Parrish et al. (2004) observed overwinter weight loss among mature, but not juvenile, salmon 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

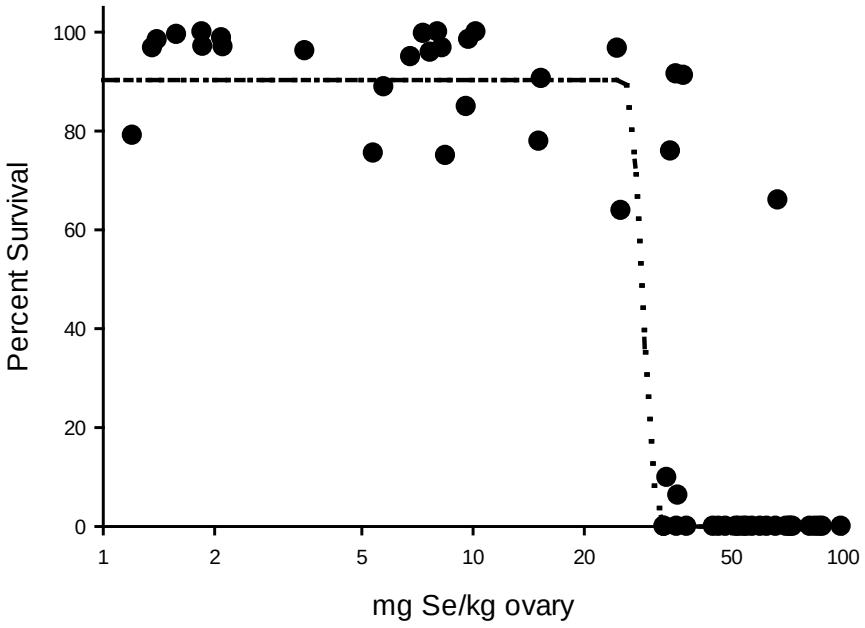


Figure 1. Largemouth bass larval survival relative to Se in ovary. TRAP interpolation was used to estimate the EC_{10} value. The higher concentration point for the interpolation was set at 32.9 mg/kg (EC_{100}) and the lower concentration point for the interpolation was set at 24.6 (NOEC) with greater 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.

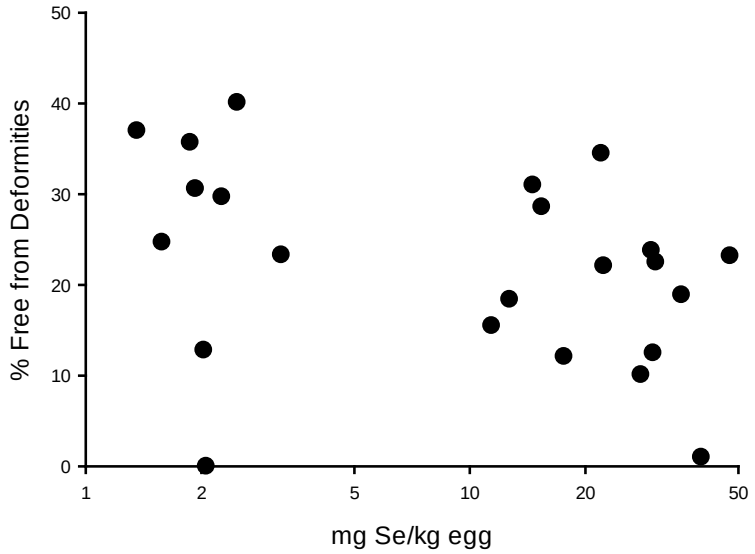


Figure E-1. Plot of percent free from deformities relative to the concentration of selenium in cutthroat trout eggs.

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

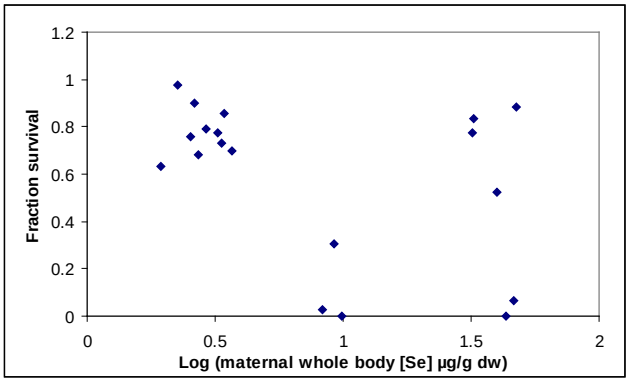
Table E-12. Fathead minnow first brood larval malformations and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

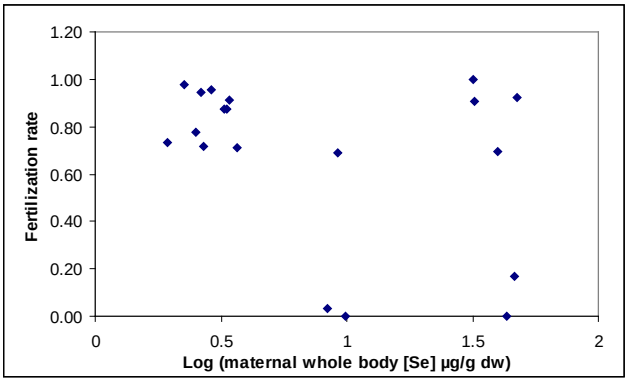
Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total Larvae	Spinal Incidence	%larvae w/o spinal deformity	%larvae w/o craniofacial deformity	%larvae w/o finfold deformity	%larvae w/o edema	Total GSI Score
T-1f-1	CON	1.94	11	9	91	100	100	100	1
T-2a-1	CON	2.25	141	3	97	99	98	96	24
T-6d-1	CON	2.52	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.





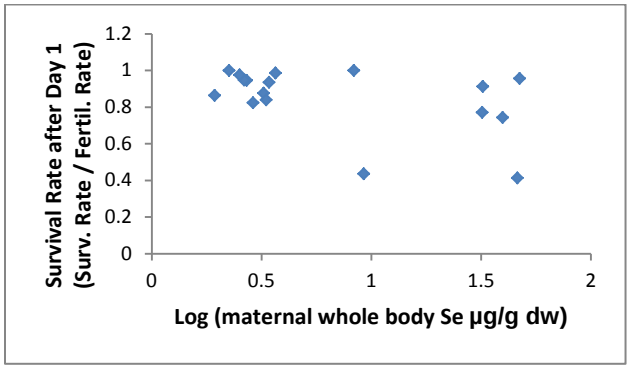


Figure E-3. Percent 2-day post-hatch larvae without edema (A), finfold deformity (B), craniofacial deformity (C), and spinal deformity (D) relative to maternal whole body selenium concentration. EC10s: 61.4 – 64.8 mg/kg dw WB.

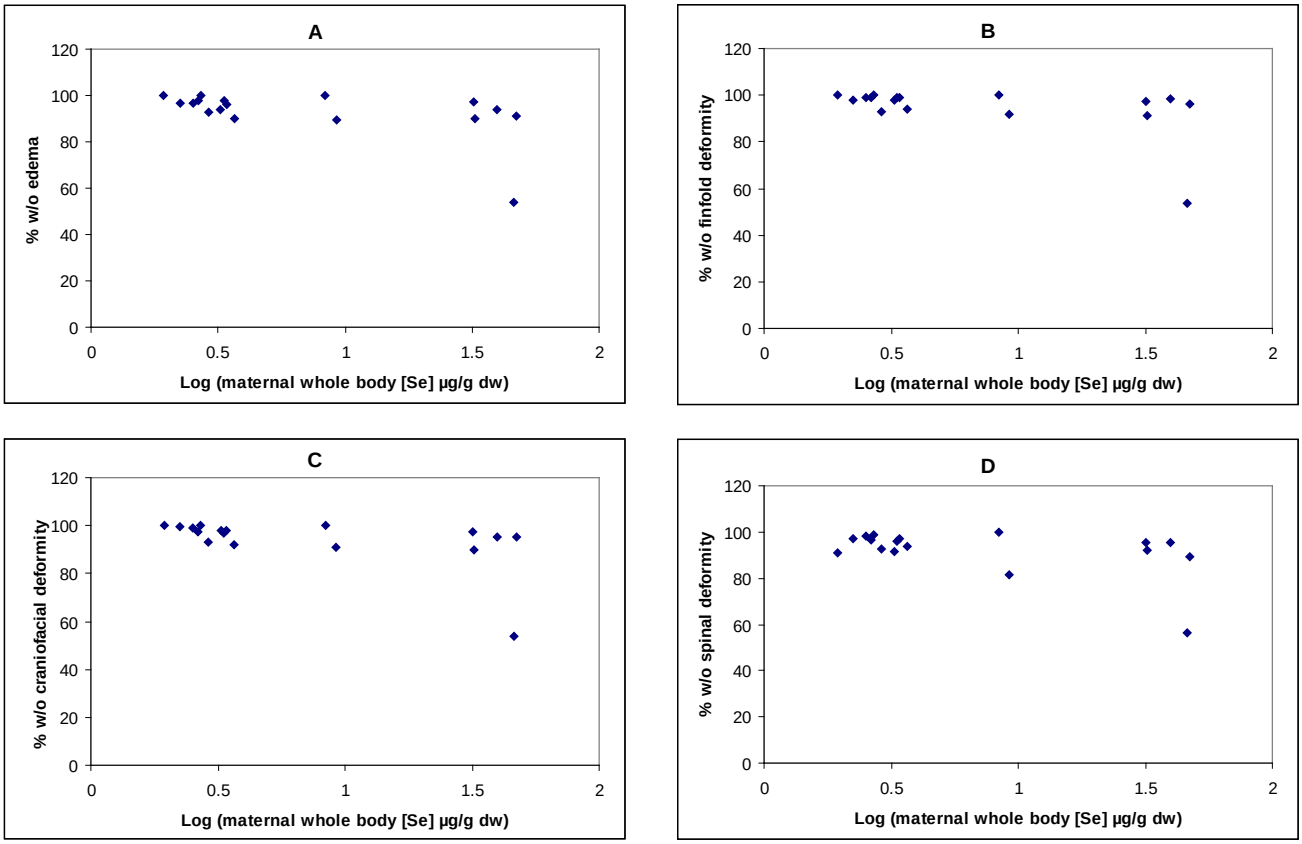
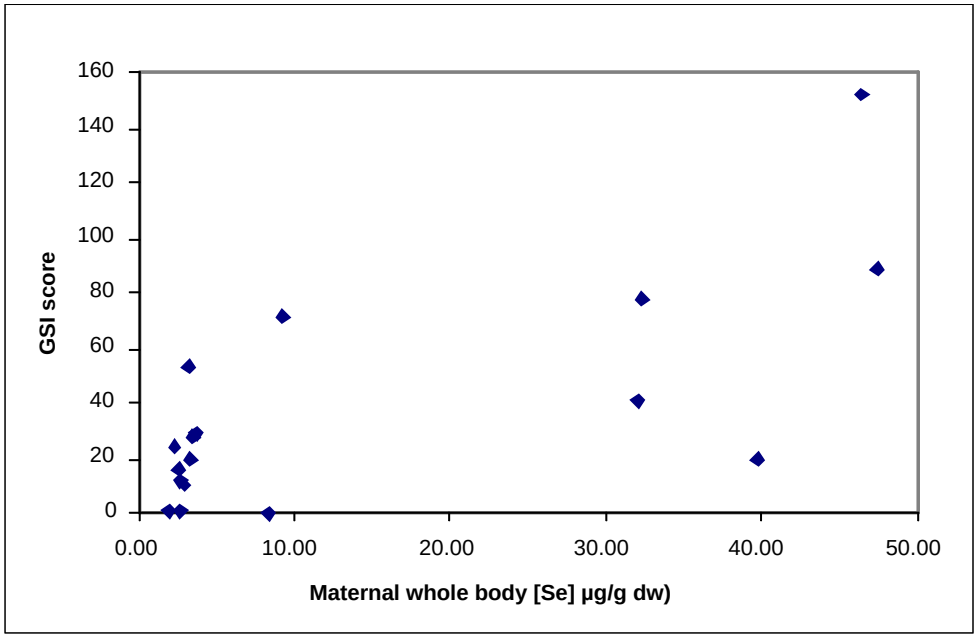


Figure E-4. Percent 2-day post-hatch larvae Graduated Severity Index (GSI) relative to maternal whole body selenium concentration



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.

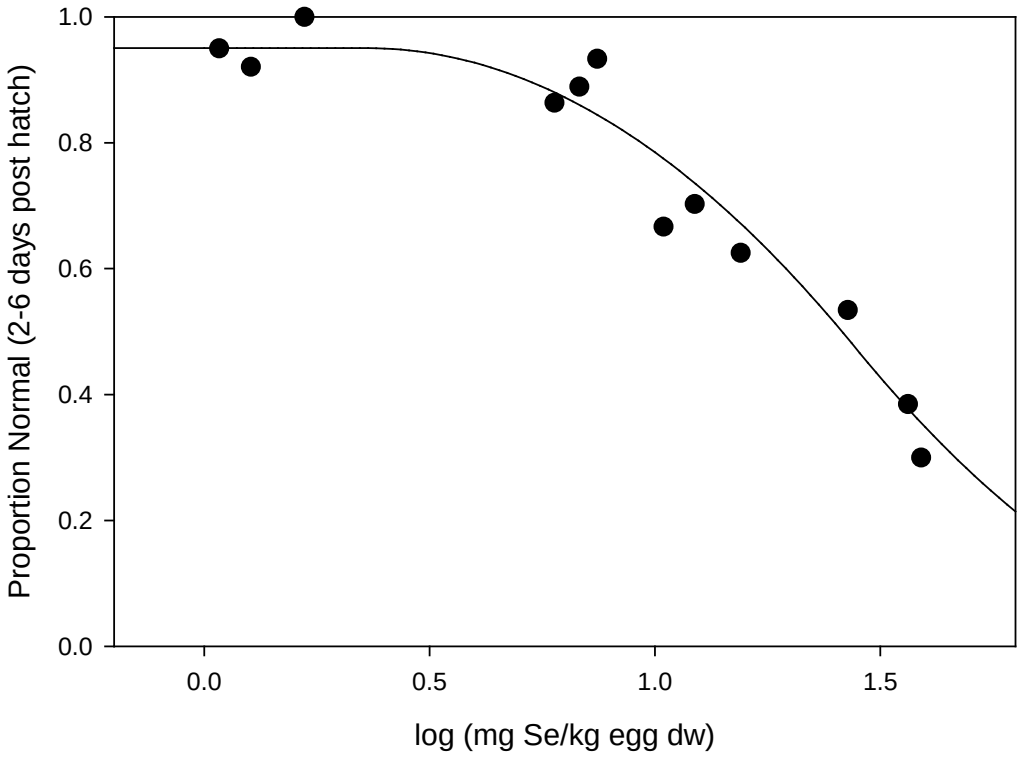
A GMCV based on this test has not been included in the Sensitivity Distribution for several reasons. Although the deformity and survival EC50s are within the range observed for a number of other species, the concentration-response curves

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.

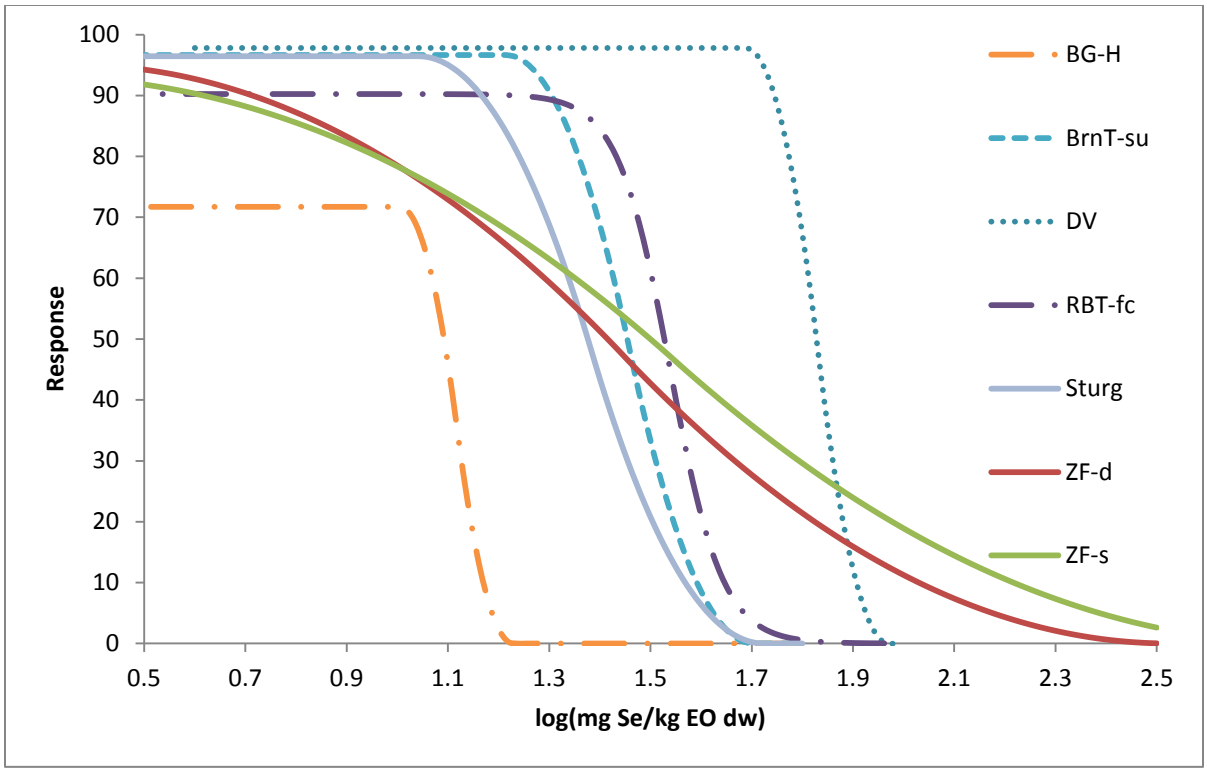


Figure E-6. Thomas and Janz (2014) zebrafish concentration-response curves for deformities and survival, ZF-d and ZF-s, compared with representative concentration-response curves for other species spanning the full range of EC10s.
BG-H: bluegill, Hermanutz et al. (1992, 1996); BrnT-su: brown trout survival 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

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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

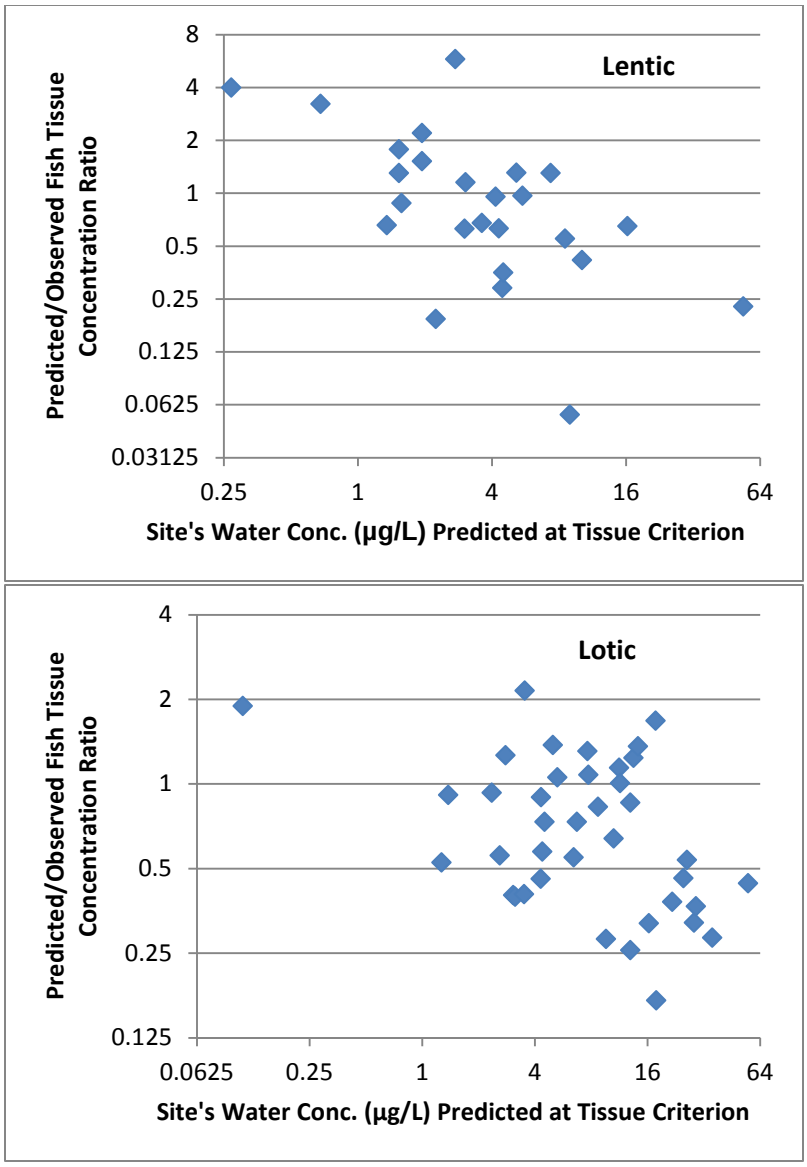
Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Lemly 1985	High Rock Lake	western mosquitofish	0.67	4.99	2.37	1.20	9.48	6.10	M
Lemly 1985	High Rock Lake	common carp	0.67	4.99	1.58	1.92	10.12	4.49	M
Lemly 1985	High Rock Lake	green sunfish	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

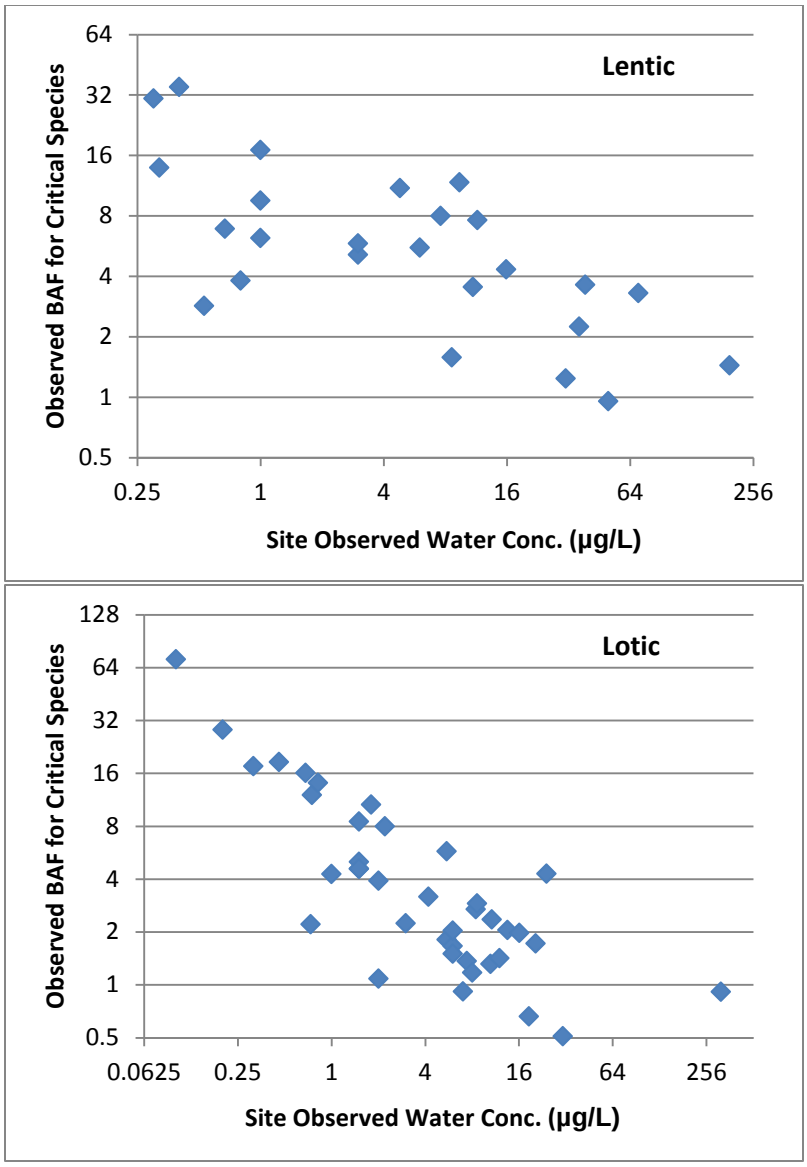
The ratio of predicted versus observed tissue concentrations in the above table can be compared against the main text Table 3.13 water concentrations that would be predicted to occur if each site's egg-ovary tissue concentration were at the criterion level. Figure I-1 shows the results. It can be seen 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).

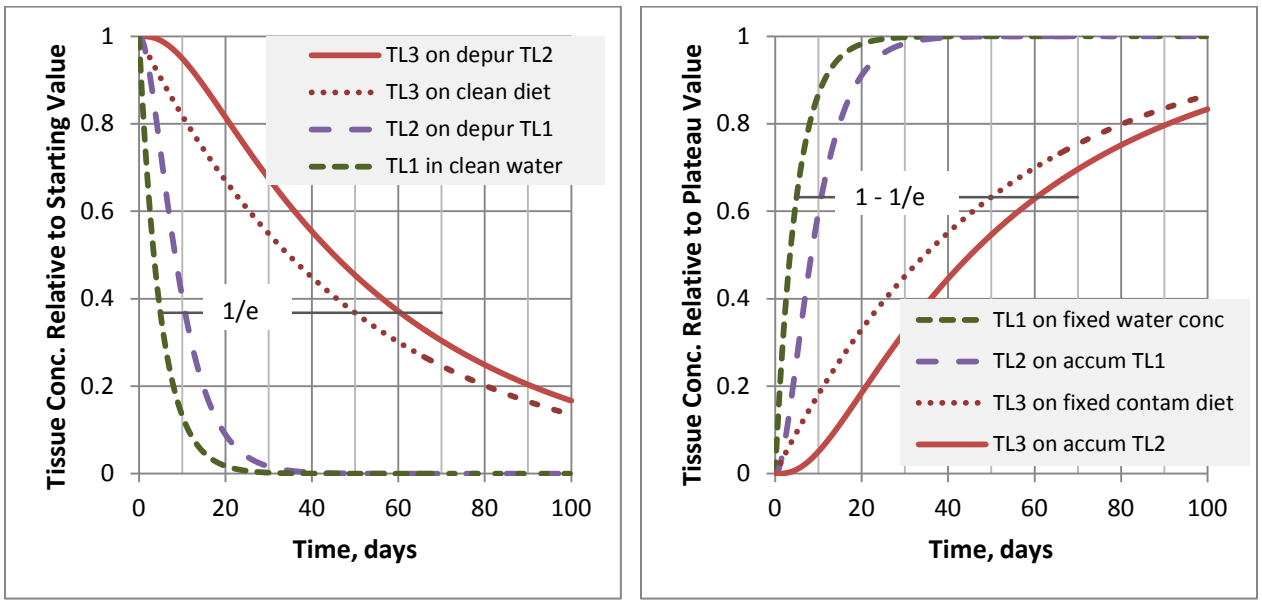
EPA's analysis showed that diet accounted for 34% - 92% of selenium bioaccumulation at trophic level 2, with a median of 74%. At trophic level 3, diet accounted for 62% - 100% of tissue selenium, with a median of 95%. Thus, disregarding aqueous uptake of selenium only resulted in a small (~5%) reduction 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:

$$C[t]water = C[t-1]water^{(\rho')} * GM^{(1-\rho')} * EXP\{\sigma * SQRT(1-\rho'^2)*NORMSINV(RAND)\}$$

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).

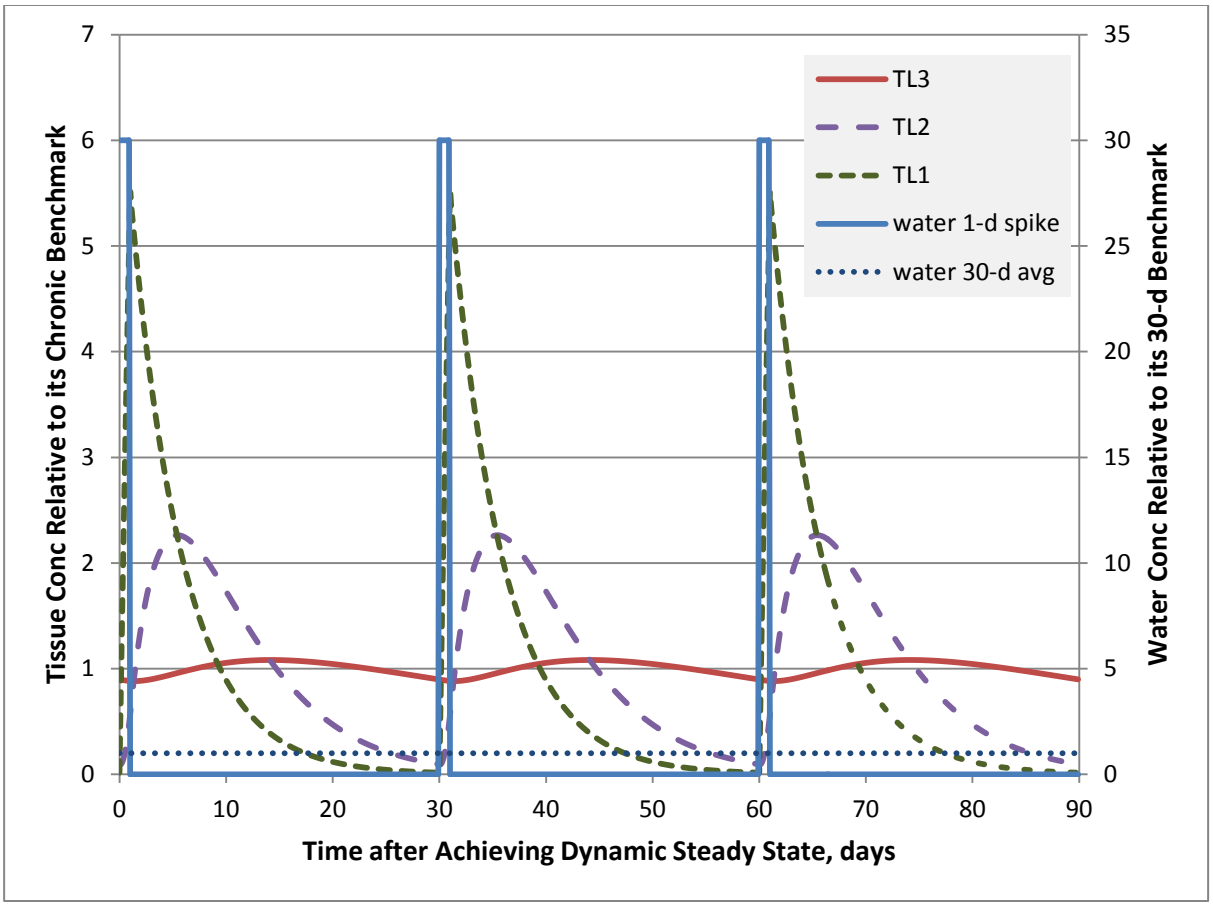


Figure J-2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion.
Tissue and water concentrations are expressed as dimensionless ratios relative to their respective criteria or benchmarks, as explained 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.

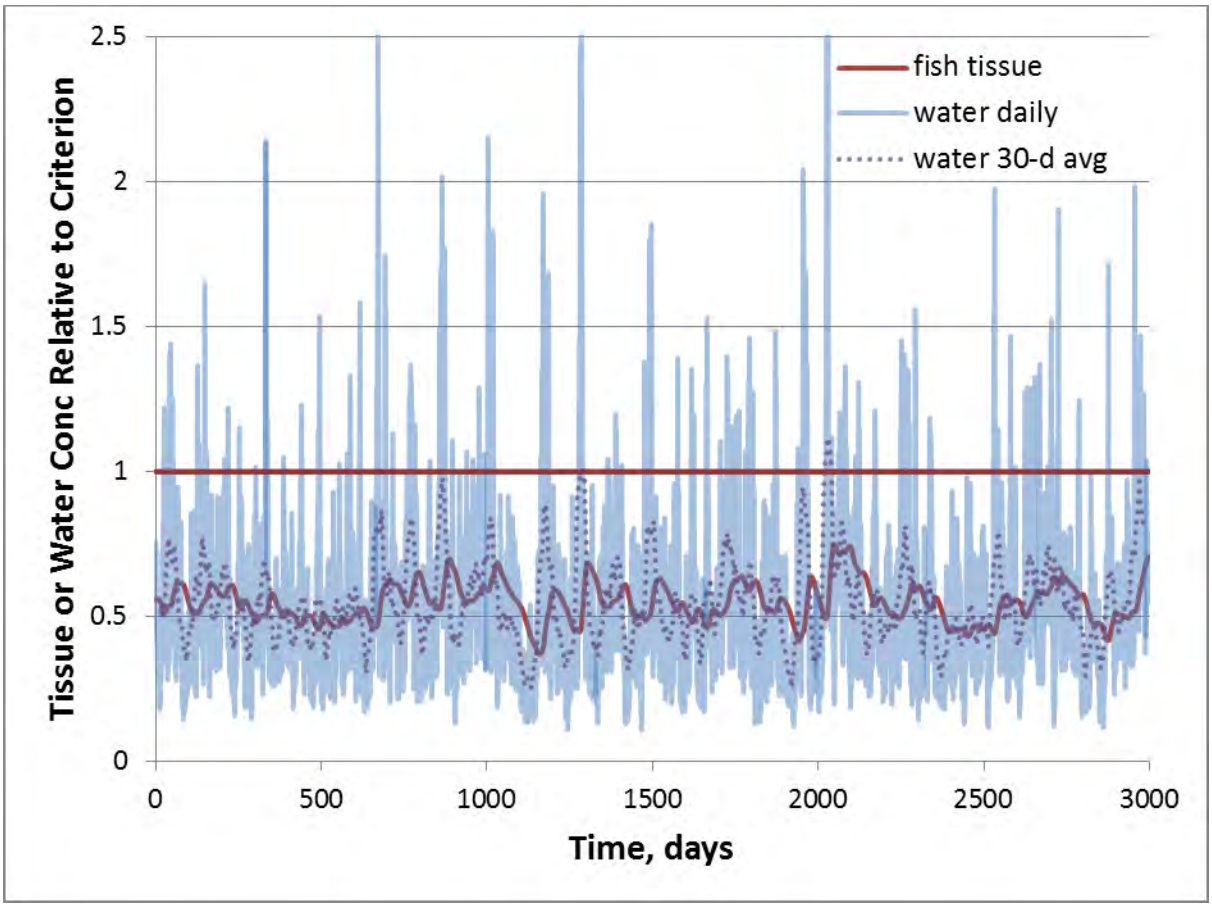


Figure J-3. A typical example of log-normally distributed, smoothly variable concentrations. The standard deviation of natural logs is 0.5 and the serial correlation coefficient of logs is 0.8 for daily values, both typical real-world situations. (The compression of 3000 days into the graph might make 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.

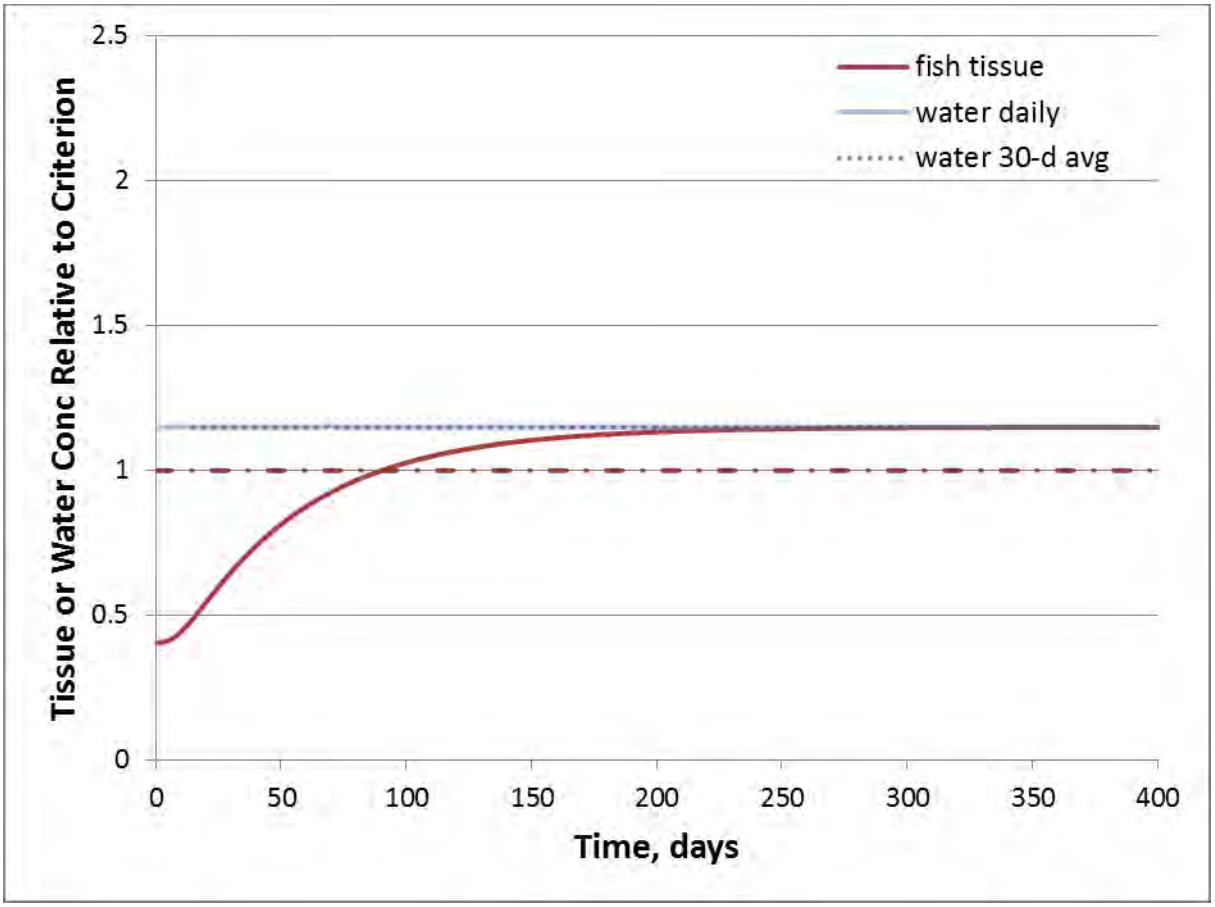


Figure J-4. TL3 concentration responding to a Time=0 step-function increase in water concentration that remains time-invariant thereafter.
Given that the water concentration is too high, ultimately yielding tissue concentrations at the hypothetical sensitive species EC20, 1.15-fold above the criterion, 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.

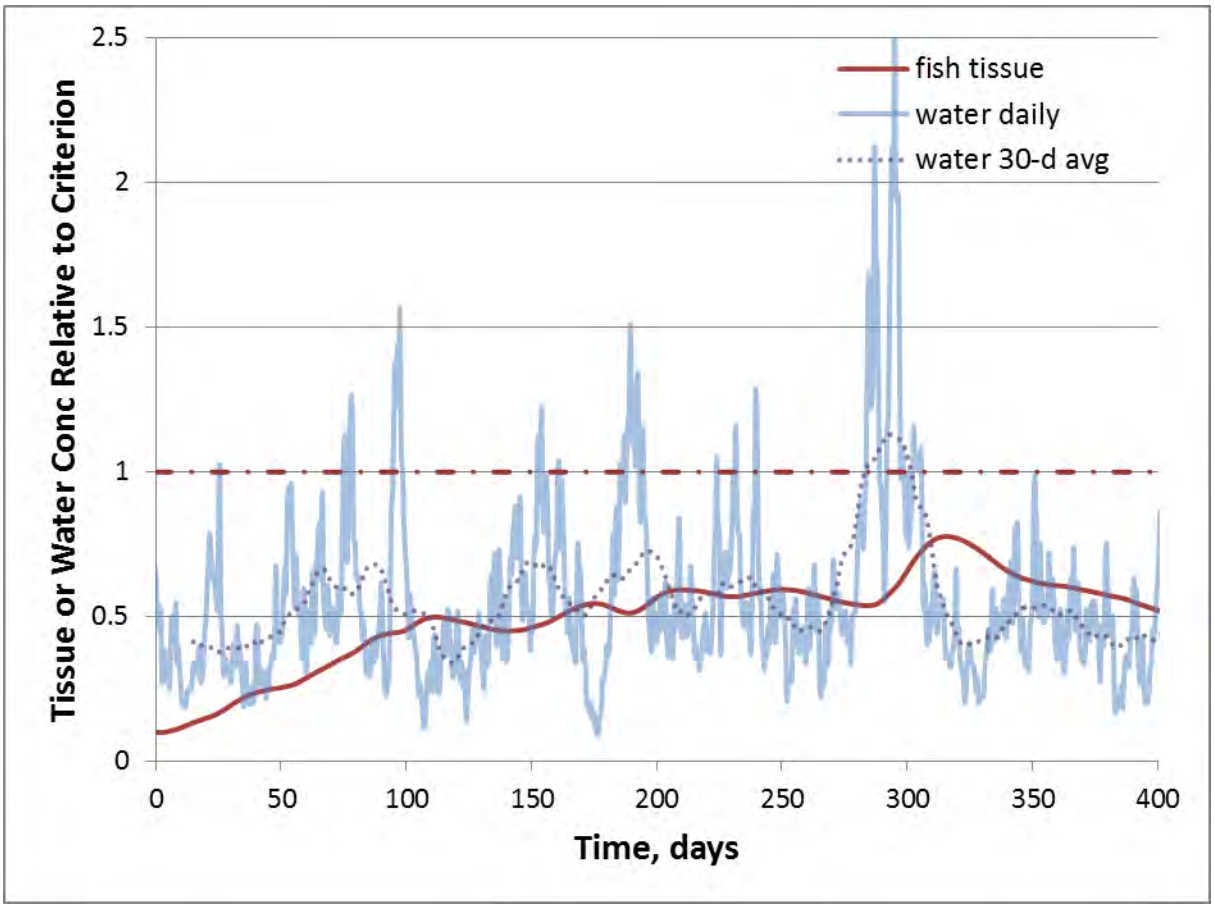


Figure J-5. Flowing water example of TL3 concentration starting at a concentration of 0.1 normalized to the criterion, and responding to randomly varying log-normally distributed water concentrations having median 0.49 (expressed as a dimensionless ratio: concentration/criterion), log standard deviation 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.

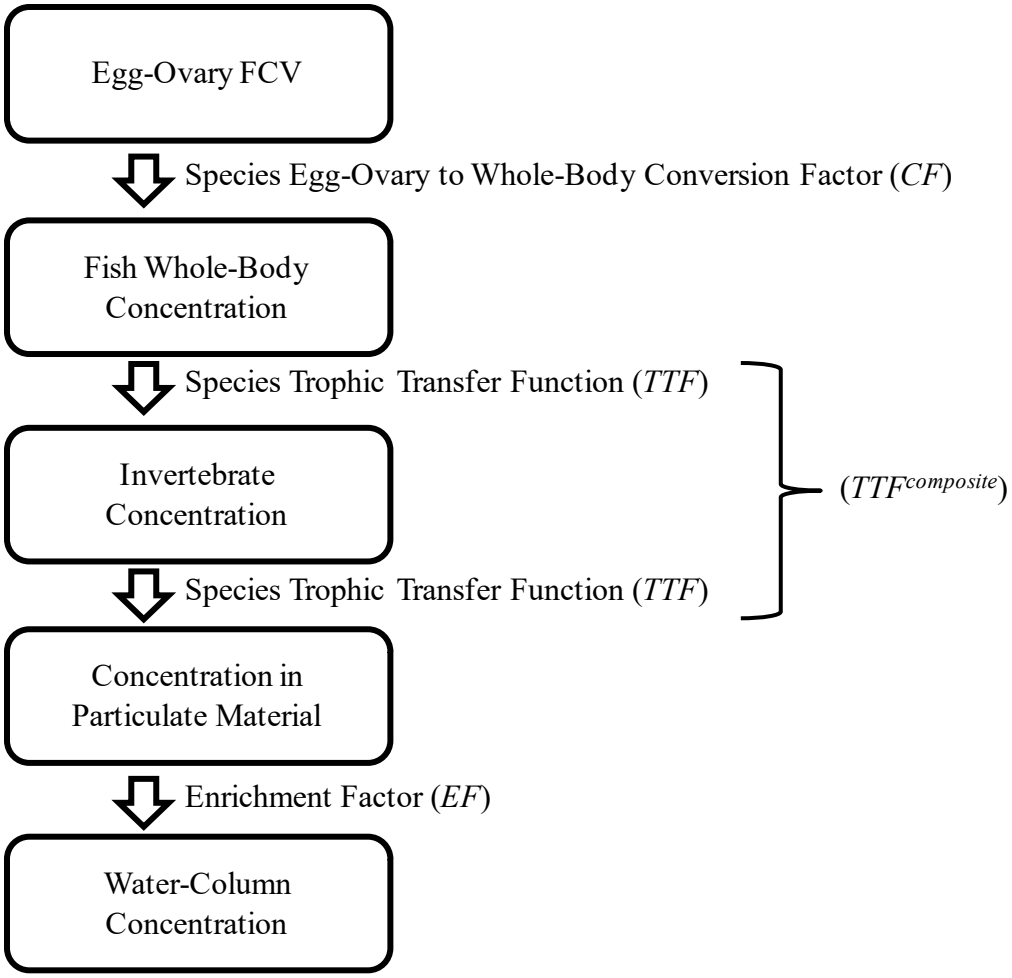


Figure K-1. Conceptual model for translating the egg-ovary FCV to a water-column concentration.
Note: States may want to use the whole body or muscle criterion elements as the starting point for site specific translation to a water column concentration.

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.

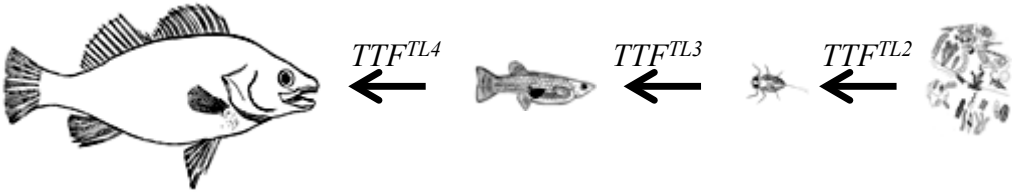
These concepts can be used to formulate a mathematical expression of $TTF^{composite}$ that models selenium bioaccumulation in a variety of aquatic ecosystems. Figure K-2 illustrates five hypothetical food web scenarios and the formulation of $TTF^{composite}$ for each of them. For each scenario, the 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):

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

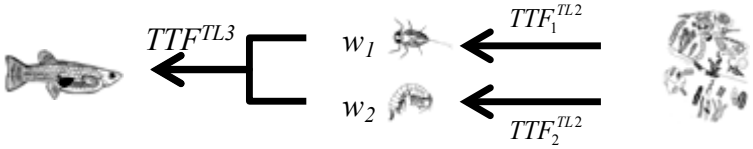
B) Four trophic levels (simple):

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



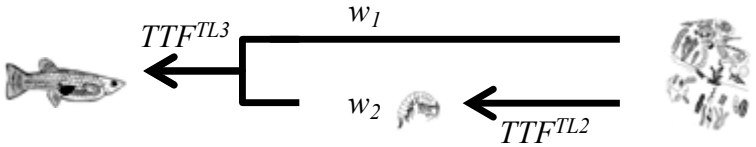
C) Three trophic levels (mix within trophic levels):

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



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):

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

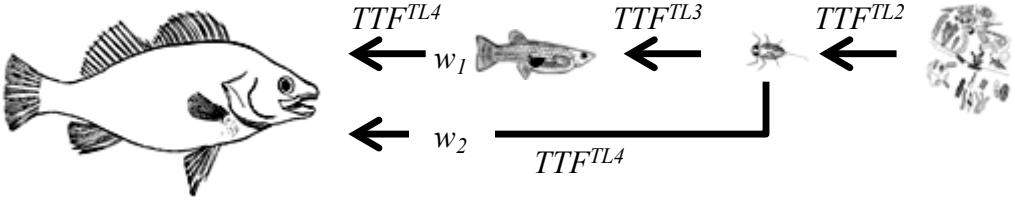


Figure K-2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios.

$TTF^{composite}$ 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.

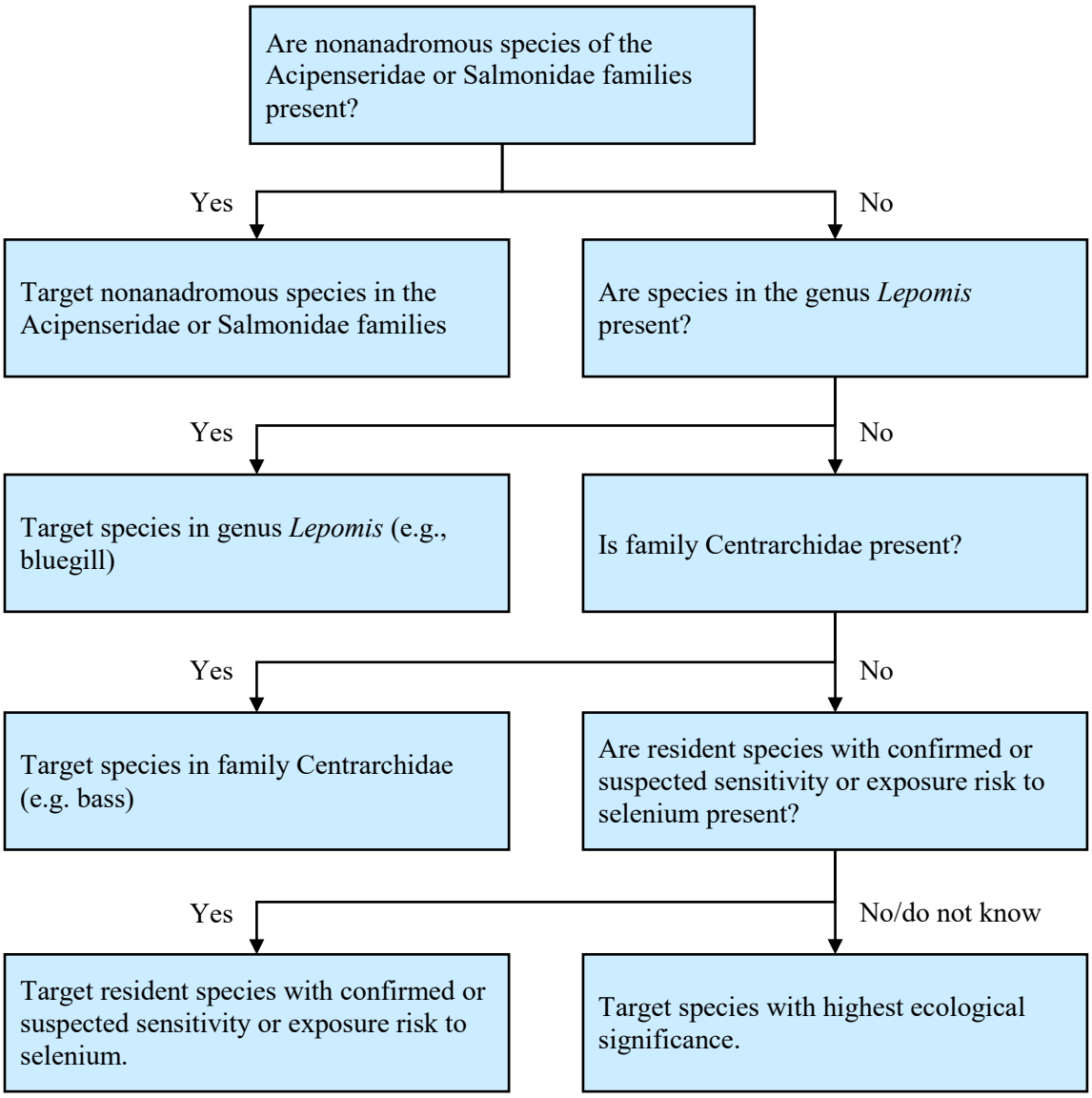


Figure K-3. Recommendeed decision process for selection of the fish species to use when deriving a water concentration from the selenium egg-ovary FCV.
This decision tree is also generally applicable when using the whole body or muscle tissue as the starting point for development of SSC, particularly 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.

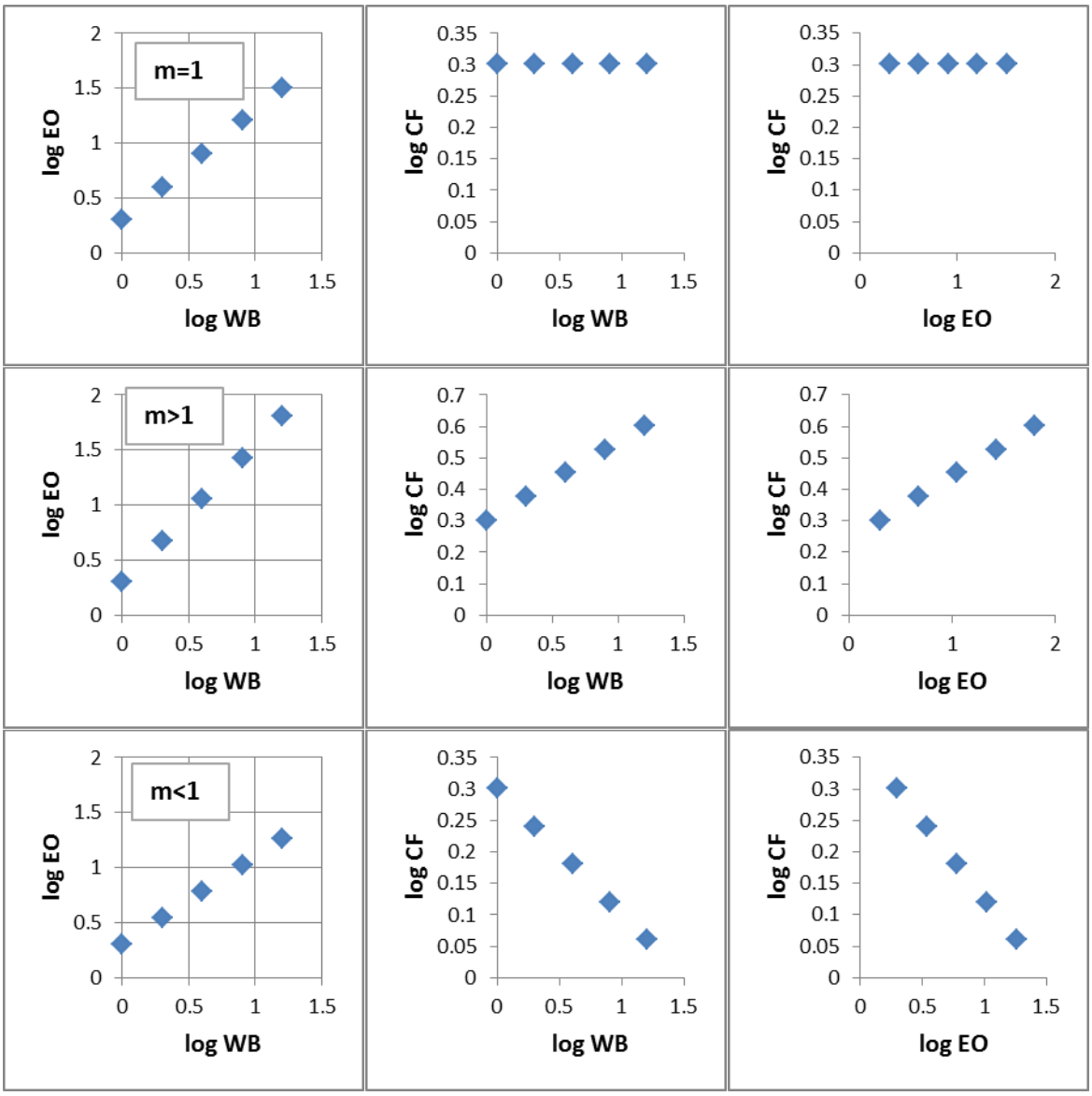


Figure N-1. Idealized illustration (sans scatter) of the effect of log-log slope, m, on whether CF is stable, increases, or decreases with concentration (whether measured as WB in column 2 or EO in column 3).

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).

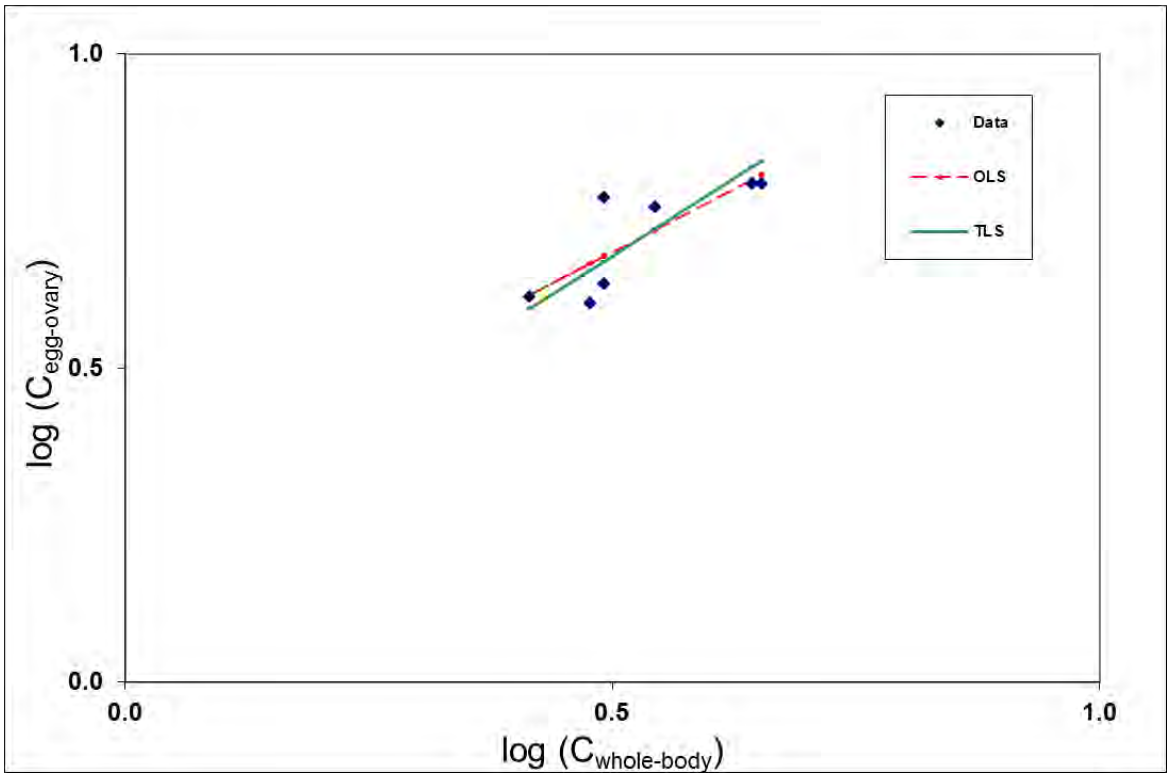


Figure N-2. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

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).

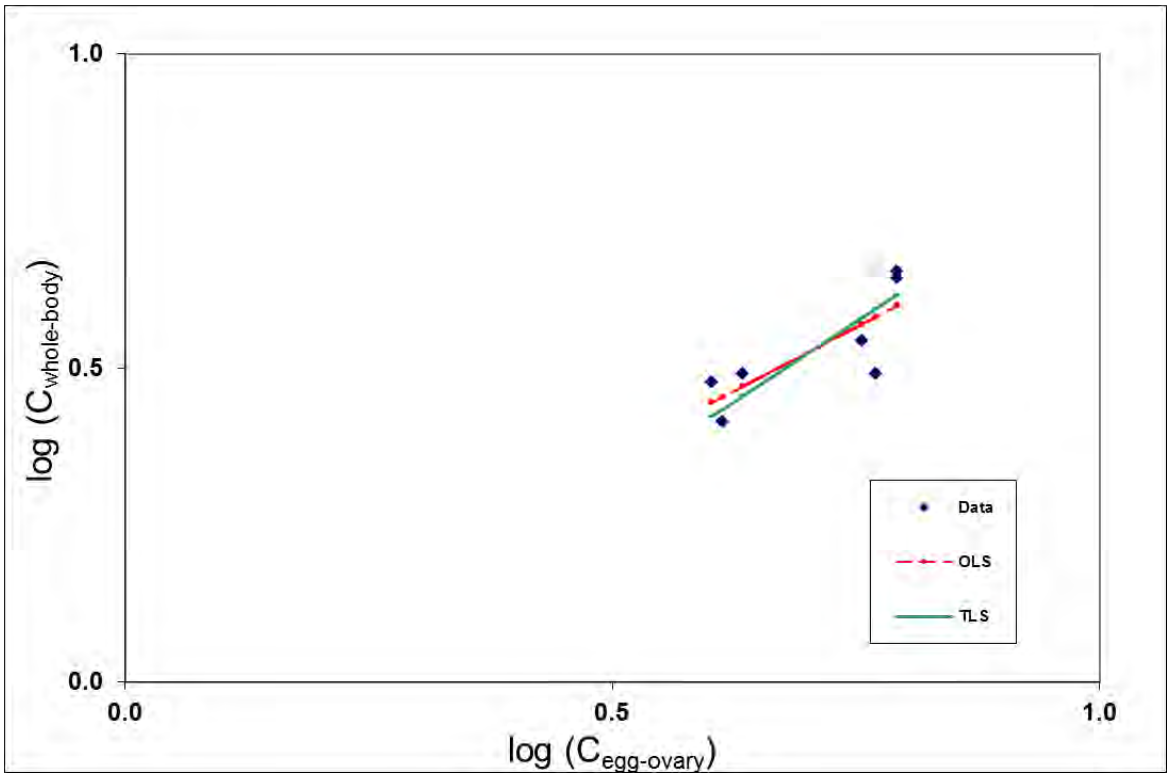


Figure N-3. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

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.

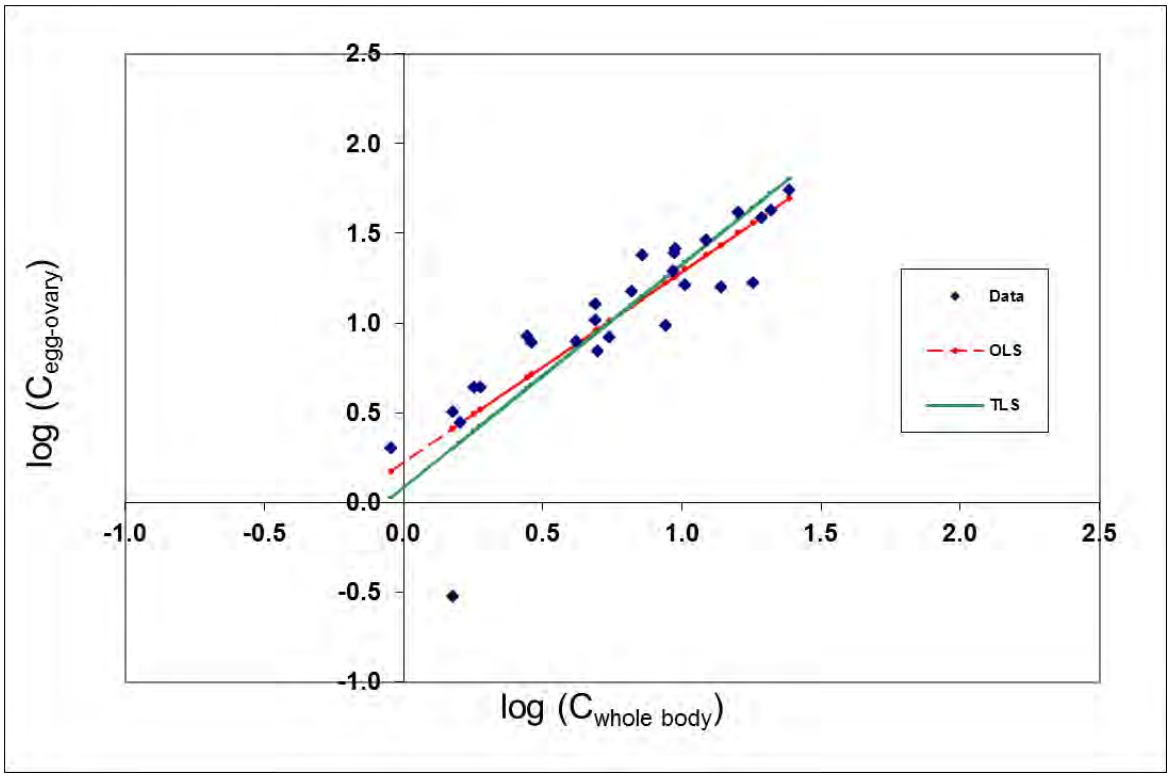


Figure N-4. OLS vs. TLS regression model fits for bluegill. Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

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.

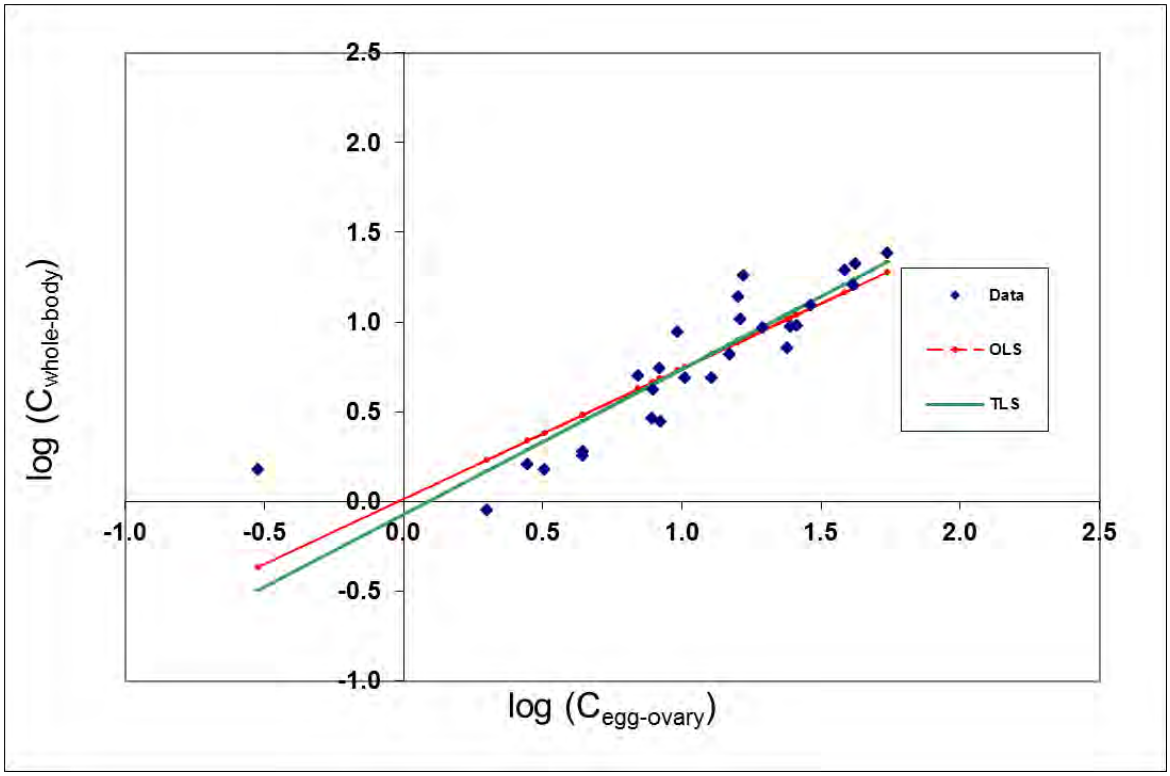


Figure N-5. OLS vs. TLS regression model fits for bluegill.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

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