BOARD OF ENVIRONMENTAL REVIEW
AGENDA ITEM
EXECUTIVE SUMMARY FOR PROPOSED NEW RULE I

Agenda Item #I.1.1

Agenda Item Summary – The Department requests the Board initiate rulemaking to amend ARM 17.30.602 (definitions pertaining to surface water quality standards) and adopt proposed New Rule I (Selenium Standards for Lake Koocanusa and the Kootenai River). State law (§§ 75-5-201, 75-5-203, and 75-5-301(2), MCA) grant the board authority to adopt water quality standards based on federal Clean Water Act (CWA) section 304(a) guidance and 304(a) guidance modified to reflect site-specific conditions. In 2016, the U.S. Environmental Protection Agency (EPA) updated the national selenium criteria guidance published pursuant to section 304(a). The guidance included a recommendation that states and tribes develop site-specific selenium standards whenever possible due to the local environmental factors affecting selenium bioaccumulation in aquatic ecosystems.

Proposed New Rule I establishes numeric selenium standards for Lake Koocanusa and the Kootenai River, geographically extending from the international boundary between Montana and British Columbia (B.C.) to the Idaho-Montana border. The standards are expressed as both fish tissue and water column concentrations and are based on EPA 304(a) guidance.

The standards are necessary because selenium loads are increasing in the reservoir due to coal mining activities in Canada, and the standards are consistent with the best available science for selenium toxicity and will protect selenium-sensitive aquatic life in this watershed. The proposed fish tissue and water column standards for the Kootenai River are based on current EPA 304(a) criteria for lotic (flowing) waters. The proposed fish tissue and water column standards for Lake Koocanusa are based on EPA 304(a) fish tissue criteria, and site-specific water column criteria derived following procedures set forth by EPA in the 304(a) guidance.

List of Affected Board Rules –The new selenium standards in proposed New Rule I will apply to Lake Koocanusa and the mainstem Kootenai River and will supersede surface water selenium standards found in Department Circular DEQ-7.

List of Affected Department Rules – The proposed rulemaking includes amendment of ARM 17.30.602 to adopt a definition of steady state. During steady state, the fish tissue standards for selenium take precedence over the water column standards. When non-steady state conditions prevail, both the fish tissue standards and the water column standards apply with neither taking precedence.

Affected Parties Summary – MPDES permit holders. ARM 17.30.632 may affect permitted dischargers in Lake Koocanusa and the mainstem of the Kootenai River with selenium limits in their MPDES discharge permits.

Scope of Proposed Proceeding –The department requests that the board initiate rulemaking and schedule a public hearing to take comments on the proposed rules.

Background – In 2015, the department began a coordinated effort with an international working group consisting of U.S. and Canadian stakeholders to develop site-specific selenium criteria for Lake Koocanusa. The department collaborated with the British Columbia Ministry of Environment and Climate Change Strategy (BC-ENV) and a selenium committee comprised of scientists recognized for their selenium expertise. There was significant stakeholder collaboration and input throughout the multi-year standards development process. The collaborative process also included a partnership with the U.S. Geological Survey to perform biodynamic selenium modeling using their peer reviewed Ecosystem-Scale Selenium Model (Presser and Luoma, 2010). The technical basis of the standards work is described in three reports (EPA, 2016; Presser and Naftz, 2020; DEQ, 2020). From this technical work, the department has identified the fish tissue and water column standards applicable to Lake Koocanusa and the mainstem Kootenai River.

The technical reports referenced above are:

DEQ (Montana Department of Environmental Quality). 2020. *Technical Support Document for the derivation of a site-specific water column standard for Lake Koocanusa.* Helena, MT: Montana Dept. of Environmental Quality.

EPA (Environmental Protection Agency). 2016. *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*. Washington DC: United States Environmental Protection Agency.

Presser, T.S., Luoma, S.N., 2010, A methodology for Ecosystem-Scale Modeling of Selenium. *Integrated Environmental Assessment and Management,* Volume 6, *Issue 4,* Pages 685-710.

Presser, T.S., Naftz D.L., 2020. *Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada*. Open-File Report 2020-1098, Helena, MT: U.S. Geological Survey.

Hearing Information – The department recommends that the board appoint a hearing officer and conduct a public hearing to take comment on the proposed new rule.

Board Options – The board may:

1. Initiate rulemaking by directing the department to file the attached proposed rulemaking notice and notice of public hearing;
2. Determine that the proposed rule is not appropriate and decline to initiate rulemaking, or;
3. Modify the proposed rulemaking notice and direct the department to file the modified proposed rulemaking notice and notice of public hearing.

DEQ Recommendation – The department recommends that the board initiate rulemaking, as proposed in the attached notice of public hearing, and appoint a hearings officer.

Enclosures –

1. Draft Administrative Register Notice of Public Hearing on Proposed Amendment

2. Four technical reports (cited above) which provide the technical foundation for the proposed standards.

BEFORE THE BOARD OF ENVIRONMENTAL REVIEW
OF THE STATE OF MONTANA

In the matter of the proposed amendment of ARM 17.30.602, and the adoption of NEW RULE I pertaining to selenium standards for Lake Koocanusa and the Kootenai River	))))))	NOTICE TO HOLD VIRTUAL PUBLIC HEARING ON PROPOSED AMENDMENT AND ADOPTION (WATER QUALITY)

TO: All Concerned Persons

1. On November 5, 2020, at 10:00 a.m., the Board of Environmental Review (board) will hold a virtual public hearing via Zoom, to consider the proposed amendment and adoption of the above-stated rules.

Due to the guidance issued by the Governor of the State of Montana on March 26, 2020, regarding the COVID-19 public health situation, the public hearing will be held virtually via the Zoom meeting platform and will be recorded. Persons wishing to attend the public hearing need to register in advance with Zoom. Registration with Zoom may be made at the following link: https://mt-gov.zoom.us/meeting/register/tJYudO6grjIrHN0MjMMCzC9gJR3is4ZkzV6d. After registering, you will receive a confirmation email containing information about joining the hearing. Please contact Sandy Scherer at the Department of Environmental Quality at (406) 444-2630 or sscherer@mt.gov should you encounter any difficulties.

2. The board will make reasonable accommodations for persons with disabilities who wish to participate in this rulemaking process or need an alternative accessible format of this notice. If you require an accommodation, contact Sandy Scherer no later than 5:00 p.m., October 29, 2020, to advise us of the nature of the accommodation that you need. Please contact Sandy Scherer at the Department of Environmental Quality, P.O. Box 200901, Helena, Montana 59620-0901; phone (406) 444-2630; fax (406) 444-4386; or e-mail sscherer@mt.gov.

3. The rule proposed to be amended provides as follows, stricken matter interlined, new matter underlined:

ARM 17.30.602 DEFINITIONS In this subchapter the following terms have the meanings indicated below and are supplemental to the definitions given in 75-5-103, MCA:

(1) through (31) remain the same.

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

(32) through (41) remain the same, but are renumbered (33) through (42).

AUTH: 75-5-201, 75-5-301, MCA

IMP: 75-5-301, 75-5-313, MCA

REASON: Proposed NEW RULE I contains two classes of selenium standards; fish tissue standards, which limit the amount of selenium allowed to accumulate in different tissues, and water column standards, which are derived from bioaccumulation modeling and intended to limit selenium accumulation in fish tissue. Fish tissue standards provide the most direct and accurate assessment of selenium impacts on aquatic life; but if selenium loading to a waterbody is increasing, it can take time (months, years) for the effect to be detected in the fish. This situation—in which there is a delay between increased selenium loading and increased levels of selenium in fish tissue—is referred to as non-steady state. When selenium loadings to the aquatic system are stable and fish selenium concentrations have leveled off, this is referred to as steady state. When steady state is achieved, selenium loading in the water body is reflected in selenium concentrations in fish tissue. It is necessary to adopt the proposed definition of steady state to determine which selenium standard will apply to protect the aquatic life beneficial use. During steady state, the fish tissue standards take precedence over the water column standards. When non-steady state conditions prevail, the fish tissue standards do not take precedence over the water column standards and both standards apply (see NEW RULE I(2)). The proposed definition of steady state provides conditions under which the water body will be determined to be in steady state. If steady state is not achieved, the water body is deemed to be in non-steady state.

4. The rule proposed to be adopted provides as follows:

NEW RULE I SELENIUM STANDARDS FOR LAKE KOOCANUSA AND THE KOOTENAI RIVER (1) For Lake Koocanusa and the Kootenai River mainstem, the standards specified in (6) and (7) supersede the otherwise applicable water quality standards found elsewhere in state law.

(2) Numeric selenium standards for Lake Koocanusa and the Kootenai River mainstem from the US-Canada international boundary to the Montana-Idaho border are expressed as both fish tissue and water column concentrations. When the aquatic ecosystem is in steady state and selenium data is available for both fish tissue and the water column, the fish tissue standards supersede the water column standard. When the aquatic ecosystem is in non-steady state, both the fish tissue and water column standards apply. The numeric selenium standards apply to the lake, to the river, or to both, as provided in this rule.

(3) As of [effective date of this rule], Lake Koocanusa and the Kootenai River aquatic ecosystems are in non-steady state. The department will reassess the status of these aquatic systems triennially and amend this rule to reflect any change.

(4) The water column standards are derived from modeling selenium bioaccumulation in fish tissue and reflect criteria that protect the aquatic life beneficial use. Permit conditions and limits developed from the water column standards comply with the fish tissue standards.

(5) No person may violate the numeric water quality standards in (6) through (7).

(6) Fish tissue standards are applicable to tissues of fish in Lake Koocanusa

from the US-Canada international boundary to the Libby Dam and in the mainstem Kootenai River from the outflow below the Libby Dam to the Montana-Idaho border. Egg/ovary tissue standards supersede any muscle or whole-body standards, as well as the water column standards in (7), when fish egg/ovary samples are available and when the aquatic ecosystem is in steady state.

Fish Tissue	Selenium Concentration
Eggs/Ovaries	15.1 mg/kg dry weight (dw)
Muscle	11.3 mg/kg dw
Whole Body	8.5 mg/kg dw

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

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

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

AUTH: 75-5-201, 75-5-301, MCA
IMP: 75-5-301, MCA

REASON: Section 75-5-301(2), MCA, grants the board the authority to adopt water quality standards under the Montana Water Quality Act. Under 75-5-203(1), MCA, the board may not adopt a standard that is more stringent than the comparable federal regulations or guidelines that address the same circumstances. In 2016, the U.S. Environmental Protection Agency (EPA) updated the national selenium criteria guidance published pursuant to section 304(a) of the federal Clean Water Act. The guidance included a recommendation that states and tribes develop site-specific selenium standards, whenever possible, due to the local environmental factors affecting selenium bioaccumulation in aquatic ecosystems.

In 2015, the department began a coordinated effort with an international working group consisting of U.S. and Canadian stakeholders to develop site-specific selenium criteria for Lake Koocanusa. The technical work was undertaken in collaboration with the British Columbia Ministry of Environment and Climate Change Strategy (BC-ENV) and a selenium committee comprised of scientists recognized for their selenium expertise. There was significant stakeholder collaboration and input throughout the multi-year standards development process. The collaborative process also included a partnership with the U.S. Geological Survey to perform bioaccumulation selenium modeling using their peer reviewed Ecosystem-Scale Selenium Model (Presser and Luoma, 2010). The technical basis of the criteria is described in three reports (EPA, 2016; Presser and Naftz, 2020; DEQ, 2020). From this technical work there is a narrow range of protective values from which the department has identified the proposed fish tissue and water column standards that would be applicable to Lake Koocanusa and the Kootenai River.

The fish tissue standards are expressed as instantaneous measurements not to be exceeded. Fish tissue standards have a hierarchy of importance; the egg/ovary standard is the most important because it is the most indicative of selenium toxicological effects on fish at the reproductive stage. Toxicological effects of selenium at the reproductive stage include, but are not limited to, mortality, deformity, growth impairment, oxidative stress, and behavioral impairment. However, fish egg/ovary tissue selenium data is not always available. Fish muscle or whole-body tissue standards can be used in the absence of fish egg/ovary tissue.

The fish tissue standards supersede the water column standard only when the lake or river is in steady state, referring to conditions whereby there are no occurrences of new activities to the lake or river that release selenium to the aquatic ecosystem (EPA, 2016). At the time of this rule adoption, Lake Koocanusa and the Kootenai mainstem river are in non-steady state (Presser and Naftz, 2020). Therefore, both the fish tissue standards and water column standards are the applicable standards for Lake Koocanusa and the Kootenai mainstem river. The department will determine when Lake Koocanusa and the Kootenai mainstem river reach steady state after review and analysis has been carried out by the department during triennial review. The proposed water column standards are chronic values. There is no acute selenium standard included since the greatest toxicity risk to aquatic life is from chronic dietary exposure.

It is necessary to adopt the proposed numeric selenium standards to incorporate the best available science for selenium toxicity and protect selenium-sensitive aquatic life in the Kootenai watershed. The proposed fish tissue and water column standards for the mainstem Kootenai River are based on current EPA 304(a) criteria for lotic (flowing) waters. The proposed fish tissue and water column standards for Lake Koocanusa are based on EPA 304(a) fish tissue criteria, and site-specific water column criteria derived following procedures set forth by EPA in the 304(a) guidance.

Montana's nondegradation rules (Administrative Rules of Montana, chapter 17.30, subchapter 7) classify certain discharge activities as nonsignificant if they meet specified conditions. For a toxic parameter, like selenium, the non-significance determination is a two-step process wherein the expected change in concentration is evaluated against a trigger value, which is usually a concentration threshold set well below the water quality standard. For the first part of the non-significance analysis, if the change does not exceed the trigger value, it is nonsignificant. For the second part of the analysis, if the change in water quality is expected to exceed the trigger value but the change will not exceed 15 percent of the standard, the activity is nonsignificant. Trigger values are housed in Department Circular DEQ-7 and there is currently a trigger value for selenium (0.6 µg/L). Although 0.6 µg/L is appropriate for selenium standards elsewhere in the state, it is too high to apply to the selenium standards for Lake Koocanusa set forth in NEW RULE I, where the site-specific standards for the Lake Koocanusa water column is 0.8 µg/L. To address this, the department will include a second selenium trigger value in DEQ-7 at a concentration

of 0.02 µg/L. This is the method detection limit (MDL) for very sensitive selenium analysis, and because it is an MDL, it is appropriate to use as a trigger value. A footnote will be added to the circular indicating the new trigger value applies only to NEW RULE I. The department is currently undertaking a triennial review of DEQ-7, which should be completed in 2021. The new trigger value and footnote will be incorporated into DEQ-7 as part of the current triennial review.

The proposed Lake Koocanusa water column standard (30-day chronic) is no more stringent than the recommended EPA 304(a) criteria because it was developed using federally-recommended site-specific procedures; therefore, it is more accurate than the generally applicable national lentic (lake) number.

The technical reports referenced above are as follows:

DEQ (Montana Department of Environmental Quality). 2020. *Technical Support Document for the derivation of a site-specific water column standard for Lake Koocanusa, Montana*. Helena, MT: Montana Dept. of Environmental Quality.

EPA (Environmental Protection Agency). 2016. *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*. Washington DC: United States Environmental Protection Agency.

Presser, T.S., Luoma, S.N., 2010, A methodology for Ecosystem-Scale Modeling of Selenium. *Integrated Environmental Assessment and Management,* Volume 6, *Issue 4,* Pages 685-710.

Presser, T.S., Naftz D.L., 2020. *Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada*. Open-File Report 2020-1098, Helena, MT: U.S. Geological Survey.

5. Concerned persons may submit their data, views, or arguments, either orally or in writing, at the hearing. Written data, views, or arguments may also be submitted to Sandy Scherer, Paralegal, Department of Environmental Quality, 1520 E. Sixth Avenue, P.O. Box 200901, Helena, Montana 59620-0901; faxed to (406) 444-4386; or e-mailed to sscherer@mt.gov, no later than 5:00 p.m., November 23, 2020. To be guaranteed consideration, mailed comments must be postmarked on or before that date. A copy of proposed NEW RULE I, as well as technical documents supporting the rules, may be viewed at the department's website: https://deq.mt.gov/water/Surfacewater/standards. Copies of any of these documents may also be obtained by contacting Lauren Sullivan at (406) 444-5226 or Lauren.Sullivan@mt.gov.

6. The board maintains a list of interested persons who wish to receive notices of rulemaking actions proposed by this agency. Persons who wish to have their name added to the list shall make a written request that includes the name, e-mail, and mailing address of the person to receive notices and specifies that the

person wishes to receive notices regarding: air quality; hazardous waste/waste oil; asbestos control; water/wastewater treatment plant operator certification; solid waste; junk vehicles; infectious waste; public water supply; public sewage systems regulation; hard rock (metal) mine reclamation; major facility siting; opencut mine reclamation; strip mine reclamation; subdivisions; renewable energy grants/loans; solar and wind energy bonding, wastewater treatment or safe drinking water revolving grants and loans; water quality; CECRA; underground/above ground storage tanks; MEPA; or general procedural rules other than MEPA. Notices will be sent by e-mail unless a mailing preference is noted in the request. Such written request may be mailed or delivered to Sandy Scherer, Paralegal, Department of Environmental Quality, 1520 E. Sixth Ave., P.O. Box 200901, Helena, Montana 59620-0901, faxed to the office at (406) 444-4386, e-mailed to Sandy Scherer at sscherer@mt.gov, or may be made by completing a request form at any rules hearing held by the department.

7. Sarah Clerget, attorney for the board, or another attorney for the Agency Legal Services Bureau, has been designated to preside over and conduct the hearing.

8. The bill sponsor contact requirements of 2-4-302, MCA, do not apply.

9. With regard to the requirements of 2-4-111, MCA, the board has determined that the amendment of the above-referenced rule will not significantly and directly impact small businesses.

Reviewed by: BOARD OF ENVIRONMENTAL REVIEW

/s/ BY: */s/*

EDWARD HAYES
Rule Reviewer

CHRISTINE DEVENY
Chair

Certified to the Secretary of State, September 29, 2020.

Derivation of a Site-Specific Water Column Selenium Standard for Lake Koocanusa.

September 2020

Prepared by:
Water Quality Standards & Modeling Section
Montana Department of Environmental Quality
1520 E. Sixth Avenue
P.O. Box 200901
Helena, MT 59620-0901

Suggested citation: Montana Department of Environmental Quality. 2020. Derivation of a Site-Specific Water Column Selenium Standard for Lake Koocanusa, Montana. Helena, MT: Montana Dept. of Environmental Quality.

EXECUTIVE SUMMARY

This document provides the Montana Department of Environmental Quality's scientific framework and recommendations for site-specific selenium water quality standards for Lake Koocanusa. The proposed standards are designed to protect fish as the most sensitive ecological endpoint, including federally listed threatened species, from effects of elevated levels of selenium. The standards described herein reflect the latest science on the toxicological effects of selenium. This document considered the United States Environmental Protection Agency's 2016 304(a) National Recommended Water Quality Criteria to develop site-specific selenium criteria, whenever feasible, and their guidance to states on developing site-specific criteria as described in Appendix K of, *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*.

The proposed criteria for Lake Koocanusa consist of a site-specific water column value and 304(a) recommended fish tissue values. The site-specific water column value is based on biodynamic selenium modeling using the United States Geological Survey Ecosystem-Scale Selenium Model. The proposed values are presented below.

Proposed Selenium Water Quality Criteria for Lake Koocanusa, Montana.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

ACKNOWLEDGEMENTS

This work was guided and directed by the collaborative state and provincial efforts of the Lake Koocanusa Monitoring and Research Working Group; in particular, the collective knowledge of the Monitoring and Research Committee and the expertise of the scientists recognized for their work in selenium who participated on the Selenium Technical Subcommittee. For the coordinated monitoring efforts, we would like to thank Montana Fish Wildlife and Parks, United States Army Corps of Engineers, United States Geological Survey, United States Fish and Wildlife Service, British Columbia Ministry of Environment and Climate Change Strategy, and Teck Resources Limited. We would like to thank the United States Geological Survey Selenium Modeling Team. We acknowledge and appreciate the British Columbia Ministry of Environment and Climate Change Strategy for the staff support and overall collaboration and participation throughout this multi-year long process. Without the joined efforts between the Department of Environmental Quality and the British Columbia Ministry of Environment staff this work would not have been possible.

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ACRONYMS

Acronym	Definition
ABMP	Area Based Management Plan
ARM	Administrative Rules of Montana
BAF	Bioaccumulation factor
BC	British Columbia
BC-ENV	Ministry of Environment and Climate Change Strategy
CWA	Clean Water Act
CSKT	Confederated Salish and Kootenai Tribes
DEQ	Department of Environmental Quality (Montana)
dw	Dry weight
EF	Enrichment factor
EO	Egg-ovary
EPA	United States Environmental Protection Agency
ESA	Endangered Species Act
EVWQP	Elk Valley Water Quality Plan
K_d	Partitioning coefficient
Kg	Kilogram
KNC	Ktunaxa Nation Council
KTOI	Kootenai Tribe of Idaho
LKMRC	Lake Koocanusa Monitoring and Research Committee
LKMRWG	Lake Koocanusa Monitoring and Research Working Group
LOEC	Low Effect Concentration
MT	Montana
ppb	Parts per billion (equivalent to micrograms per liter in aqueous solutions)
ppm	Parts per million (equivalent to milligrams per liter in aqueous solutions)
Se	Selenium
SOP	Standard Operating Procedure
SETSC	Selenium Technical Subcommittee (of the LKMRC)
SOP	Standard Operating Procedure
TAC	Technical Advisory Committee
TTF	Trophic transfer factor
TSI	Trophic state index
USACE	United States Army Corps of Engineers
USGS	United States Geological Survey
µg/L	Micrograms per liter
WQG	Water Quality Guideline
WQPB	Water Quality Planning Bureau
WQSM	Water Quality Standards and Modeling Section

1.0 INTRODUCTION

This document presents the scientific basis used for the development of the site-specific water column selenium standard for Lake Koocanusa, MT. This work was a collaborative effort between the Montana Department of Environmental Quality (DEQ), the British Columbia (BC) Ministry of Environment and Climate Change Strategy (BC-ENV), the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), and a Selenium Technical Subcommittee (SeTSC).

1.1 BACKGROUND

In 2010, a Memorandum of Understanding and Cooperation (MOUC) between BC and MT was drafted to end the decades-long dispute over transboundary mining in the Flathead Valley. The MOUC expanded collaboration on environmental protection and assessments that have bi-national significance. Coordinated efforts began between BC-ENV and DEQ to address regional transboundary water quality issues including those in the Elk Valley, BC.

In 2012, DEQ added Lake Koocanusa to the 303(d) list of impaired or threatened waterbodies, as threatened by selenium (DEQ, 2012). In April 2013, the BC Minister of Environment issued a Ministerial Order (No. M113) under the Environmental Management Act to remediate water quality effects of past mining activities and to guide environmental management of future mining activities in the Elk Valley, including the Canadian portion of Lake Koocanusa.

This Order mandated the development of an area-based management plan (ABMP) due to evidence of increasing concentrations of water quality constituents of potential concern, including but not limited to selenium (Se), from numerous sources related to mining activity in the Elk Valley watershed https://www2.gov.bc.ca/gov/content/environment/waste-management/industrial-waste/mining-smelting/teck-area-based-management-plan. As the sole operator of the five coal mines in the Elk Valley, Teck Resources Limited (Teck) was required by the Order to develop the Elk Valley ABMP according to requirements outlined in the Order. Agencies from Canada and the US participated in a Technical Advisory Committee (TAC)—established as a condition under the Order—to provide science-based technical advice to Teck during the development of the Elk Valley Water Quality Plan (EVWQP). The EVWQP was submitted by Teck to BC-ENV in July 2014 and revised by BC-ENV in 2019. The EVWQP established short, medium, and long-term water quality targets for Se (and other constituents) at specific order stations in the Elk Valley, including a station in Lake Koocanusa (LK2) where the target for Se was set at 2 µg/L (equivalent to the BC Water Quality Guideline (WQG)). These water quality targets were incorporated into enforceable limits under Permit No. 107517, issued to Teck on November 19, 2014 (**Table 1-1**).

Table 1-1. Effluent limits for Order Stations in the Elk Valley (Teck, 2014).

Management Unit	Order Stations	Selenium (µg/L)
1	FR4	57
2	FR5	40
3	ER1	19
4	ER2	19
5	ER3, ER4	19
6	LK2	2

At the conclusion of the EVWQP development process, the TAC recommended that a site-specific ecological effects assessment be completed to evaluate whether the BC WQG set at 2 µg/L for Se is protective for Lake Koocanusa. Although BC WQGs are designed to be protective of aquatic life and wildlife, WQGs do not account for site-specific factors, so the TAC recommended that an ecological effects assessment be conducted for Lake Koocanusa. The TAC concluded that Se bioaccumulation in organisms is affected by site-specific factors and has potentially irreversible consequences (i.e. extirpation of species). In addition, current concentrations of Se in Lake Koocanusa were recorded above the BC alert threshold of 1 µg/L promulgated by BC-ENV during the same time period that draft EPA 304(a) Se criteria—released in 2015 and finalized in 2016—showed that a protective Se water quality concentration for aquatic life in lentic waters was 1.5 µg/L (**Table 1-2**). EPA also provided a recommendation that site-specific Se criteria be developed whenever possible.

TAC recommendations on Lake Koocanusa and government-to-government discussions on transboundary impacts resulted in formal commitments by BC-ENV to DEQ and the EPA to establish a process to assess whether a Se target of 2 µg/L at Lake Koocanusa is protective, and to provide a forum for discussing other water quality issues relevant to Lake Koocanusa. To meet this commitment, BC-ENV proposed the establishment of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG).

As directed by the Steering Committee of the LKMRWG, a Selenium Technical Subcommittee (SeTSC) was established in 2015 with selenium experts from both the US and Canada. In 2019, technical representatives from the Ktunaxa Nation Council (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI) joined the SeTSC. The SeTSC multi-agency, multi-institutional, international team of experts worked with DEQ and BC-ENV to develop the scientific basis for a site-specific Se criterion in Lake Koocanusa. The multi-year collaborative effort included research goals and methodology, coordinated monitoring plans, Se biodynamic modeling, and Se criteria recommendations. The technical work of the SeTSC was routinely reported to the Monitoring and Research Committee (MRC) and the Steering Committee. **Figure 1-1** describes the structure of the LKMRWG.

The goal of this coordinated effort, is a MT and BC co-developed site-specific Se criteria for Lake Koocanusa detailed in this document.

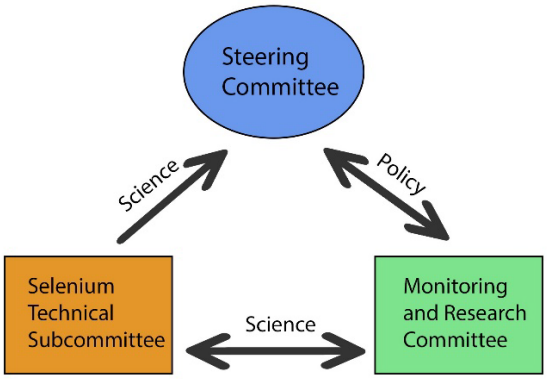

Figure 1-1. Structure and information flow of the LKMRWG.

1.2 PURPOSE

The purpose of this document is to provide the technical framework for the derivation of a site-specific selenium standard for Lake Koocanusa, MT. Montana is required under section 303(c)(2)(B) of the federal Clean Water Act (CWA) to establish water quality criteria for toxic pollutants, for which selenium is listed in section 307(a)(1). In adopting criteria, Montana is authorized to establish numeric values based on CWA Section 304(a) guidance modified to reflect site-specific conditions.

1.3 EXISTING SELENIUM WATER QUALITY STANDARDS

This section details the existing Se water quality standards for Montana, the 304(a) criteria, and British Columbia's Se WQG's defined in **Table 1-1**.

Table 1-2. Selenium water column thresholds applicable to Lake Koocanusa.

Agency	Target (µg/L)	Description
EPA	1.5	2016 updated criterion for dissolved Se in lentic systems
ENV	2	2014 Established guideline for aqueous Se
ENV	1	2014 Established Alert level for aqueous Se
DEQ	5	Chronic standard for dissolved Se established based on 1987 EPA guidance
DEQ	20	Acute standard for dissolved Se established based on 1987 EPA guidance

1.3.1 Montana's Surface Water Quality Standards for Selenium

Existing Se water quality standards are found in Department Circular DEQ -7 (June 2019 edition) which is incorporated by reference into the Administrative Rules of Montana (ARM) title 17, chapter 30, subchapter 6. The current acute (20 µg/L) and chronic (5 µg/L) water column standards were established based upon the 1987 EPA Ambient Water Quality Criteria for Selenium (U.S. EPA, 1987).

Water quality standards are designed to protect the beneficial uses of a given waterbody. The state of Montana has classified waterbodies based on the beneficial uses they are expected to support, as found in ARM 17.30.621 through 17.30.629. Lake Koocanusa is classified as a B-1 waterbody (ARM 17.30.609). State law requires that waterbodies in a B-1 use class be suitable for drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply. The most sensitive beneficial use for Se in Lake Koocanusa is growth and propagation of salmonid fishes and associated aquatic life.

Section 305(b) of the federal CWA requires states to assess waterbodies to determine whether the waterbodies are supporting their beneficial uses. In 2012, DEQ listed Lake Koocanusa as threatened by Se. Montana defines a threatened waterbody as currently meeting water quality standards and supporting the beneficial uses, but standards are likely to be exceeded and beneficial uses threatened if current trends continue.

1.3.2 National Ambient Water Quality Criteria for Selenium

The CWA section 304(a) requires the EPA to develop water quality criteria using the best available science. While the EPA requires states to consider their recommendations (40 CFR part 131) when adopting water quality standards, it is a recommendation only and the state must adopt the criterion into state water quality standards for it to become a regulation.

The science on Se toxicology has significantly advanced over the last thirty years. The EPA first issued recommended water quality criteria for Se in 1980, with revised criteria issued in 1987. In 1996, the acute criterion was updated to account for toxicity of two Se species, selenite and selenate. In 2004, EPA released the first draft update to the 1996 Se criterion which included fish tissue criteria to account for a dietary exposure pathway. In 2015, EPA released a draft update to the 2004 criteria, and it was finalized in 2016 as *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater* (U.S. EPA, 2016).

The 2016 document considers the latest scientific information and provides guidance to states and tribes to protect freshwater aquatic life from toxic effects of Se. It is based on dietary exposure and accounts for reproductive effects in fish. The 2016 recommended criteria—which are derived for the protection of 95% of species nation-wide—is comprised of four elements (**Figure 1-2**). Two elements are fish tissue concentrations and two are water column concentrations. The elements are defined below:

1) a fish egg-ovary element,
2) a fish whole-body or muscle element,
3) a water column element (one for lentic and one for lotic) and
4) an intermittent element for short term exposures

EPA's 2016 guidance document recognizes that selenium bioaccumulation and toxicity in an ecosystem are based on site-specific environmental factors, therefore, the EPA provides additional guidance and methodology in Appendix K for states and tribes to follow when deriving site-specific criteria.

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

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

Figure 1-2. Selenium water quality criteria (U.S. EPA, 2016).

1.3.3 British Columbia's Water Quality Guideline for Selenium

BC-ENV is responsible for developing province-wide ambient WQGs. Per BC-ENV Policy 6.10.03.02, WQG's are defined as:

"A maximum and/or minimum value for a physical, chemical or biological characteristic of water, sediment or biota, applicable province-wide, which should not be exceeded to prevent specified detrimental effects from occurring to a water use, including aquatic life, under specific environmental conditions."

BC's aquatic WQGs are science-based and intended for provincial-wide application. BC-ENV applies an appropriate assessment factor determined on a case-by-case basis, according to the toxicity and bioaccumulation potential of the constituent (B.C. ENV, 2014). The assessment factor is the best estimate of a no-effect concentration, for the protection of 100% of species during all life stages. WQGs are not legally enforceable themselves but are used to provide scientific basis for best management practices and may be incorporated into permits (such as Elk Valley Permit No. 107517).

BC-ENV updated their Se Water Quality Guidelines (WQGs) in 2014 which includes an assessment factor of 2 pertaining to Se. (**Table 1-3**).

Table 1-3. BC Provincial WQG for selenium (B.C. ENV, 2014).

Water Use		Updated 2012 BC Se WQG	2001 Approved BC Se WQG	Guideline Derivation Method/Approach
Source Drinking Water		10 µg/L	10 µg/L	*Source Drinking Water:* Adopted from Health Canada; a maximum acceptable concentration of 10 µg/L to protect against adverse effects in humans from excessive exposure.
Human Consumption Screening Values				
High fish intake (0.22 kg/day)		1.8 µg/g (ww), 7.3 (dw)[1]	None proposed	*Tissue Consumption*: Values were derived using Health Canada's recommended equation for ingestion of Se-contaminated fish and the dietary reference value's tolerable upper intake.
Moderate fish intake (0.11 kg/day)		3.6 µg/g (ww), 14.5 (dw)	None proposed	
Low fish intake (0.03 kg/day)		18.7 µg/g (ww), 75.0 (dw)	None proposed	
Aquatic Life				*Water column*: Review of previous WQG (uncertainty factor (UF) applied to toxicity threshold); weight of evidence including food web modelling and reported relationships between impacts and Se concentrations in water.
Water column freshwater & marine				
Alert concentration	1	µg/L	None proposed	
Guideline	2	µg/L	2 µg/L	
Sediment - Alert concentration		2 µg/g (dw)	None proposed	*Sediment*: Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time.
Dietary				*Dietary:* Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time. Invertebrate tissue as surrogate for aquatic dietary tissue.
Invertebrate tissue (interim)		4 µg/g (dw)	2 µg/g (dw)	
Tissue (fish)				*Egg/ovary:* Combination weight of evidence and mean of published effects data with an UF of 2 applied; *Whole-body*: previous WB guideline compared with published literature, mean of published effects data with UF (2) applied and weight of evidence; *Muscle*: WB translation to derive muscle WQG, no additional UF applied to muscle guideline.
Egg/ovary		11 µg/g (dw)	None proposed	
Whole-body (WB)		4 µg/g (dw)	4 µg/g (dw)	
Muscle/muscle plug (interim)		4 µg/g (dw)	None proposed	
Wildlife				The *water column* guideline for aquatic life (fish) is adopted for wildlife since dietary accumulation is most critical. *Bird eggs* were used as surrogate for all wildlife; weight of evidence; egg Se most direct/sensitive measure; mallard EC10 with UF of 2 applied.
Water column		2 µg/L	4 µg/L (maximum)	
Bird egg		6 µg/g (dw)	7 µg/g (dw)	
Recreation and Aesthetics		None proposed	None proposed	No data
Irrigation Water				Not updated at this time
2001 guideline not updated		10 µg/L	10 µg/L	
Livestock Watering				Not updated at this time
2001 guideline not updated		30 µg/L	30 µg/L	
Industrial Water		None proposed	None proposed	No data

1.4 SELENIUM

Selenium is a member of Group 16, the chalcogen group on the periodic table of elements. Classified as a nonmetal, Se has properties of both metals and nonmetals. Selenium is considered chemically similar to other nonmetals in Group 16, for example sulfur (S) (Chapman et al., 2010). Selenium, a naturally occurring trace element essential for life, has a narrow margin between the amount necessary for proper functioning of organisms and the amount considered toxic (Janz, 2011). The toxicological potential of Se is strongly related to its chemical form (speciation).

1.4.1 Physical-chemical properties

Selenium exists in four oxidation states; elemental Se (0), selenite (+4), selenate (+6), and selenide (-2) (**Table 1-4**). Three primary transformation mechanisms occur for Se; 1) oxidation/reduction, 2) mineralization/immobilization, and 3) volatilization with Se speciation, with microbial activity and pH-redox conditions driving the kinetics of each function.

In aquatic ecosystems there exists three main fates for Se; it can 1) remain in solution, 2) be assimilated by organisms, and 3) it can be sorbed to suspended sediment. The oxyanions, selenate and selenite, are the dissolved inorganic forms of Se most commonly present in waters. It is generally understood that

selenite is less soluble, more easily transformed, assimilated by organisms, and considered to have a greater toxicity in aquatic systems than selenate. When taken up by organisms, Se can replace S in two amino acids, thought to contribute to toxicity in organisms particularly in oviparous (egg-laying) vertebrates (EPA, 2016). Finally, selenate and more commonly selenite can sorb to organic matter, clays, and oxides and hydroxides most frequently associated with the ions of iron (Fe), manganese (Mn), and sodium (Na). EPA (2016) provides significant detail on both the biochemical and the geochemical pathways of selenium in aquatic systems. It is clear that an understanding of the biogeochemical transformations of selenium is essential to understanding the fate, transport, and toxicological effects of selenium in the environment.

Table 1-4. Examples of the forms of selenium found in the environment (B.C. ENV, 2014).

Name	Valence/ Oxidation State	Forms/Se Species	Occurrence
Selenides	-II, Se^{II-}, Se^{2-}	Inorganic selenides, (Se^{2-}, HSe^-)	Found in reducing environments, sorbed onto soil/mineral particles, e.g., ferroselite ($FeSe_2$), chalcopyrite ($CuFeSe_2$)
		Hydrogen selenide, H_2Se	Unstable highly toxic gas, converts to Se^0 in H_20
		Organic selenides, R_2Se	
		Volatile organic selenides:	
		dimethyl selenide (DMSe), $(CH_3)_2Se$;	Gas, volatilization from soil/sediment bacteria and fungi
		dimethyl diselenide (DMDSe), $(CH_3)_2Se_2$;	Gas, volatilization from soil/sediment plants
		dimethyl selenone $(CH_3)2SeO_2$	Volatile metabolite, intermediate form between DMSe and DMDSe
		Biochemical intermediates, amino acids	Many forms, but most common are the amino acids selenomethionine (SeMet) and selenocysteine (SeCys)
Elemental selenium	0, Se^0		Insoluble, fairly stable, unweathered mineral form of Se, found in water, soil, sediment and biological tissue
Selenium dioxide	+II, Se^{+II}, Se^{+2}	SeO_2	Gas, not a naturally occurring form, product of fossil fuel combustion (coal, oil, gas), and smelting, soluble, forms selenous acid with water
Selenites/selenous acid	+ IV, Se^{+IV}, Se^{+4}	SeO_3^{2-}	Soluble, found in mildly oxidizing conditions in air, water, soil/sediment,
		Hydrogen selenite ($HSeO_3^-$)	Common form of selenites in soils, easily sorbed onto iron(hydr)oxide minerals $Fe(OH)SeO_3$, or other ions e.g., sodium selenite Na_2SeO_3, highly mobile and available to plants
		Selenous acid (H_2SeO_3)	
Selenates/selenic acid	+ VI, Se^{+VI}, Se^{+6}	SeO_4^{2-}	Common form of Se in surface water and soils, very soluble in water, stable in well-oxygenated water, not easily transformed biologically to more reduced forms, reduction reactions slow. In plants, selenate is actively transported against electrochemical potential gradient.
		Hydrogen selenate $HSeO_4^-$	
		Selenic acid H_2SeO_4	

1.4.2 Selenium Toxicity to Wildlife

Selenium bioaccumulates in wildlife primarily through a dietary pathway with egg-laying vertebrates determined to be the most sensitive. EPA (2016) outlines the differences in bioaccumulation between lentic (lake-like) and lotic (flowing-water) systems. Retention time, dissolved oxygen, and carbon content result in greater bioaccumulation in lentic systems. It is understood that while Se is nutritionally required in small quantities, it becomes highly toxic in slightly greater amounts with the potential to cause rippling effects through both aquatic and terrestrial food webs (Naslund et al., 2020). **Figure 1-3** describes selenium toxicity for fish and birds. Fish are considered the most sensitive ecological end point in Lake Koocanusa as determined by the SeTSC (see **Section 3.7**), therefore, fish are the focus of this report and the development of the Se standards for Lake Koocanusa.

Selenium toxicity in fish is most severe at the reproductive stage where newly hatched larval fish may experience teratogenic deformities and death while feeding off yolk sacs enriched in Se (Lemly 1993, Skorupa 1998). Extremely high concentrations of Se can be lethal to adult fish but this is not common; rather, more commonly, fish are exposed to various sublethal effects (**Figure 1-3**).

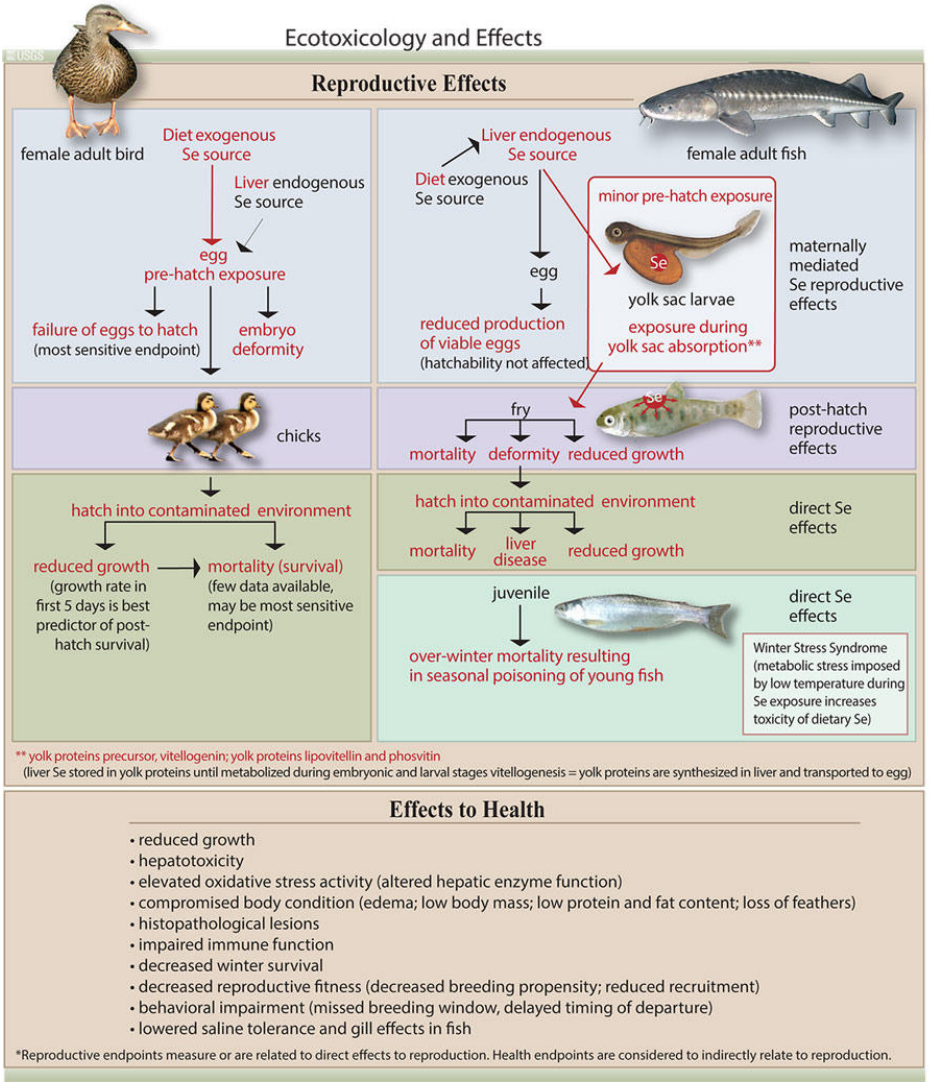

Figure 1-3. Details of general ecotoxicological pathways of selenium for fish and birds (top) and effects of concern for selenium (bottom). As represented here, birds and fish differ in how selenium is taken up in the diet and distributed among tissues (Presser and Skorupa, 2019).

1.4.3 Sources of Selenium

The primary source of Se to Lake Koocanusa is anthropogenic release to the environment from historic and present-day mining operations in the Elk Valley, BC. Coal in the Elk Valley belongs mainly to the Mist Mountain Formation of the Jurassic-Cretaceous Kootenay Group and is part of the East Kootenay coalfields (Grieve, 1952). Carbonate bedrock in the Elk Valley is excavated to access coal seams underneath for metallurgical steelmaking coal production. The excavation process creates a by-product

called overburden (waste rock) which becomes exposed to oxidation, increasing selenium mobilization through infiltration and runoff to nearby groundwater and surface water including the Elk River, BC.

Currently, Teck is the sole operator of five active coal mines in the Elk Valley and permitted under Elk Valley Permit No. 107517 (**Figure 1-4**). That permit authorizes Se concentrations at sites throughout the Valley (**Table 1-1**). **Figure 1-5** illustrates the location of Teck's existing coal mines in relation to coal bearing strata in the Kootenai (Kootenay) River Watershed.

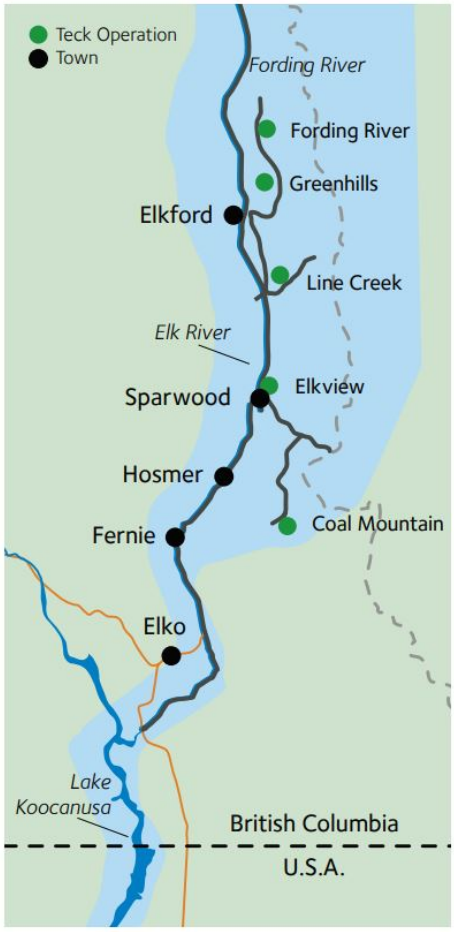

Figure 1-4. Teck's Operations in the Elk Valley Watershed (Teck, 2017).

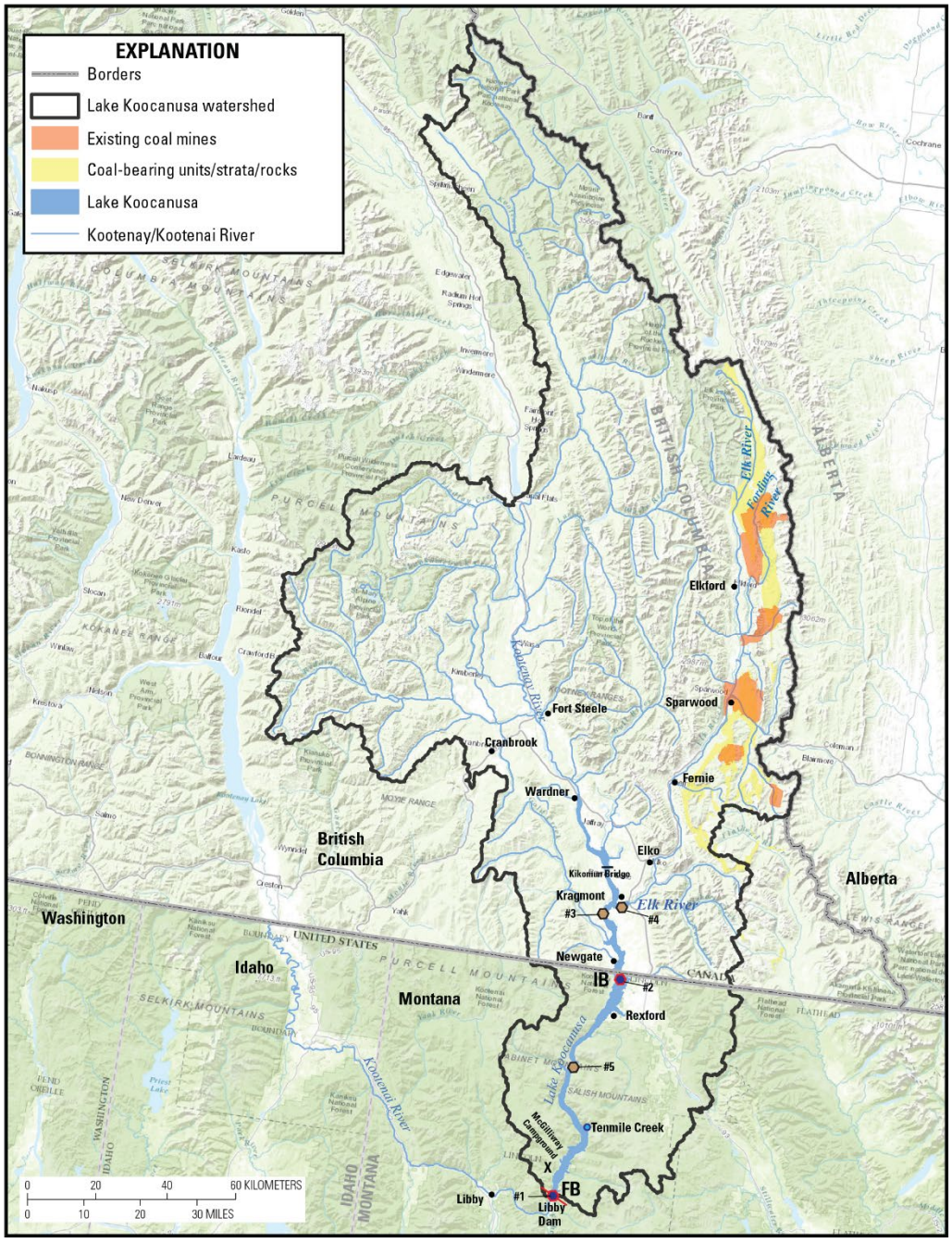

Figure 1-5. Teck's existing coal mines and location of coal bearing strata within the Kootenai (Kootenay) River Watershed (Jenni et al., 2017).

From 1984 to 2019, concentrations ranging from below detection level (DL) to greater than 8 µg/L have been recorded 2.2 miles upstream from Lake Koocanusa on the Elk River, a tributary (**Figure 1-6**). Selenium contributions from the Kootenay River are minimal and described in **Figure 1-7**, and **Table 1-5**.

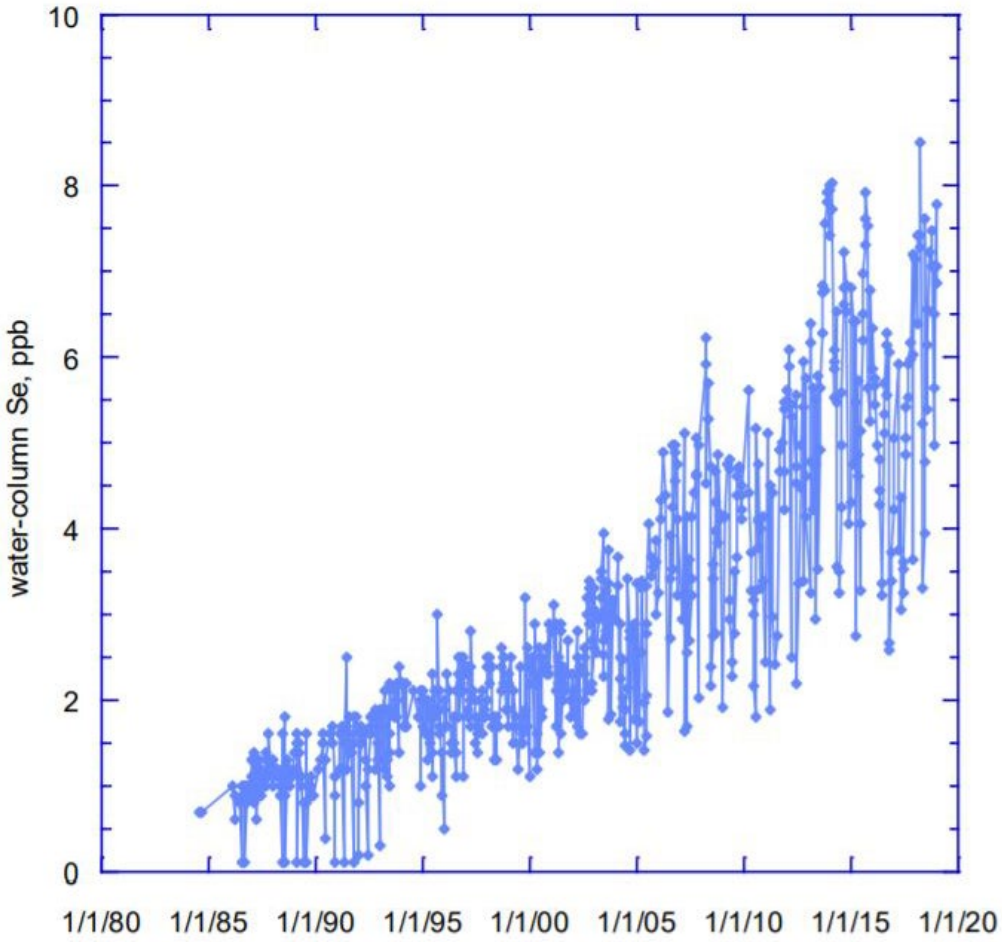

Figure 1-6. Se concentrations from 1984-2019 recorded at station BC08NK003 on the Elk River, a tributary to the Kootenay River located 2.2 miles upstream of its confluence with Lake Koocanusa (Presser and Naftz, 2020).

The Elk River contributes over 95% of the selenium to Lake Koocanusa with the Kootenay River and Bull River delivering together less than 5%. McDonald (2009) reported that in 2008 the total selenium loads were 23,720 lb/yr (10,759 kg/yr). The Elk River contributed 22,450 lb (10,183 kg) Se, the Kootenay River provided 1,078 lb (489 kg) Se, and the Bull River added only 192 (87 kg) Se. In 2012, it was determined that from 1992 to 2012, the amount of Se entering the lake each year increased fivefold, from 5,732 lb (2,600 kg) in 1992 to more than 28,660 lb (13,000 kg). **Figure 1-7** shows the comparison of Se load estimates between the Kootenay River and Elk River. The values presented in **Figure 1-7** were calculated and incorporate scaled flow values, and do include some uncertainty and are not direct measurements of load or water quality and quantity at the reported locations. **Table 1-5** describes the loading comparisons and Se concentration comparisons between upstream and downstream sites as reported in Teck's 2019 Monitoring report.

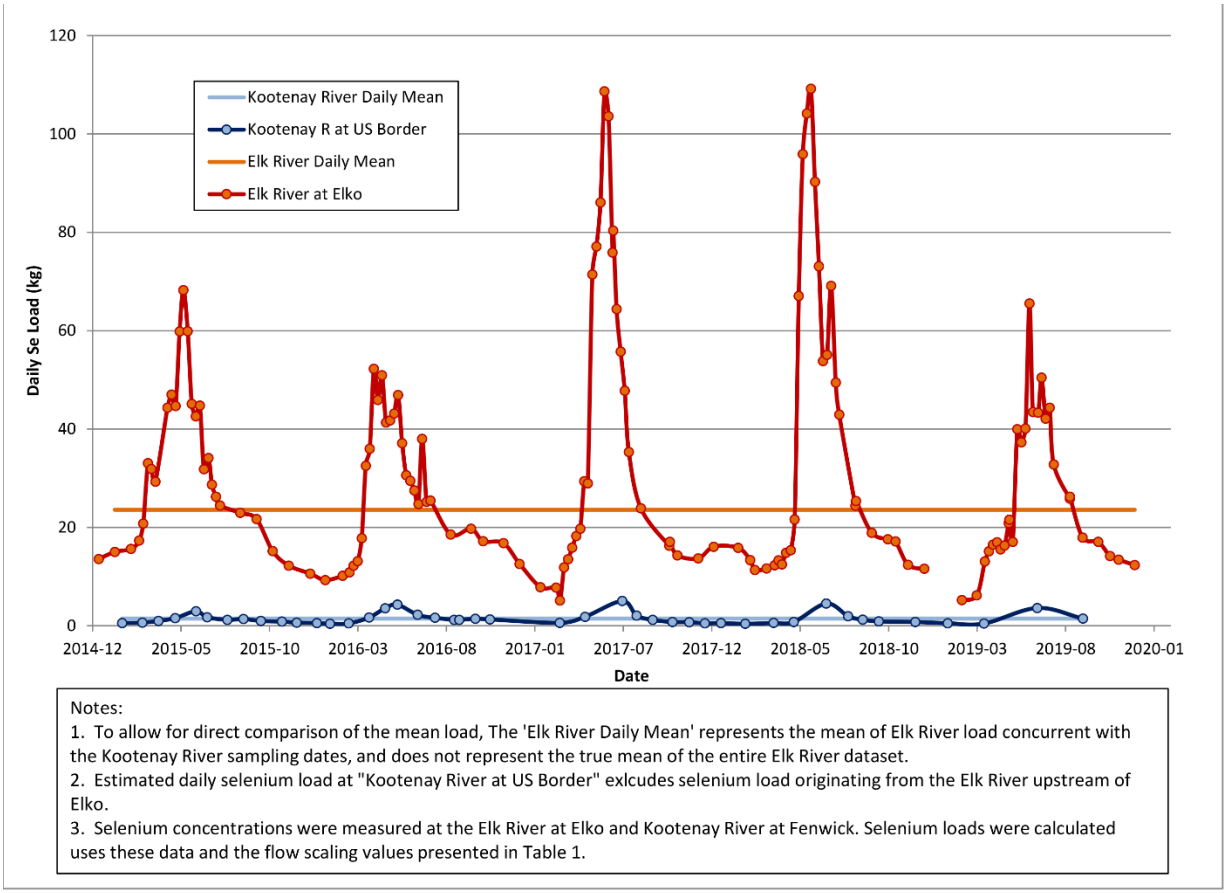

Figure 1-7. Selenium loads from the Kootenay River and Elk River (Sheldon Reddekopp, BC-ENV, personal communication, 8/4/2020).

Table 1-5. Average monthly selenium concentrations, selenium loadings, and total water volume at two Teck monitoring sites; 1) the mouth of the Elk River (RG_ELKMOUTH), and 2) the upstream site on the Kootenay River (RG_WARDB) (Minnow, 2019).

Source	Month	Average Selenium µg/L	Total Volume (m^3)	Selenium Loadings (kg/day)
Elk River (RG_ELKMOUTH)	January	6.66	41,006,976	7
	February	7.43	25,089,073	6
	March	7.21	46,239,045	11
	April	5.62	106,686,508	18
	May	3.84	276,188,619	32
	June	3.57	421,070,214	51
	July	4.64	297,267,032	46
	August	5.92	149,747,763	30
	September	6.1	98,566,165	20
	October	6.62	82,005,592	19
	November	6.54	54,291,842	14
	December	6.57	60,114,499	12
Kootenay River (RG_WARDB)	January	0.14	112,819,052	0
	February	0.14	77,486,057	0
	March	0.12	116,675,008	0
	April	0.11	267,887,635	1
	May	0.09	947,360,464	3
	June	0.08	1,370,615,445	4
	July	0.11	941,856,710	3
	August	0.11	482,055,684	2
	September	0.1	359,498,669	1
	October	0.1	267,152,361	1
	November	0.11	171,424,041	1
	December	0.15	147,008,197	1

2.0 SITE DESCRIPTION

Lake Koocanusa, sometimes referred to as Koocanusa Reservoir, is a 90-mile long transboundary body of water that lies in northwest Montana and southeast British Columbia (**Figure 2-1**). It is within the international Kootenai (Kootenay) River watershed, draining an area of approximately 19,420 square miles (50,298 km^2). Around 70% of the watershed is located within BC with 23% in Montana and 6% in Idaho (USFWS, 2006). The Kootenai River is the second largest tributary to the Columbia River in volume and third in drainage area (USACE, 1972). The total river length is 485 miles (781 km), originating in southeast British Columbia, extending through Montana and Idaho, returning back into BC where it flows through Kootenay Lake, and finally reaches the Columbia River at Castlegar, BC. (**Figure 2-2**).

Lake Koocanusa was formed by the impoundment of the Kootenai (Kootenay) River upon construction of Libby Dam, approximately 17 miles (27 km) upstream of Libby, Montana, and was completed in 1972. The reservoir occupies lands on the territories of the Ktunaxa First Nations (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI). Lake Koocanusa was created under the Columbia River Treaty (CRT) between the United States and Canada to provide power and flood control (Storm et al., 1982). Construction of the Libby dam resulted in the inundation of approximately 90 miles (145 km) of the Kootenay River and 40 miles (65km) of low-gradient tributary habitat. The reservoir operations of Lake Koocanusa are managed by the U.S. Army Corps of Engineers (USACE) outlined in the CRT and hydroelectric power is sold by Bonneville Power Administration (BPA).

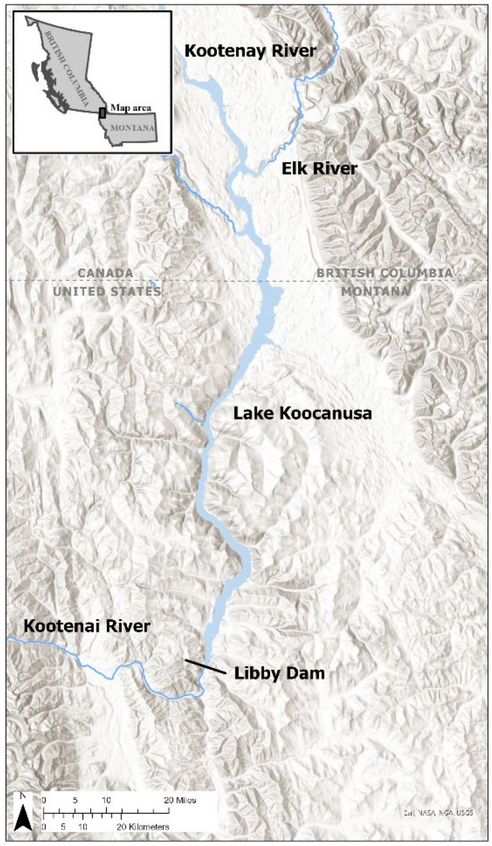

Figure 2-1. Transboundary Lake Koocanusa in northwest Montana.

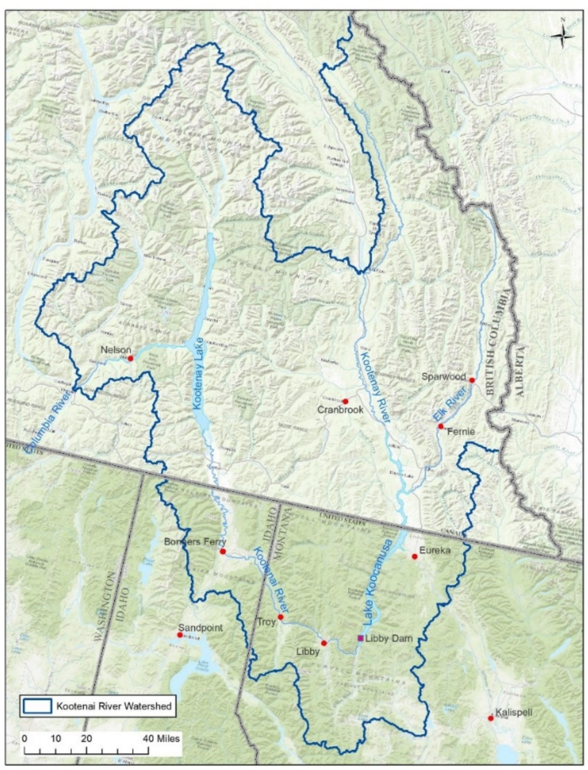

Figure 2-2. Kootenai (Kootenay) River Watershed.

2.1 RESERVOIR HYDROLOGY

A complete hydrological description of the Kootenai (Kootenay) River watershed, Lake Koocanusa and its tributaries, hydrology, climate, and physical properties can be found in the Lake Koocanusa Data Compilation Report (Lotic, 2019).

The construction of the Libby Dam in 1972 converted the Kootenai (Kootenay) river from a lotic to a lentic system. The aquatic community, dryland ecosystems, waterfowl species, and other human uses and values were affected.

Three major rivers supply water to Lake Koocanusa. The mean annual flow contribution from the Kootenay River is 56%, the Elk River provides 22%, and the Bull River contributes 11%. The Tobacco River provides 2% while the remaining 9% coming from ungauged flows (Lotic, 2019).

Lake Koocanusa water-level elevations are managed primarily for power and flood control purposes (Storm et al., 1982). **Table 2-1** describes the hydrological characteristics of Lake Koocanusa. Maximum surface area is 46,500 acres (188 km^2) with 28 sq. miles (72km^2), or 38% within BC. Maximum depth at full pool is approximately 350 ft (107 m) and mean bulk water retention time is 6 months. Typical draw down is 98 ft (30 m) with a maximum draw down capacity of 170 feet (52 m).

Table 2-1. Lake Koocanusa hydrological characteristics (Chisholm, 1989)

Surface elevation		
	maximum pool	2,459 ft (749.5 m)
	minimum operational pool	2,287 ft (697.1 m)
	minimul pool (dead storage)	2,222 ft (671.2 m)
Area		
	maximum pool	46,500 acres (188 km^2)
	minimum operational pool	14,487 acres (58.6 km^2)
Volume		
	maximum pool	5,869,400 acre-ft (7.24 km^3)
	minimum operational pool	890,000 acre-ft (1.10 km^3)
Maximum legnth		90 mi (145 km)
Maximum depth		350 ft (107 m)
Mean depth		126 ft (38 m)
Shoreline length		224 mi (360 km)
Shoreline development		4.6 mi (7.4 km)
Drainage Area		8,985 sq. mi (23,271 sq. km)

Water levels in Lake Koocanusa are generally lowest in late winter and early spring (i.e., February through April) and highest in summer and early fall (i.e. August through October; Minnow, 2014). Power generation drawdown begins in November with maximum draws during April. Flow is dependent on hydro-related demands, where spring flows have been attenuated, winter flows are above normal, and daily and hourly flows can change drastically (FWP, 2019b). Residence times have been reported as

variable. Presser and Naftz (2020) describe reports ranging from 1.7-7.5 months during initial construction (1972-1980). While more recent reports range between 5.5 and 9 months.

The USACE manages Libby Dam with a selective withdrawal system to provide flows through Libby Dam for downstream Kootenai River fish. Specifically, flows are provided for federally listed white sturgeon (*Acipenser transmontanus*), bull trout (*Salvelinus confluentus*), and salmon (*Oncorhynchus nerka*) during spring; for salmon during summer, and for bull trout and resident fish in September (BPA, 2018). The selective withdrawal system was installed on Libby Dam to control temperature of water releases from the dam. This system is intended to maximize the probability of allowing significant white sturgeon recruitment, provide a year-round thermograph that approximates normative conditions, while also meeting flood damage reduction objectives (BPA, 2018). The ecological flow plan followed by USACE was developed to restore ecological function in support of Kootenai River white sturgeon recovery while also maintaining flood control (USFWS, 2006). These functional normative flows (ecological flows) are designed to more closely mimic pre-dam hydrographs (**Figure 2-3**). Annual variability for flow and elevation for the reservoir and river from 2006 to 2019 are shown in **Figure 2-4**.

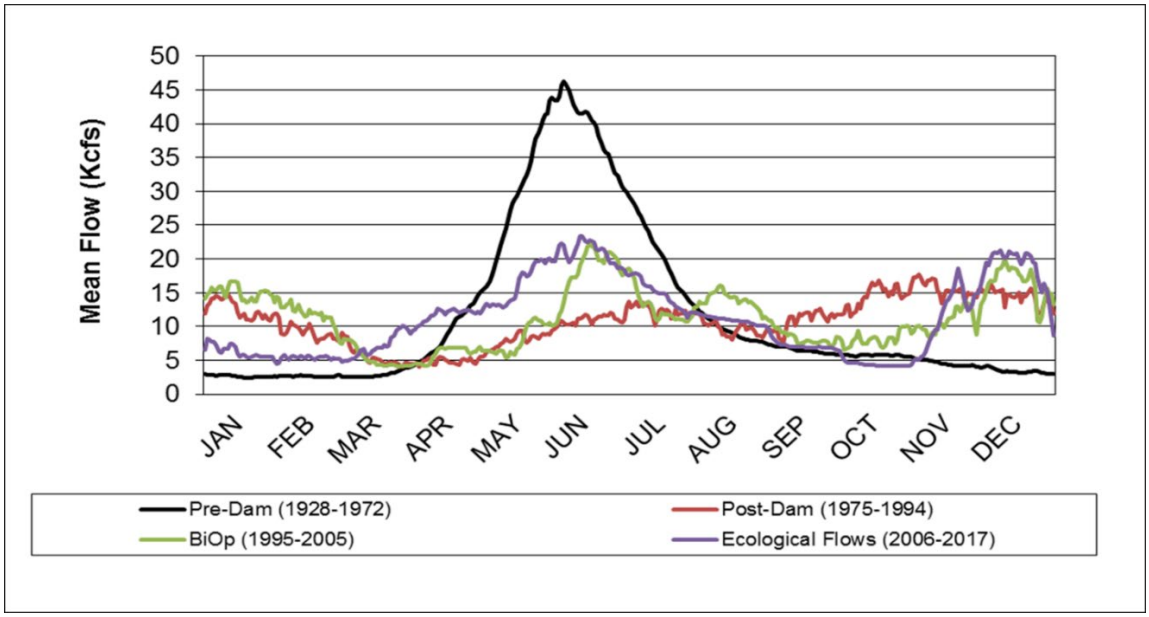

Figure 2-3. USACE stream flow management out of Libby dam from 1976-2017. Ecological flows are displayed in purple and are designed to more similarly mimic pre-dam flows displayed in black (U.S. ACE, 2019).

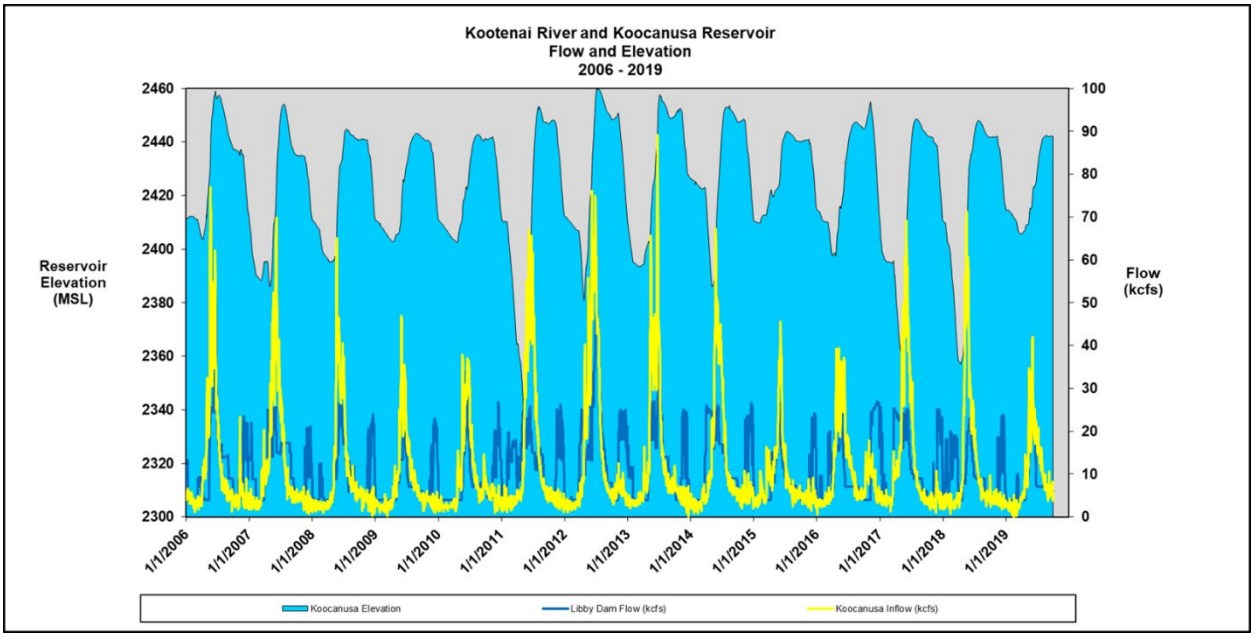

Figure 2-4. Fluctuations in reservoir elevation and flow from 2006-2018 (U.S. ACE, 2019).

The seasonal fluctuation of water levels affects the hydrology of the waterbody and associated aquatic life by impeding the establishment of riparian vegetation or aquatic macrophytes which has left the composition of the littoral zone to be that of cobble, mud, and sand substrates with limited habitat structures. Variable water levels have also affected bank stability and can impact spawning success of certain fish such as burbot (Lotic, 2019).

2.2 PHYSICOCHEMICAL CHARACTERISTICS

Routine monitoring has been conducted on the US portion of the reservoir beginning in the early 1970s with detailed summaries included in reports by USACE found at https://www.nws.usace.army.mil/About/Offices/Engineering/Hydraulics-and-Hydrology/Water-Quality/Water-Quality-Documents/ and USGS publicly available data found at https://www.waterqualitydata.us/provider/NWIS/USGS-MT/USGS-12301919/. The USACE maintains three main monitoring stations in Lake Koocanusa (**Figure 2-5**).

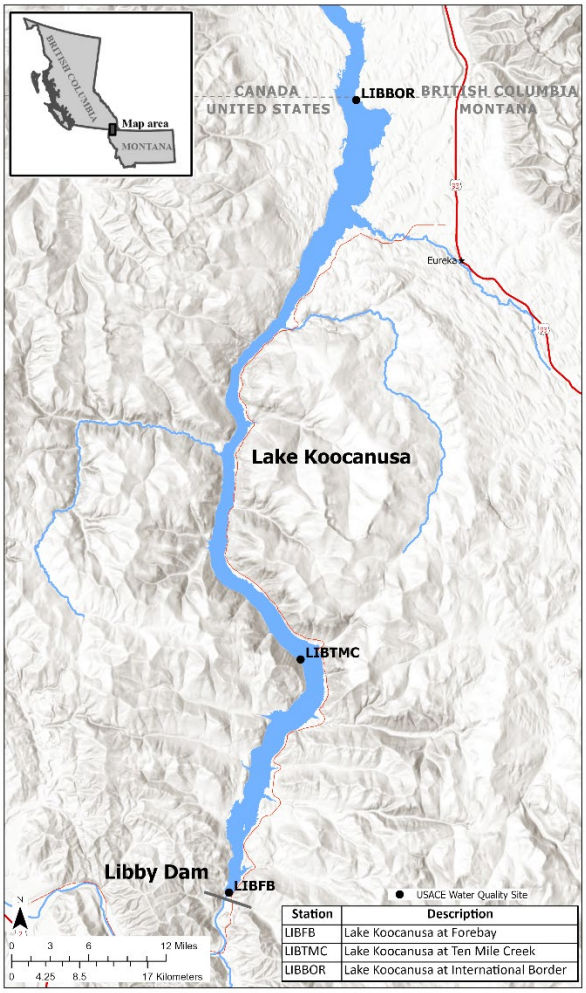

Figure 2-5. Locations of USACE's routine monitoring stations in Lake Koocanusa.

In BC, routine physicochemical monitoring is done by the federal department of Environment and Climate Change Canada (ECCC) which has a long-term monitoring station (BC08NK0003) on the Elk River approximately 2.2 miles upstream from the confluence with the Kootenay River. Additionally, under the EVWQP, Teck is required to conduct comprehensive physico-chemical and biological monitoring which first began in 2014. Monitoring sites for Teck are located both upstream and downstream of the Elk River. Monitoring reports by Teck are published annually and are vetted by BC-ENV scientists, the KNC, and independent scientists. Reports from 2014-2019 can be found on at https://www.teck.com/responsibility/sustainability-topics/water/water-quality-in-the-elk-valley/.

2.2.1 Nutrients & chlorophyll *a*

Nutrients and chlorophyll *a* routinely monitored by the USACE and Teck and are detailed extensively in USACE reports found at https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/ and Teck reports found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Briefly, Lake Koocanusa in Montana is considered oligotrophic and at times mesotrophic as defined by Lake Koocanusa's trophic State Index (TSI). TSI measures transparency and presence of nutrients and chlorophyll *a* (Carlson, 1977). The Canadian section of the reservoir experiences periods of eutrophic conditions in spring (April – June) but qualifies as oligotrophic the remainder of the year. The majority

of the phosphorus (P) entering the reservoir is P sorbed to soil particles, entering the reservoir during spring runoff which is likely not biologically available. The years with the highest runoff are associated with greater total phosphorus (TP). The P-bound sediment settles from the water column to the sediment layer as it travels through the 90-mile reservoir towards the dam. As a result, Lake Koocanusa acts as a nutrient sink, trapping an estimated 63% of incoming phosphorus (Wood, 1982). Soluble Reactive Phosphorus (SRP) is consistently lower than the 1 µg/l detection limit (DL). While low levels of TP and SRP are recorded in the reservoir, increasing concentrations of nitrogen (N) have been detected (**Figure 2-6 and Figure 2-7**). Elevated nitrate concentrations entering the reservoir are linked to blasting practices during coal production (Mahmood et al., 2017). The high N concentrations and low P concentrations have resulted in a N:P ratio far from what is considered healthy for an aquatic system and has had deleterious effects below Libby Dam by encouraging the presence of the nuisance diatom *Didymosphenia geminata* (didymo) (Dunn et al., 2015).

Chlorophyll *a* concentrations are greatest during the spring and early summer with similar concentrations recorded at all three USACE monitoring stations. Higher concentrations of chlorophyll *a* are associated with high flow years. Chlorophyll *a* measurements supported the stratification trends identified with dissolved oxygen, temperature and pH (Minnow, 2018).

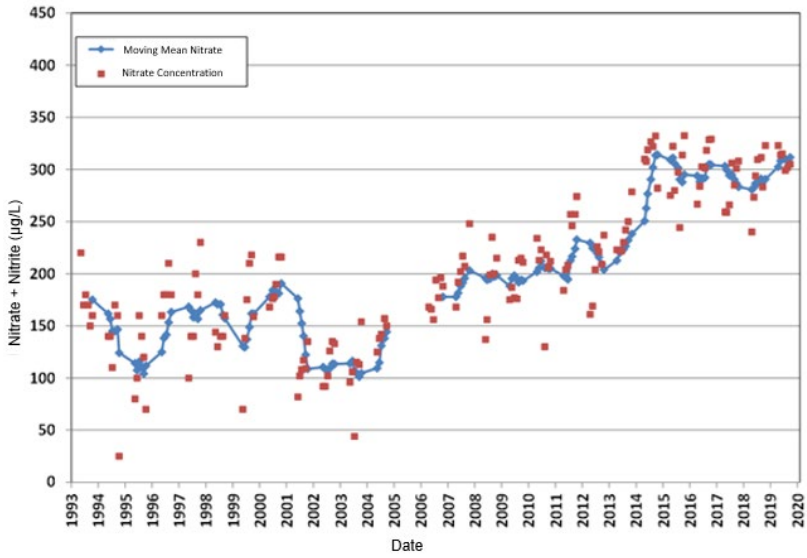

Figure 2-6. Nitrate + nitrite concentrations in the hypolimnion at the LIBFB (Forebay) monitoring station (USACE, 2018).

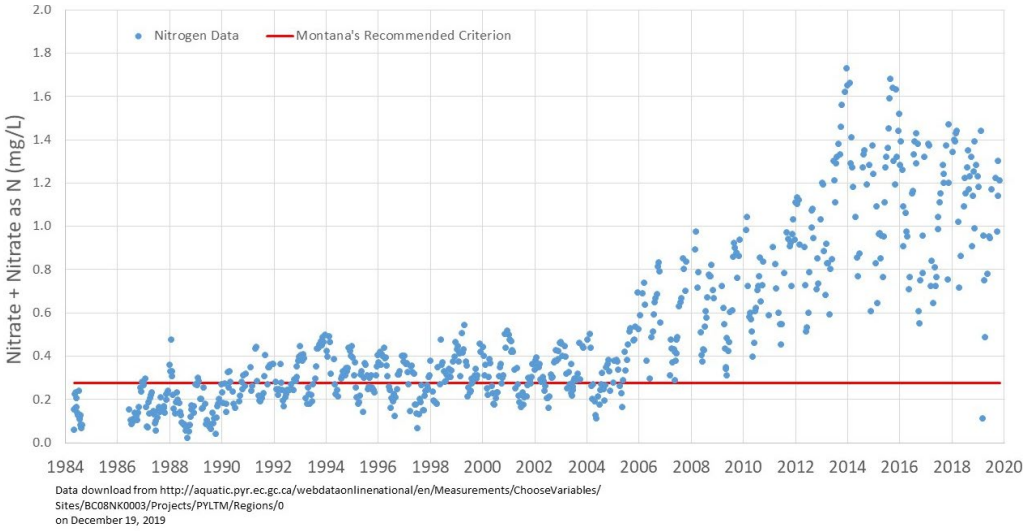

Figure 2-7. Nitrate + nitrite from 1984-2019 recorded at station BC08NK003 located 2.2 miles upstream of Lake Koocanusa (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020).

2.2.2 Metals and Metalloids

Lake Koocanusa has been monitored for a suite of major and trace metals and metalloids since the 1970s by USACE and USGS. Trend data for each metal and metalloid can be found in the USACE reports https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/. In BC, metal(loid) data are included in the Teck monitoring reports found in at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

The MT data show below DL or low levels of metals with the exception of the metalloid selenium. Selenium has been routinely monitored on the US side of the reservoir since 2013. Dissolved selenium concentrations in MT range from 0.04-2.29 µg/L from 2013-2018.

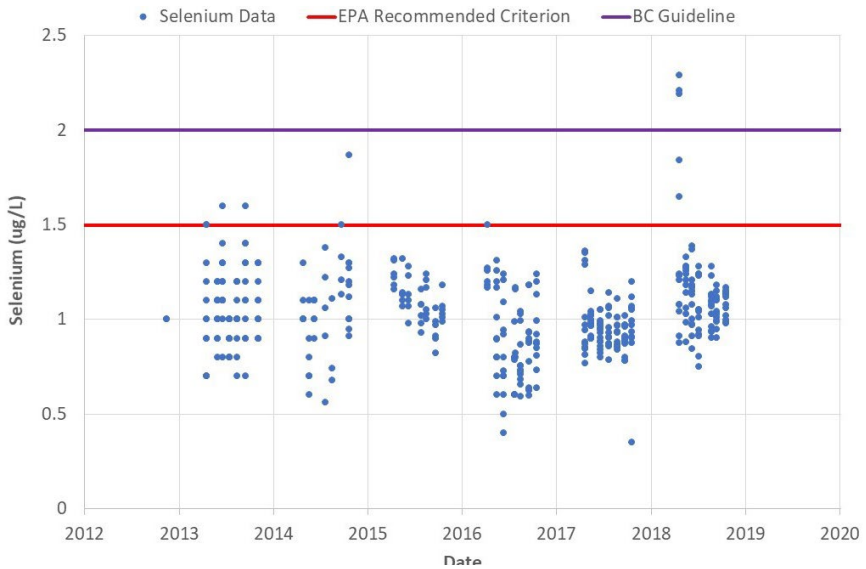

Figure 2-9. Selenium Data in Lake Koocanusa, U.S. Stations, all depths 2012-2018. (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020)

2.3 BIOLOGICAL CHARACTERISTICS

This section details baseline biological characteristics of Lake Koocanusa to provide a basic understanding of the system.

2.3.1 Phytoplankton

USACE has included phytoplankton in their routine monitoring from 2006-present. Phytoplankton densities vary from year to year and by location within the reservoir. A more detailed report of USACE's findings can be found in https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/.

Peak concentrations occur between May and August. The dominant algal group are Diatoms followed by Chrysophytes. Cyanobacteria have been measured since 2015 with *Planktolynbia* documented as the dominant type of cyanobacteria. The diatom species dominance has changed since sampling began in 2006. The most abundant diatoms in the reservoir include: *Asterionella, Cyclotella, Fragilaria, Stephanodiscus, Syndedra,* and *Nitzschia*. From 2008-2012 diatoms were recorded at low density with diverse composition (**Figure 2-10 and Figure 2-11**). In 2014 a shift was recorded in which *Cyclotella* and *Fragilaria* dominated. In 2017 USACE recorded high density and low diversity with near total dominance of *Cyclotella* and *Fragilaria*. There have been no differences in the phytoplankton community compositions between upstream and downstream monitoring locations on the Elk River (Minnow, 2019).

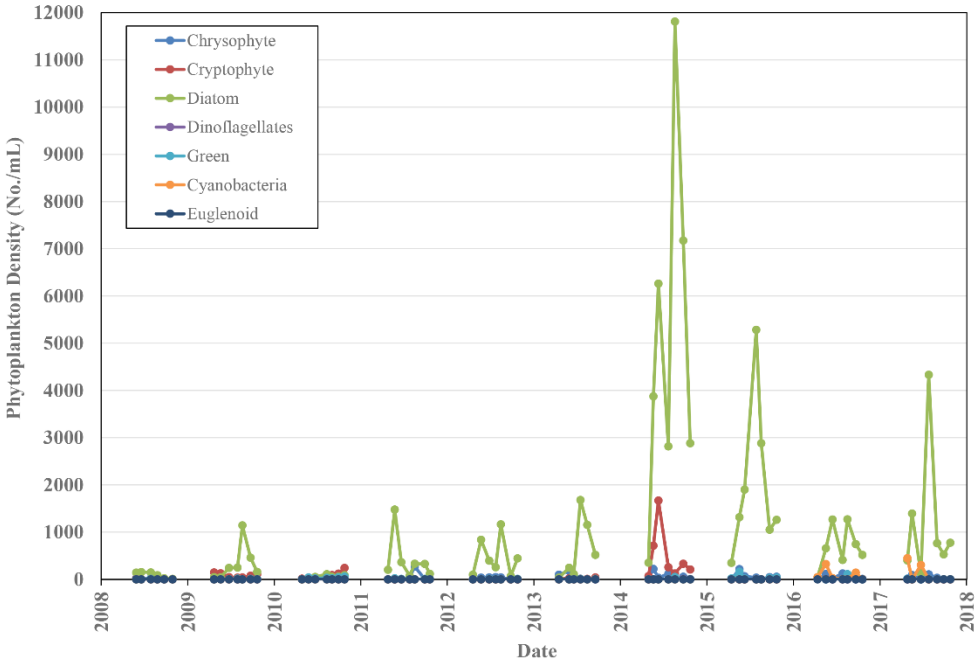

Figure 2-10. Phytoplankton density at LIBFB (Forebay) from 2008-2017 (U.S. ACE, 2019).

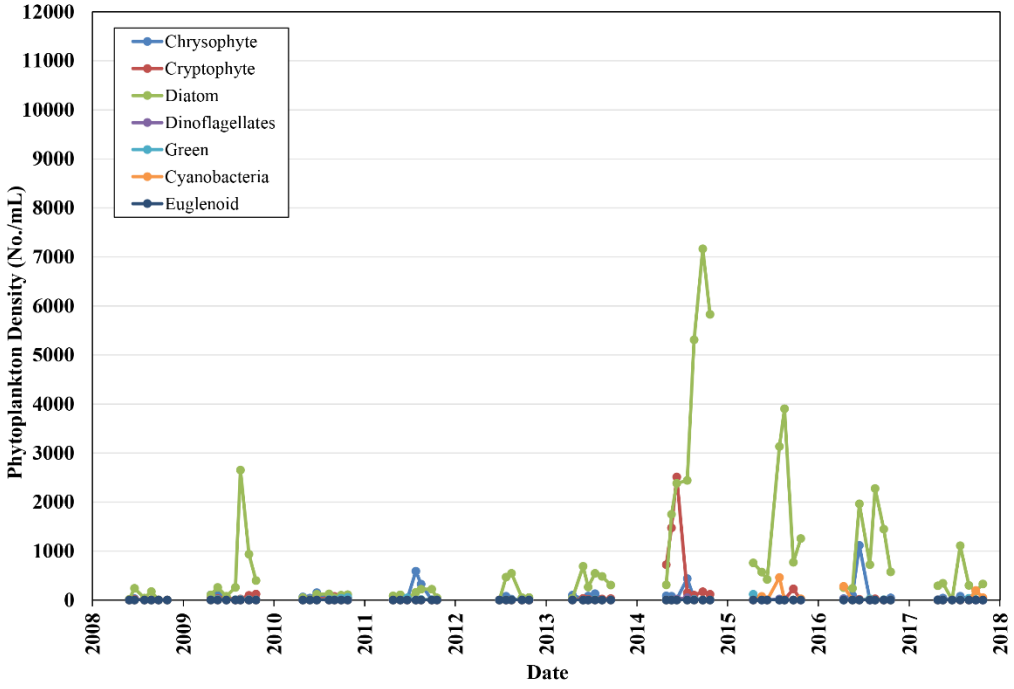

Figure 2-11. Phytoplankton density at LIBBOR (International Border) from 2008-2017 (U.S. ACE, 2019).

2.3.2 Zooplankton

Zooplankton abundance and composition recorded in Montana by USACE has been variable between the years of record 2006-2017. Copepods dominated between 2006 and 2010 while Rotifers dominated between 2011 and 2017 (**Figure 2-12 and Figure 2-13**). Within Rotifers, *Keratella* and *Keilicottia*

dominate. Cyclopoid and *Diacyclops* are the dominant copepods. *Daphnia* and *Bosmina* are the dominant Cladocerans. Seasonal succession in zooplankton community is likely in response to food source availability, changes in temperature, and grazing pressure by fish.

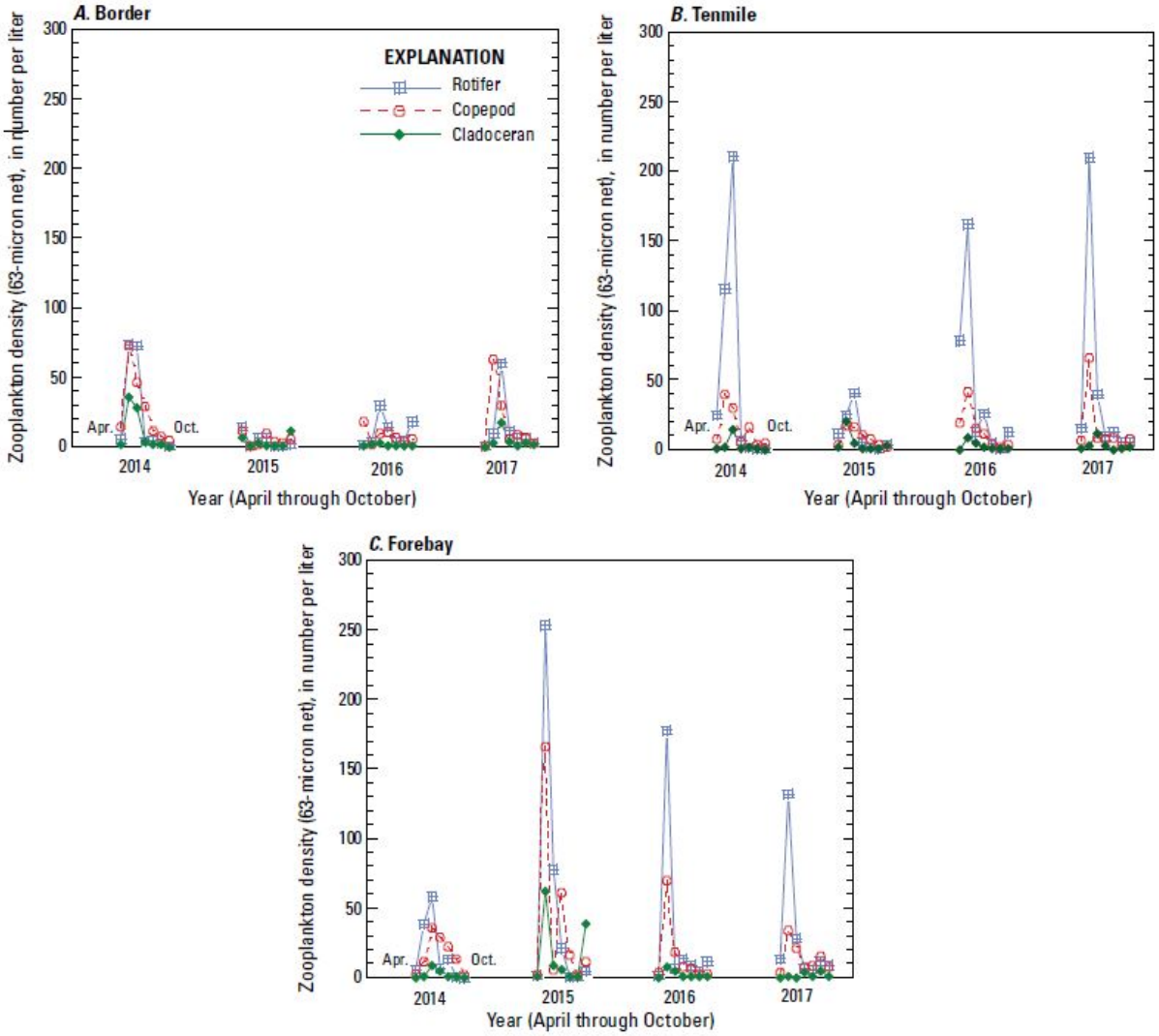

Figure 2-12. Zooplankton densities 2014-2017 (63 micron net) at sites A) Border, B) Tenmile, and C) Forebay (Presser and Naftz, 2020).

Figure 2-13 describes the Se concentrations in Zooplankton collected by Teck, USACE, and USGS between 2008 and 2019.

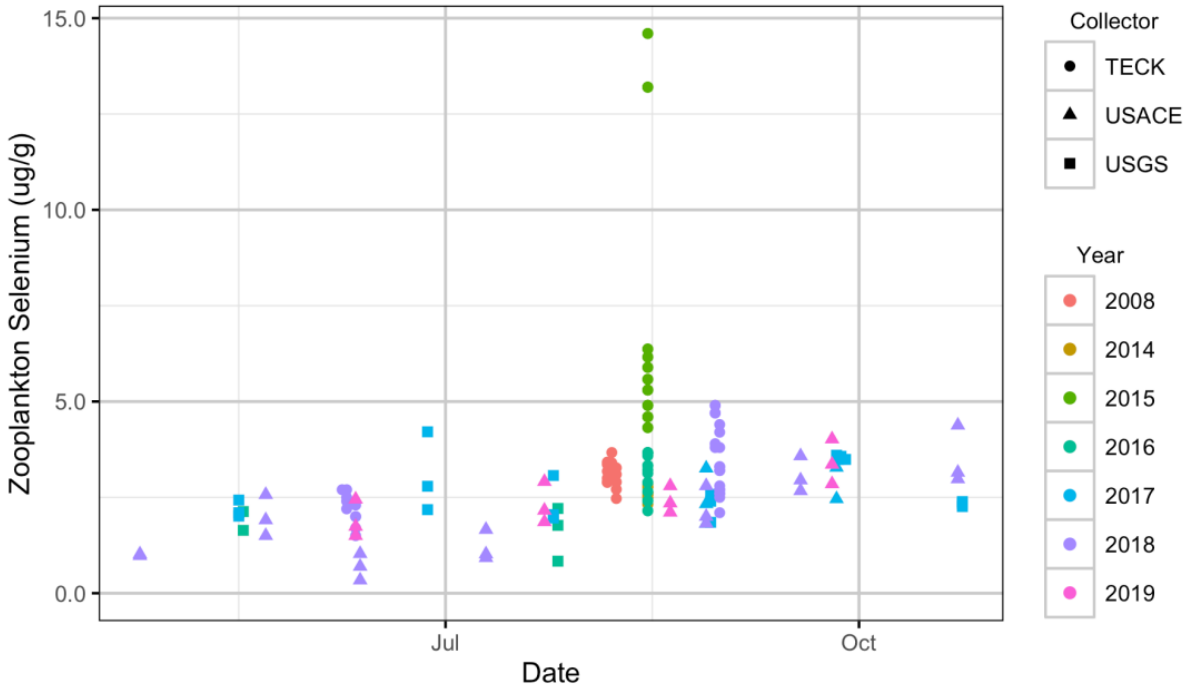

Figure 2-13. Zooplankton selenium concentrations by date, collector, and year (Thorley, 2020).

2.3.3 Macroinvertebrates

Routine data on richness and abundance for macroinvertebrates on the Montana portion of Lake Koocanusa do not exist. Montana Fish Wildlife and Parks (FWP) in collaboration with DEQ conducted benthic and surface macroinvertebrate sampling during 2018 (see **Section 3.0**). The results for Se concentration in macroinvertebrates showed chironomid dominance.

In BC, Teck conducted benthic community data collection and detailed reports on richness and abundance can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf. Dominant taxa found at their sampling sites were *Chironomus, Procladius* and *Tanytarsus*. Additionally, Teck has monitored for Se in macroinvertebrate tissues as required by their permit. Their 2019 monitoring report showed Se concentrations in benthic tissues were higher at the mouth of the Elk River compared to upstream (Kootenay River) and downstream locations. Moreover, average selenium concentrations in invertebrate tissues collected both upstream and downstream of the Elk River have oscillated between being below BC guidelines (4 µg/g dw) during spring samples and above guidelines in summer and fall samples (Minnow, 2019). Between sites, there were significant differences found in selenium concentrations from Kootenay River upstream concentrations (lower Se) to downstream concentrations (higher Se) regardless of season.

2.3.4 Fish

Numerous biological inventories have been conducted on the MT portion of the reservoir both pre-and-post dam construction. Reports detailing fish monitoring results can be located in Libby Mitigation Reports located at http://fwp.mt.gov/fwpDoc.html?id=95385 and results from fish monitoring conducted by Teck can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Figure 2-13 shows the relative abundance of the following fish species found in Lake Koocanusa: bull trout, burbot (*Lota lota*), Kokanee (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), westslope cutthroat trout (*Oncorhynchus clarkia lewisi*), mountain whitefish (*Prosopium williamsoni*), northern pikeminnow (*Ptychocheilus oregonensis*), redside shiner (*Richardsonius balteatus*), peamouth chub (*Mylocheilus caurinus*), longnose sucker (*Catostomus catostomus*), and largescale sucker (*Catostomus macrocheilus*) (Presser and Naftz, 2020). An accidental release of 250,000 kokanee fry into Lake Koocanusa from Kootenay Trout Hatchery in the late 1970s (Richards, 1997) led to the establishment of Kokanee in the reservoir. MT FWP stocks rainbow trout under their fisheries management program.

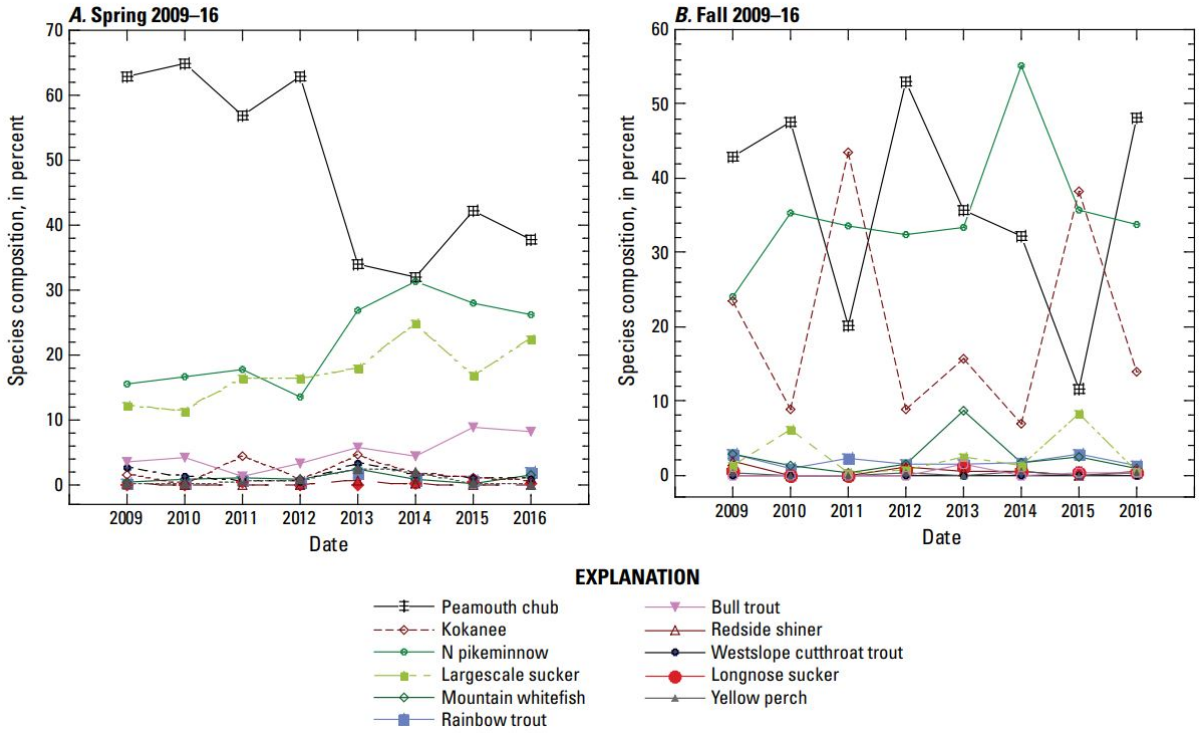

Figure 2-13. Fish species composition during A) Spring 2009-2019 and B) Fall 2009-2016 (Presser and Naftz, 2020).

Figures 2-14 and 2-15 show the results of MT FWP's 2018 fish sampling. All three redside shiner egg/ovary samples exceeded the EPA 304(a) criterion of 15.1 mg/kg dw along with one sample from peamouth chub. The other samples all remained below the EPA criterion. The 2018 fish muscle tissue samples were found to be at a comparable concentration as MT FWP's 2008 results. All samples were below the EPA fish muscle criterion of 11.3 mg/kg dw. Fish tissue samples from the BC portion of the reservoir vary in concentrations for egg/ovary ranging from 4-5 mg/kg dw for kokanee and yellow perch to higher levels for redside shiner, peamouth chub, and northern pikeminnow, ranging between 5 mg/kg to 40 mg/kg with one red side shiner reaching 80 mg/kg. The BC WQG for egg/ovary is 11 mg/kg dw.

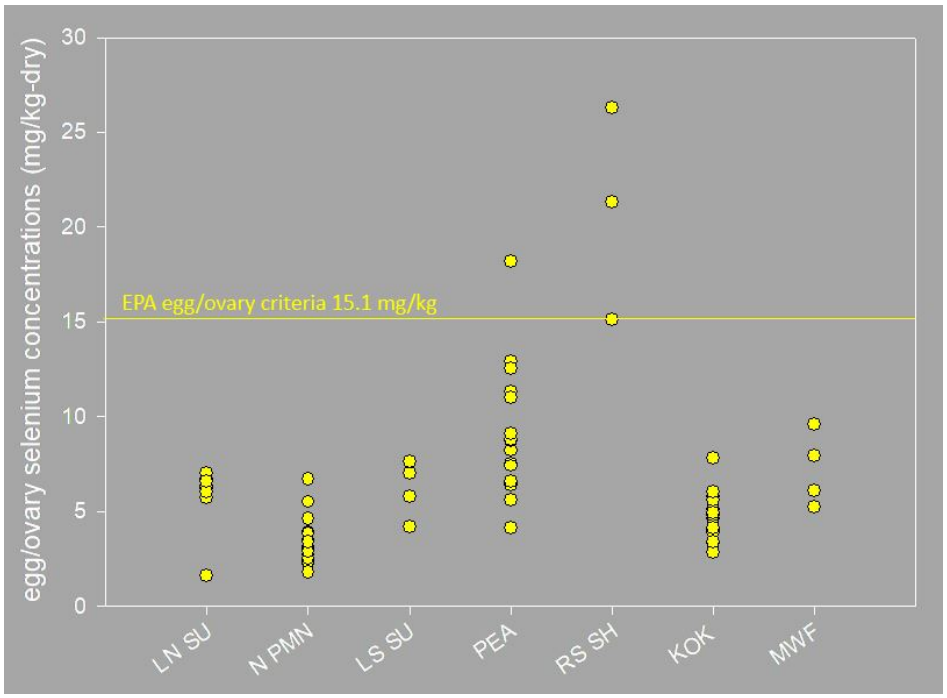

Figure 2-14. Montana Fish Wildlife and Parks 2018 egg/ovary Se concentrations found in LN SU (longnose sucker), N PMN (northern pikeminnow), LS SU (largescale sucker), PEA (peamouth chub), RS SH (redside shiner), KOK (kokanee), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

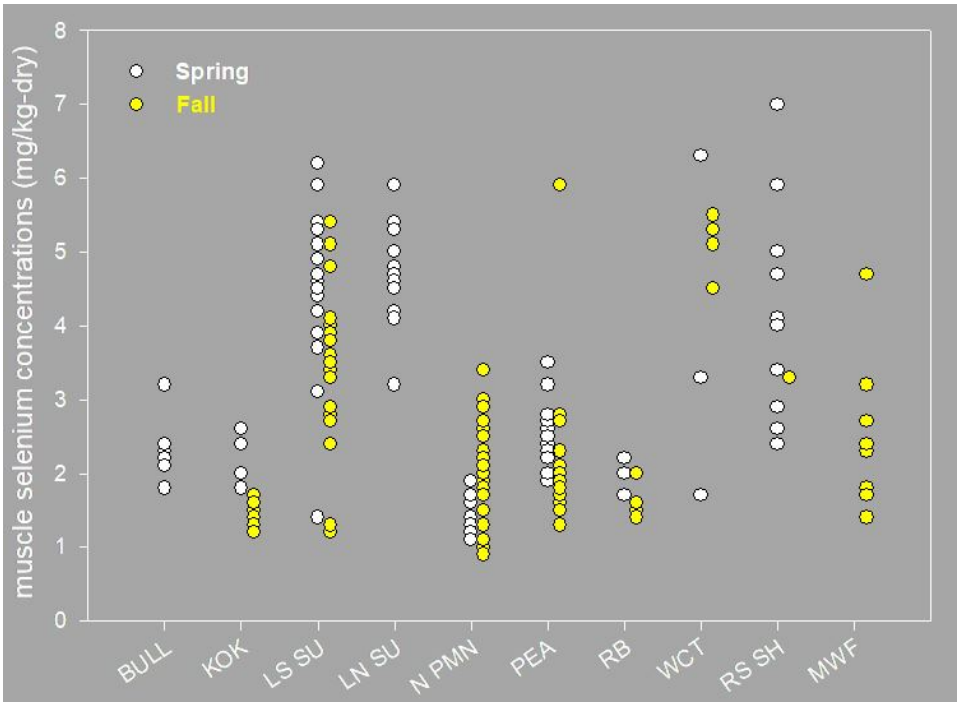

Figure 2-15. Montana Fish Wildlife and Parks 2018 muscle selenium concentrations found in BULL (bull trout), KOK (kokanee), LS SU (largescale sucker), LN SU (longnose sucker), N PMN (northern pikeminnow), PEA (peamouth chub), RB (rainbow trout), WCT (westslope cutthroat trout), RS SH (redside shiner), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

2.3.5 Birds

Lake Koocanusa encompasses transboundary migratory routes and the Pacific Flyway. On the Montana portion of the reservoir, the most common shore bird on Lake Koocanusa is spotted sandpiper (*Actitis macularius*). Less common during the summer months along the shore are killdeer (*Charadrius vociferus*) but they are regulars on the mud flats at Rexford in fall and into winter. Other shore birds are limited on Lake Koocanusa because of the steep shoreline, a result of dam management. Presser and Naftz (2020) reports that the common resident birds on Lake Koocanusa are Bald Eagle (*Haliaeetus leucocephalus*) and Osprey (*Pandion haliaetus*), Common Loon (*Gavia immer*), Ring-billed Gull (*Larus delawarensis*), Canada Goose (*Branta canadensis*), Mallard (*Anas platyrhynchos*), Common Goldeneye (*Bucephala clangula*), Common Merganser (*Mergus merganser*), Western Grebes (*Aechmophorus occidentalis*), and swallow species (*Hirundinidae spp.*). They further detail avian species which pass through Lake Koocanusa during migration, including Eared Grebe (*Podiceps nigricollis*), Lesser Yellowleg (*Tringa flavipes*), Wilson's Phalarope (*Phalaporpus tricolor*), and several species of ducks including American Wigeon (*Mareca americana*), Gadwall (*Mareca strepera*), Redhead (*Aythya americana*), Ring-necked Duck (*Aythya collaris*), Green-winged Teal (*Anas carolinensis*), Blue-winged Teal (*Anas discors*), Hooded Merganser (*Lophodytes cucullatus*), and Lesser Scaup (*Aytha affinis*).

3.0 DATA COLLECTION FOR CRITERIA DEVELOPMENT

This section presents details on enhanced Se data collection efforts conducted to inform criteria development. In addition to routine monitoring (see **Section 2.0**), the state, federal, provincial, and industry monitoring entities followed recommendations provided by the SeTSC to the extent feasible. Specifically, coordinated cross-border monitoring protocols were developed, additional parameters collected, and monitoring sites added. **Table 3-1** describes the parameters collected, years of record, responsible monitoring entity, and if the sampling was associated with routine monitoring or added.

Significant advances in coordinated monitoring efforts occurred between 2015-2019, but as a result of the transboundary nature of the reservoir and dual jurisdictions, perfectly matched datasets for target parameters were not possible. Presser and Naftz (2020) detail data collection efforts specific to the biodynamic modeling and utility of data while explanation on sample collection and analysis are described in the Presser and Naftz (2020) data release.

Table 3-1. Monitoring data, years of collection, and monitoring entities.

Data	Sampling (routine or added for criteria development)	USGS	USACE	Teck	ENV	FWP	USFWS
phyiscochemical parameters	routine		2015-2019	2015-2019			
dissolved selenium	routine	2012-2019	2015-2019	2015-2019	2017		
selenium speciation	added	2015	2016				
particulate selenium	added	2015-2019	2017-2019	2017-2019	2017		
sediment selenium	routine			2015-2019			
periphyton tissue selenium	added				2017	2017	
invertebrate tissue selenium	added			2016; 2018-2019		2018-2019	
zooplanktoon selenium	added	2016-2017	2016-2019	2018-2019			
fish muscle tissue selenium	routine			2015-2019		2015-2019	
fish whole body tissue selenium	routine			2015-2019		2015-2019	
fish egg/ovary tissue selenium	routine			2015-2019		2015-2019	
fish food habits	added					2017-2018	
bird egg selenium	added						2016

3.1 DISSOLVED AND PARTICULATE SE

The ratio of Se concentration of suspended particulate matter (SPM) to dissolved Se in the water is the environmental partitioning coefficient (K_d). For the most accurate understanding of the K_d, it is necessary to measure SPM and dissolved Se values across multiple years and multiple seasons. Throughout spring-fall months, large volume water samples were collected at two depths (epilimnion and hypolimnion), centrifuged, and analyzed for Se. Dissolved Se samples, defined as passing through a 0.45 µm filter, were collected at the same time and location as SPM samples (these are considered matched samples). USGS and USACE each collected matched dissolved and SPM samples at the main MT monitoring sites, the International Boundary, Tenmile, and Forebay. Teck and BC-ENV collected dissolved and SPM Se samples on the BC portion of the reservoir (**Figure 3-1**). A more robust matched dataset exists for the Montana portion of the reservoir.

Biogeochemical processes have the potential to influence Se distribution between particulate and dissolved phases. At each matched sampling site, high resolution vertical profile data was collected including, temperature, pH, specific conductance, DO, and fluorescent dissolved organic matter (fDOM). Details on these parameters are located in Presser and Naftz (2020).

A concerted effort was made to analyze SPM samples for Se speciation but due to limited sample mass as a result of low lake productivity, the laboratory was unable to complete Se speciation on all SPM data. Only a small subset of the SPM data includes Se speciation.

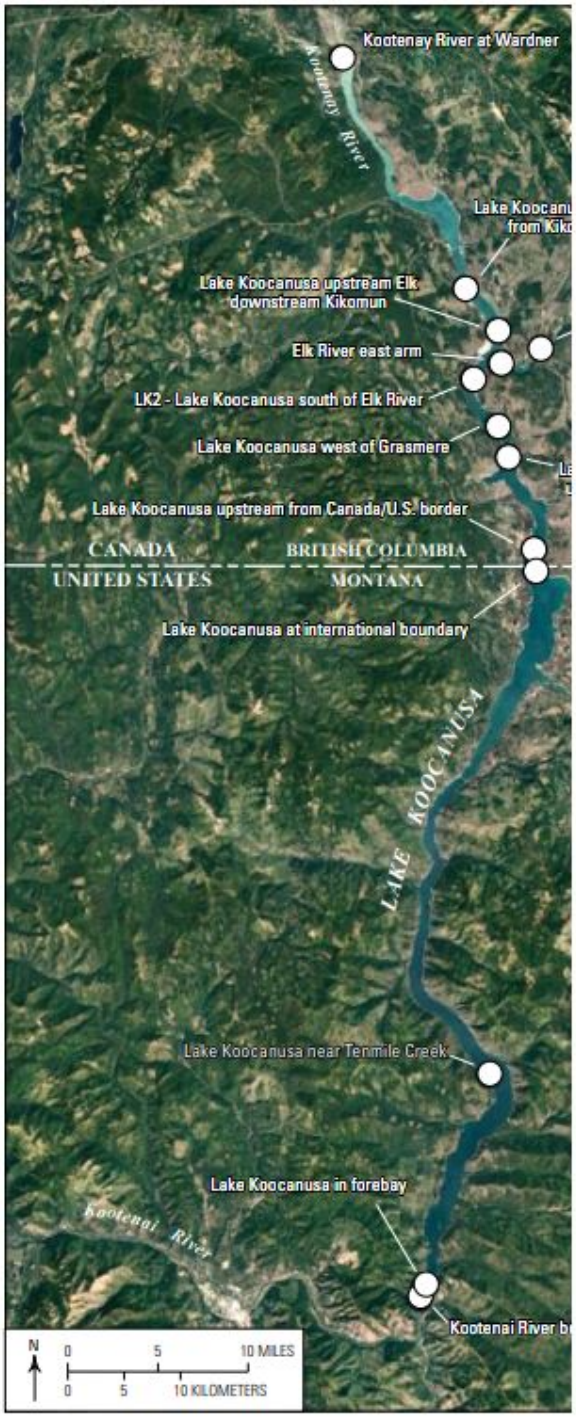

Figure 3-1. Location of sites where water quality and (or) SPM samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3. 2 PERIPHYTON

DEQ and FWP conducted a pilot study to determine how Se moves through the food chain at the periphyton level, subsequently transferred to periphyton-associated macroinvertebrates and, if present, periphyton feeding fish. Sampling of shoreline periphyton occurred at Tenmile and Rexford sites in MT

during September 2017. Teck additionally sampled for periphyton on the BC portion of the reservoir. Full details on these sampling methods are found in Lotic (2019).

3.3 ZOOPLANKTON

Zooplankton is routinely sampled by USACE and Teck for density and identification. In addition, zooplankton selenium concentrations were measured to help understand the trophic transfer function from SPM to zooplankton. Zooplankton was sampled concurrently with water and SPM samples in MT and BC. **Figure 3-2** details the transboundary zooplankton sampling sites.

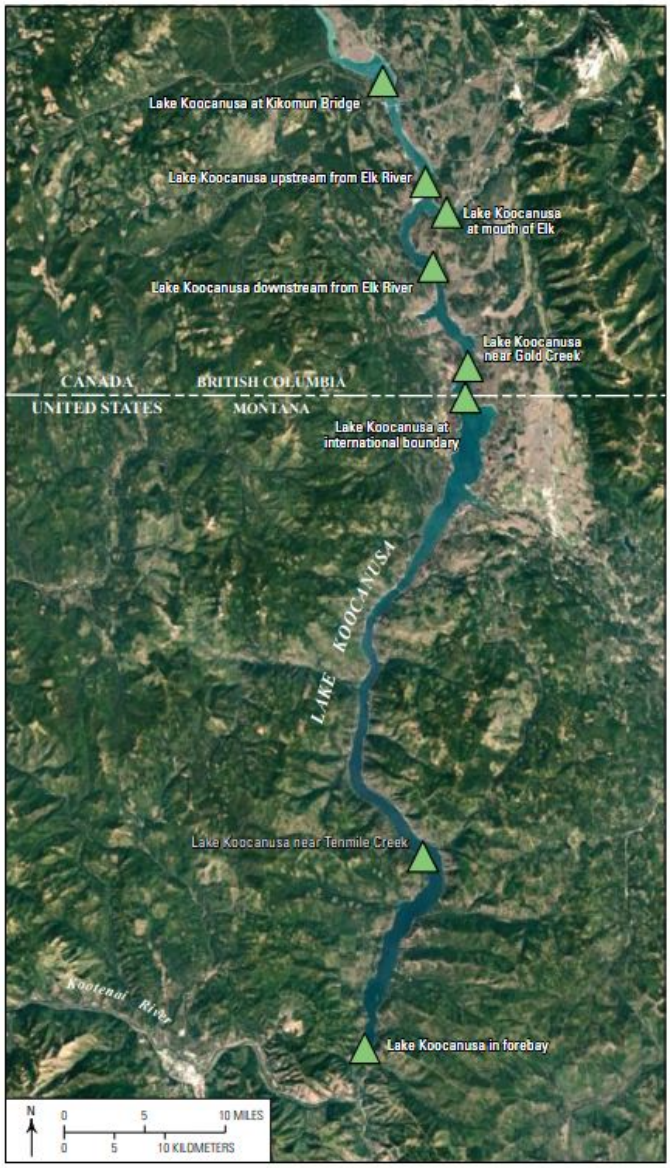

Figure 3-2. Location of sites where zooplankton samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.4 INVERTEBRATES

To better understand the trophic transfer of selenium, FWP collaborated with DEQ to collect and analyze surface and benthic invertebrates in Lake Koocanusa. As part of Teck's monitoring requirements, sampling for invertebrates for selenium analysis continued as routine monitoring on the BC portion of the lake. **Figure 3-3** displays the macroinvertebrate sampling sites in MT and BC.

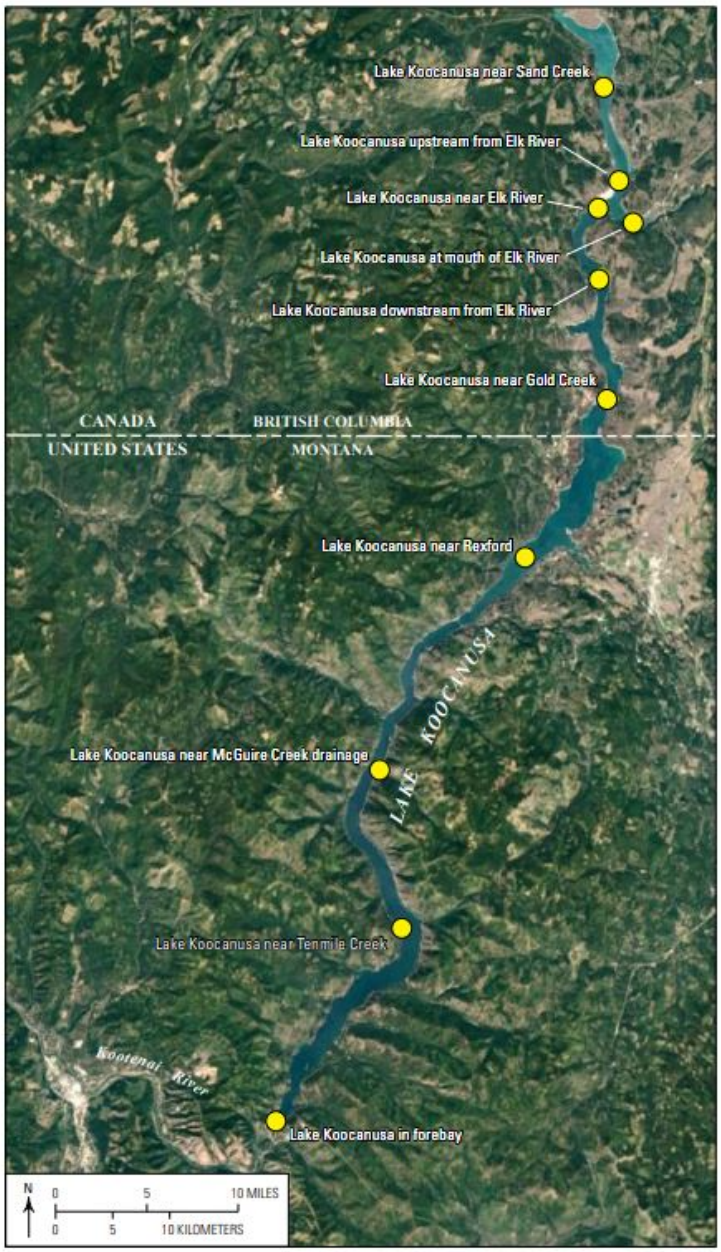

Figure 3-3. Locations of sites where invertebrate samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.5 FISH

FWP and DEQ worked collaboratively to sample fish tissue and continue baseline monitoring of fish tissue Se in Lake Koocanusa and evaluate concentration trends. Since 2008, fish tissues have been collected and analyzed for Se. In 2008, 2013, and 2018 bull trout, longnose sucker, northern pikeminnow, kokanee, peamouth, rainbow trout, and westslope cutthroat trout were targeted by FWP for tissue and opportunistic egg/ovary sampling. The three locations for this effort were; 1) near the mouth of the Elk River in Canada, 2) Rexford, and 3) McGillivray (Tenmile). Egg/ovary samples were taken if ovaries were with eggs, but the stage of development was not noted. In 2018-2019 FWP expanded their Tenmile site further south to the Forebay to give a more complete spatial representation of the lower portion of the reservoir.

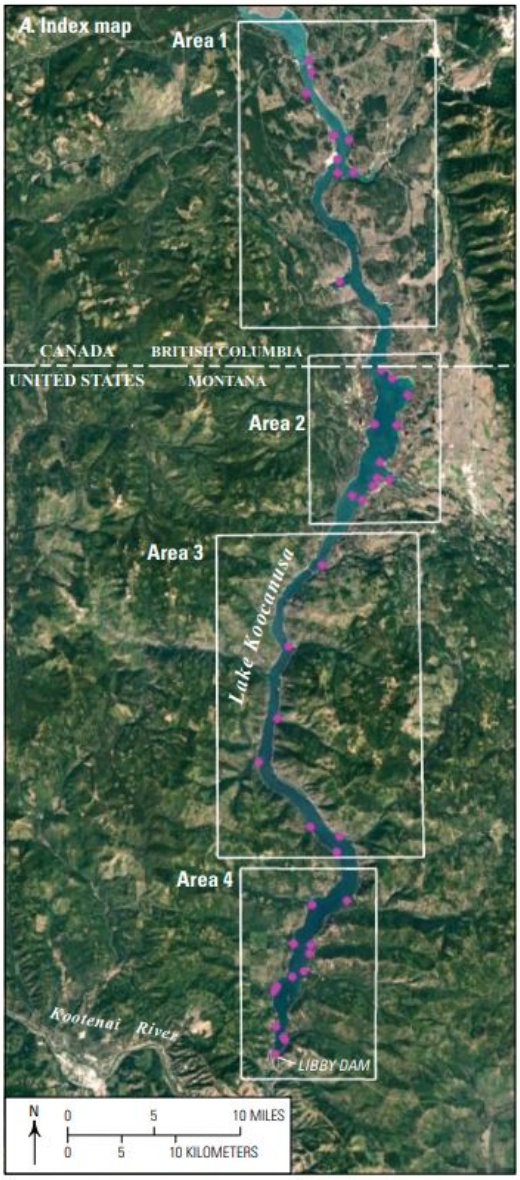

Figure 3-4. Fish collection sites for Montana and British Columbia. Explanations of collections within area 1 (South of Elk), area 2 (International Boundary), area 3 (Tenmile), and area 4 (Forebay) are further defined in Presser and Naftz, 2020.

A fish food habit study was conducted by FWP in 2017 to determine any differences from previous food habit information collected from fish in Lake Koocanusa from 1983-1992. Target species for the food habit study were westslope cutthroat trout, rainbow trout, longnose suckers, kokanee, and burbot. Stomach contents were analyzed and no major differences in food habits were determined.

Teck conducted fish sampling and analysis for Se as outlined in their permit requirement. Similarly, Teck sampled whole body, muscle, and fish egg/ovary tissue. Detailed information can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

3.6 BIRDS

USFWS conducted a preliminary risk assessment for avian exposure to Se in Lake Koocanusa. Data were collected in June 2016 for killdeer. Methods and results are described in Skorupa and Nelson (2018). The concentrations found for killdeer were well below the known toxicity for this species. The results of this study suggest birds in the MT portion of Lake Koocanusa are not currently experiencing Se-induced reproductive impairment. The SeTSC determined that fish are the most sensitive endpoint for consideration for modeling, and so fish became the focus for future data collection efforts to inform criteria development. No additional bird studies were conducted on the US portion of the reservoir, although Teck continues to monitor birds, primarily spotted sandpiper in BC, as part of their permit requirement.

4.0 SELENIUM MODELING

This section provides an overview of information on the USGS biodynamic selenium modeling utilized for derivation of a protective water column criterion for Lake Koocanusa. Consistent with the approach used by EPA in developing 304(a) criteria, DEQ partnered with the USGS to employ the mechanistic Ecosystem-Scale Selenium modeling approach for Lake Koocanusa (Presser and Luoma, 2010). The work by Presser and Naftz (2020) tailors the Presser and Luoma (2010) model to the Lake Koocanusa ecosystem. The peer-reviewed report and data release can be found at https://pubs.er.usgs.gov/publication/ofr20201098.

DEQ worked with the SeTSC and the USGS while concurrently following the EPA guidance in Appendix K for criteria derivation.

4.1 ECOSYSTEM-SCALE MODEL OVERVIEW

Presser and Luoma (2010) describe the Ecosystem-Scale Model and its use in understanding bioaccumulation and trophic transfer as essential to managing ecological risks from Se. The modeling process, using key components outlined below, provides a basis for understanding and quantifying dietary uptake and linkages among food webs. **Figure 4-1** illustrates the processes regulating movement of Se through an ecosystem.

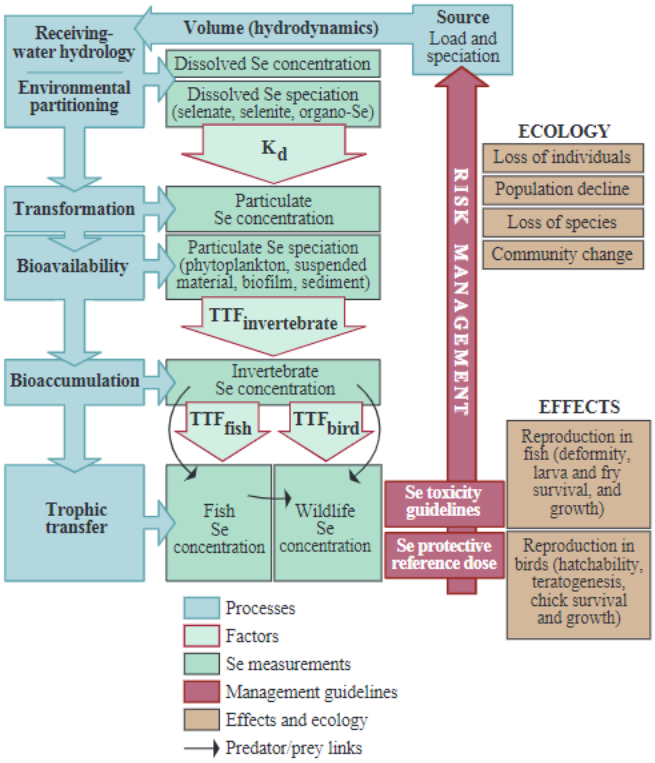

Figure 4-1. Conceptual illustration of the Selenium Ecosystem Scale Model (Presser and Luoma, 2010).

Jenni et al. (2017) first described tailoring the Presser and Luoma (2010) conceptual model to the Lake Koocanusa ecosystem. The key factors of the modeling include a tissue criterion element, a trophic transfer factor (TTF), and a partitioning coefficient (K_d). All of these are required to derive a protective dissolved water column number, as described by the equation below:

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{TTF^{composite} X\ K_d}$$

Where,

C_{target}	= translated site-specific water criterion element (µg/L)
$C_{tissue\ criterion\ element}$	= fish tissue criterion element (mg/kg dw)
$TTF^{composite}$	= product of the species-specific trophic transfer factor (TTF) values in each trophic level of the food web of the target fish species related to the tissue criterion element (no units of measurement)
K_d	= environmental partitioning factor

If the desired tissue criterion element is for an egg-ovary concentration, Jenni et al. (2017) prescribe translating this into a whole body concentration using either EPA guidelines or species-and site-specific data. Each of the key modeling factors incorporated as model inputs are described in more detail below.

4.2 Fish Tissue Criterion Element

It is widely understood that Se toxicity is manifested through chronic dietary exposure to Se. A growing body of research has described egg-laying vertebrates as the most sensitive ecological endpoint for selenium (see **Section 1.4.2**). The SeTSC scientific recommendation was to consider fish as the most

sensitive endpoint in the Lake Koocanusa ecosystem (see **Section 3.7**). Limited toxicity data exists for fish species in Lake Koocanusa, only species in the genus *Onchorhynchus* (rainbow trout and westslope cutthroat trout) and species within *Salvelinus* (bull trout) have known Se toxicity thresholds. **Table 4-1** describes the toxicity thresholds based on 10% effect concentrations (EC10's) described in EPA (2016). Laboratory analysis whereby the effect concentrations results in 10% mortality is referred to as an EC10. This is also referred to as a low effect concentration (LOEC).

Table 4-1. Tested reproductive-effect whole body (WB) concentrations measured directly or converted to WB concentrations from egg-ovary (EO) concentrations (EPA, 2016). Taxon resident in Lake Koocanusa *are Salvelinus*, *O. mykiss*, *Onchorhynchus*.

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

EPA (2016) describes Se concentrations in egg or ovaries as the best predictors of Se toxicity but explains the vulnerability of a species is the product of its propensity to accumulate Se from its environment through diet and transfer the Se from its body into the eggs. Therefore, EPA includes guidance in Appendix K to use whole body tissue as a reasonable alternative for modeling site specific criteria derivation. Presser and Naftz (2020) provide details outlining the necessity to model from whole body rather than eggs or ovaries in Lake Koocanusa. Moreover, given the limited toxicity data for the resident species in Lake Koocanusa, the EPA guideline for whole body calculated to 8.5 mg/kg dw was used for the criterion element in modeling (Presser and Naftz, 2020). This value was calculated by EPA using OLS regression based on the known four most-sensitive species in the nation, including the most sensitive species, white sturgeon, which is resident in the downstream Kootenai River. A criterion element of 8.5 mg/kg dw is considered protective of white sturgeon across the US. While Presser and Naftz (2020)

applied the 8.5 mg/kg 304(a) whole body criterion in the modeling, they describe how other values may be applied depending on the level of protection desired and the goals of the modeling.

4.3 TROPHIC TRANSFER FACTORS

Selenium trophic transfer factors (TTFs) describe uptake and efflux of prey and predator species (Presser, 2013). Chapman et al. (2010) discusses the similarity of TTFs found within groups of related species and species with a similar trophic status (level). Chapman further describes an important concept in understanding Se TTFs is that the majority of Se enrichment occurs at the lowest trophic levels through particulates and primary consumers. The implication of this is that secondary and tertiary consumers may not always experience substantially higher Se exposure than lower trophic levels. This differs from contaminants such as mercury (Hg) that consistently bio-magnify at higher trophic levels.

Presser and Naftz (2020) utilized TTFs established from laboratory experiments. Field derived TTFs have greater uncertainty than laboratory derived TTFs and Lake Koocanusa had limited data; therefore, USGS determined it most appropriate to model with laboratory derived TTFs as described in Presser and Luoma (2010). The following TTFs were applied; 2.8 (aquatic insects), 1.5 (zooplankton), and 1.1 (fish). Presser and Naftz (2020) present two choices for bioavailability (100% and 60%). The 60% bioavailability effectively reduces the TTF's by 60% to match observed data.

4.4 FOOD WEB MODELS

Two primary food web models were presented in Presser and Naftz (2020) and described in Tables 6 and 7 of their report. Included are the invertebrate to fish model (IFM) and trophic fish model (TFM).

The IFM model is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish guideline wb}/TTF_{fish}\ /[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

The TFM modeling is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish tissue guideline wb}/TTF_{fishTL4}/TTF_{fishTL3}[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

Focal fish, previously selected by the SeTSC, were grouped into categorized food webs representative of variations in diet of modeled fish. The categorized food webs described for the IFM model are located in **Table 4-2** and for the TFM model are in **Table 4-3**. These tables present different model scenario options, in which the invertebrate fraction (invert faction) in the above equations are modified based on the percentage consumed. The adjustment to the food web, in turn, modifies the bioaccumulation potential (BAP). The BAP is the combined effect of diet and TTFs. The food web consisting of 100% chironomids (aquatic insects) has the greatest bioaccumulation potential.

Table 4-2. Invertebrate to fish (IFM) model: fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
RBT, WCT, RSS, LNS	100% chironomid	3.08	1.85
PMC, LSS, MWF	50% chironomid 50% zooplankton	2.37	1.42
rainbow trout: Dec-Mar	25% chironomid 75% zooplankton	2.01	1.20
kokanee	100% zooplankton	1.65	0.99

Table 4-3. Trophic level fish model (TFM): fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
BT, burbot (winter, benthic), NPM	100% TL3 fish species (100% insectivores)	100% chironomid or aquatic insect	3.39	2.03
BT, burbot, NPM	100% TL3 species (50% IV; 50% PV)	50% chironomid 50% zooplankton	2.60	1.56
[1)]BT, burbot (summer fish), NPM	100% TL3 species (100% planktivores)	100% zooplankton	1.82	1.09

4.5 PARTITIONING COEFFICIENT (K_D)

The partitioning coefficient (K_d) describes the relationship between Se concentrations in particulate and dissolved phases (Presser, 2010). The term K_d has been used interchangeably with enrichment factor (EF), a term more commonly used by EPA. The K_d is a simple ratio described below which could be expanded to include a more complex enrichment function incorporating saturation kinetics. In this modeling process the equation below was used. The collection of matched particulate Se and dissolved Se is described in **Section 3.1** using the terminology suspended particulate matter (SPM) and particulate interchangeably.

$$K_d = [\text{particulate Se}]/[\text{dissolved Se}]$$

K_d values calculated from 2015-2019 ranged from 424.2 to 7,474.5 L/g. Rather than statistically reducing the K_d dataset down to a single representative value to use in the model equation, Presser and Naftz (2020) present each K_d calculation as an independent scenario (n=87). The result of this is that each model scenario includes 87 predicted dissolved selenium concentrations.

4.6 MODELING CONCLUSIONS

Presser and Naftz (2020) present a report and accompanying data release which provide the data, rationale, food web modeling structure, and interactive spreadsheets for the quantitative derivation of a site-specific selenium guideline for Lake Koocanusa.

Model predictions of protective dissolved selenium concentrations were specific to the EPA national guideline of 8.5 mg/kg whole body criterion, while recognizing that this whole-body concentration could be changed to meet the protection goals of BC-ENV. Modeling choices and assumptions used for the modeled scenarios were guided by the goals described in the report, and previously defined by the SeTSC.

Those goals are summarized here as:

- Consideration of ecologically significant species and those important to stakeholders,
- Protection of ecosystems during maximum dietary exposure (i.e., feeding within a benthic food web),
- Protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding in an ecosystem that functions as a lentic reservoir, and
- Long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all Se loading profiles, and all water years.

The IFM model based on the food web with maximum BAP (maximum dietary exposure) was through a 100% chironomid (aquatic insect) diet and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice. As noted in **Section 4.5**, Presser and Naftz (2020) recommend that model runs be undertaken for each measured K_d, in order to provide the full range of candidate criteria for a specified model scenario.

The TFM model was based on the food web with maximum BAP (TL3: trophic level 3) was through a piscivorous diet of 100% forage fish which had a diet of 100% chironomid (aquatic insect), and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice.

Presser and Naftz (2020) did not provide a final recommended protective water column Se concentration. Rather, as described above, the report provided the foundation from which DEQ and BC-ENV were able to co-develop a protective water column Se standard.

5.0 CRITERIA DEVELOPMENT AND IDENTIFICATION

The scientific expertise of the SeTSC guided the development of Lake Koocanusa's site specific water column Se standard, from the early stages of data collection to the final recommendations for ecosystem-scale model factors. The USGS modeling results described in **Section 4.0** describes the foundation from which a protective water column value would be co-developed between DEQ and BC-ENV. This section presents the scientific recommendations of the SeTSC and analysis by DEQ and BC-

ENV to co-develop a numeric water column standard that prevents impairment of the aquatic life beneficial uses of Lake Koocanusa.

5.1 SeTSC Recommendations

As part of the criteria development process and different protection goals between BC and the US, the LKMRWG Steering Committee (comprised of a BC and DEQ representative) solicited individual SeTSC recommendations on model input parameters (whole body tissue criterion, food web, TTF, and K_d). The SeTSC was additionally requested to provide recommendations on a final protective water column Se standard for Lake Koocanusa. Recommendations were discussed at length throughout a half-day teleconference held August 25, 2020. A subset of the members submitted additional written recommendations (**Appendix A**). The three USGS SeTSC members recused themselves from providing recommendations beyond what is provided in Presser and Naftz (2020) and Jenni and Schmidt (2020).

5.1.1 Fish Tissue Criterion Element

Presser and Naftz (2020) applied the US EPA criterion of 8.5 mg/kg whole body tissue threshold, yet describe that modification of this value may be appropriate depending on the level of protection desired and modeling goals. The derivation of the national whole body fish tissue threshold includes white sturgeon, the species with the known greatest sensitivity to selenium (EPA, 2016). In spite of that, five out of seven participating SeTSC members recommended applying a lower whole body value. Their recommendations ranged from 4.6 – 7.0 mg/kg dw. Primary rationale for selecting a lower whole body value included; 1) consistency with the lower British Columbia guideline which is considered protective of 100% of species at all life stages, 2) uncertainty around potentially sensitive species and species of cultural importance for which no toxicity data exists, 3) providing assurance for sensitive aquatic dependent wildlife which may become resident in the future, 4) based on selenium toxicity expertise and understanding of the reservoir.

5.1.2 Trophic Transfer Factors (TTFs)

Recommendations for TTFs are intertwined with the bioavailability. As described in **Section 4.3** Presser and Naftz (2020) included a 2.8 TTF for aquatic insects and 1.5 for zooplankton. Additionally, Presser and Naftz (2020) present two bioavailability choices, 100% and 60% bioavailability. The 60% bioavailability was recognized to be the option better tailored to Lake Koocanusa. The 60% bioavailability effectively reduces the 2.8 TTF (aquatic insects) to 1.7 and the 1.5 TTF (zooplankton) to 0.9. However, there was general agreement among the SeTSC that the 2.8 and 1.5 TTFs at the 60% bioavailability may still be over predictive. The SeTSC individual members had varying recommendations for approaching TTFs including; 1) applying the 2.8 and 1.5 at 60% as a conservative measure particularly if using a less conservative whole body threshold (8.5 mg/kg dw), 2) use site specific TTFs (recommendations ranged from 1.1 – 1.2 (aquatic insects) and 0.52-0.85 (zooplankton), 3) combine the Presser and Luoma (2010) and EPA (2016) TTF data to produce a more robust dataset from which to derive a TTF based on the central tendency.

5.1.3 Food Web Model

Presser and Naftz (2020) included two models, the IFM and TFM. Within each are categorized food webs representative of variations in diet of modeled fish. Recommendations focused on using a piscivorous food web at 100% aquatic insects. This food web is referred to as TFM with TL3 100% Aquatic Insects (**Section 4.4**). The rationale for TFM with TL3 100% aquatic insects, the most

conservative food web, was for protection for potentially sensitive piscivorous species and species of cultural importance.

5.1.4 Partitioning Coefficient (Kd)

Two of the SeTSC members recommended additional analysis be done to sub-set the K_d dataset to include only K_d values from the epilimnion. The epilimnion showed slightly greater K_d values overall, and the rationale to include only epilimnion K_d data was to include a conservative approach which may be more representative of Se entering the food chain, specifically through primary producers.

Ultimately, a protective water column value must be selected from the distribution of observed K_d values. Recommendations ranged from the 50th percentile (median) to the 90th percentile depending on the model assumptions (model inputs). The recommendations as to which K_d percentile to select was reliant upon the level of conservatism incorporated into other model parameters, particularly the whole body tissue criterion. There was overall agreement that if applying a lower (more conservative) whole body value, then a median K_d would be protective of the beneficial use. However, if a less conservative whole body value was used, such as the 8.5 mg/kg, then a more protective percentile from the distribution would be recommended to ensure adequate protection.

5.1.5 Water Column Concentration Recommendations of the SeTSC

Recommendations on a final Se water column criterion were presented from four of the seven participating SeTSC members. Final values presented ranged from 0.6 μg/L to 1.5 μg/L, with one committee member describing a range for consideration between 0.73 μg/L to 0.8 μg/L. Three of the four SeTSC members proposed criteria recommendations less than 0.9 μg/L with one recommendation of 1.5 μg/L. The 1.5 μg/L recommendation was proposed with the rationale to follow the EPA 304(a) criteria, rather than utilize the work presented in Presser and Naftz (2020).

5.2 DEQ & BC-ENV SUPPLEMENTAL ANALYSIS

As previously stated, the goal of this work was to co-develop a site-specific water column standard for Lake Koocanusa. A challenge of that work has been the differing protection goals between BC-ENV and DEQ. However, the two agencies worked collaboratively, giving consideration to SeTSC recommendations and the protection goals of each agency, to co-develop three additional model scenarios to consider in conjunction with the model assumptions presented in Presser and Naftz (2020). This section describes the collaborative analysis and the DEQ proposed dissolved Se standard. **Table 5-1** describes model inputs (model assumptions) representative of a balance between the SeTSC recommendations and BC-ENV and DEQ agency goals for the water body.

The scenarios described in **Table 5-1** apply a whole body tissue value of 5.6 mg/kg. As this work was a collaborative process between BC-ENV and DEQ, the 5.6 value was incorporated to remain consistent with BC-ENV's more stringent guidelines. This value falls within the range recommended by five out of seven SeTSC members (see **Section 5.1.1**). This value was derived by applying a westslope cutthroat (a resident species within the known most Se sensitive genus) egg-ovary to whole body conversion factor of 1.96 (EPA, 2016) to the BC provincial egg ovary guideline of 11.0 mg/kg dw.

Based upon recommendations by some SeTSC members to include site specific TTFs, BC-ENV and DEQ applied a bioavailability of 45%. This work was guided both by analysis by Thorley (2020) and the

recommendations by two of the seven SeTSC members. While the 45% bioaccumulation incorporates less conservatism, it was found to be more representative of the observed concentrations. It was determined by BC-ENV and DEQ that this 45% bioavailability would only be appropriate if a more conservative whole body tissue value was also applied (5.6 mg/kg). The 45% effectively reduces the aquatic insect TTF from 2.8 to 1.26 and the zooplankton from 1.5 to 0.68. For aquatic insects, this value is very close to site-specific TTFs recommended by two SeTSC members which ranged from 1.1-1.2 and is within the range of site specific zooplankton TTFs recommended that ranged from 0.56-0.85. The live Excel spreadsheets for the three model scenarios presented in **Table 5-1** are available from DEQ upon request (please contact DEQ's Water Quality Standards & Modeling Section).

Table 5-1. Three additional model scenarios developed by BC-ENV and DEQ incorporating SeTSC recommendations.

Scenario	Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
1	5.6	IFM	100% Aquatic Insects		2.8		45%	50th (median)	0.89
2	5.6	TFM	75% Aquatic Insects/ 25% Zooplankton	1.1	2.8	1.5	45%	50th (median)	0.91
3	5.6	TFM	100% Aquatic Insects	1.1	2.8		45%	50th (median)	0.8

With consideration of the SeTSC recommendations and supplemental DEQ and BC-ENV analysis, DEQ determined Scenario 3 from **Table 5-1,** resulting in a dissolved water column numeric standard of 0.8 µg/L, to be protective of the aquatic life beneficial uses of Lake Koocanusa. Scenario 3 ensures all ecosystem food webs are protected, a stated goal of the SeTSC.

Additionally, DEQ considered the SeTSC recommendation to use the 8.5 mg/kg tissue threshold. Through working collaboratively with BC-ENV, the following model assumptions as described in Presser and Naftz (2020) were applied; IFM 100% Aquatic Insects, 60% bioavailability, and DEQ selected a more conservative percentile from the upper quartile of the K_d distribution (75th percentile). This modeling scenario also meets the protection goals defined by the SeTSC and DEQ. This scenario resulted in a water column Se value of 0.8 µg/L protective of the aquatic life beneficial uses of Lake Koocanusa.

Table 5-2. Model inputs DEQ considered following the two SeTSC member recommendations to apply 8.5 mg/kg and following the recommendations to then identify a more conservative K_d percentile.

Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
8.5	TFM	100% Aquatic Insects	1.1	2.8		60%	75th	0.8

6.0 PROPOSED CRITERIA FOR LAKE KOOCANUSA

Proposed Se standards for Lake Koocanusa contain two classes of selenium standards; fish tissue standards, which limit the amount of Se allowed to accumulate in different fish tissues, and a water column standard which was derived from bioaccumulation modeling also intended to limit Se accumulation in fish tissue.

Table 6-1. Proposed water column and fish tissue Se standards for Lake Koocanusa.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

The national fish tissue standards have a hierarchy of importance; the egg/ovary standard is the most takes precedence because those data are the most indicative of selenium toxicological effects on fish at the reproductive stage. However, fish egg/ovary tissue is not always available. Fish muscle or whole body tissue standards can be used in the absence of fish egg/ovary tissue. The fish tissue standards supersede the water column standard only when the lake or river is in steady-state, referring to conditions whereby there are no activities resulting in new, increasing, or changing selenium loads to the lake, and selenium concentrations in fish have stabilized. Lake Koocanusa is not currently in steady state (Presser and Naftz, 2020). Therefore, both the fish tissue standards and water column standards are applicable standards for Lake Koocanusa. The department will determine when Lake Koocanusa reaches steady state after review and analysis has been carried out by the department during triennial review. The proposed water column standards are chronic values. There is no acute selenium standard included since the greatest toxicity risk to aquatic life is from chronic dietary exposure.

6.1 FREQUENCY AND DURATION

The proposed recommendation for return frequency is consistent with EPA's current 304(a) guidance based on the 1985 Guidelines for water column criteria. The proposed return frequency for a water column criteria exceedance of not more than once in three years, on average, is based on EPA's determination of the resiliency of the aquatic ecosystem to recover from a toxin when the impacts are associated exclusively with a water column exposure (Stephan et al., 1985).

The duration component of the criteria describes the exposure time-period and restricts the length of time that the concentration in the receiving water can be continuously above a criterion concentration, in order to protect aquatic life. The proposed durations are consistent with the current 304(a) guidance such that the numerical fish tissue criterion elements are specified as instantaneous. The use of an instantaneous measurement (duration) not to be exceeded (frequency) is because fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish population(s) at a given site. Selenium concentrations in fish tissue are generally expected to only change gradually over time in response to environmental fluctuations. The duration for the chronic water column standard is a 30-day average.

6.2 PROTECTION OF DOWNSTREAM WATERS

Federal regulation at 40 CFR 131.10(b) requires the State to consider and ensure the attainment and maintenance of downstream (intra-and interstate waters) WQS. The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream Endangered Species Act (ESA) listed, white sturgeon.

The Kootenai River is in a B-1 use class, identical to what was outlined for Lake Koocanusa (**Section 1.3.1**). The designated use class for the Kootenai River in Idaho is outlined in Idaho's regulations found at 58.01.02.100 as, "*water quality appropriate for the protection and maintenance of a viable aquatic life community for cold water species*." Idaho defines viable aquatic life as communities that are functioning and intact. Additionally, the Kootenai River Native Fish Conservation Program includes a Tribal Sturgeon Hatchery managed by the Kootenai Tribe of Idaho (KTOI) to prevent extinction, preserve the existing gene pool, and rebuild a healthy age class of the ESA listed endangered white sturgeon.

The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream ESA listed, white sturgeon. DEQ modeled white sturgeon using the bioaccumulation model by Presser and Naftz (2020). Applying the white sturgeon whole body sensitivity of 9.2 mg/kg, the food web of TFM with TL3 100% aquatic insects, a TTF of 2.8, and a bioavailability of 60%, the results showed 0.8 μg/L to be protective at the 90th percentile of the Kd distribution. As white sturgeon are not resident within Lake Koocanusa, DEQ finds this to meet the protection of downstream use including protection of white sturgeon.

6.5 PROTECTION OF FEDERALLY LISTED SPECIES

The bull trout was listed as threatened under the ESA on November 1, 1999 (64 FR 58910). Bull trout are native to Lake Koocanusa and the Kootenai river, representing a geographically distinct and important population within the broader bull trout range. In Montana, the management of fisheries including that of bull trout is executed by FWP. Montana FWP biologists monitor spawning sites (redds) annually as a metric for measuring fish reproduction, recruitment, and fisheries management. The monitoring takes place during the fall as most bull trout spawn between late August and early November. Current trends for bull trout in Lake Koocanusa are common abundance with a stable population. The 2002 FWP Bull Trout Report lists current threats to bull trout in Lake Koocanusa (above Libby Dam) as identified by the Montana Bull Trout Scientific Group as including introduced fish species, rural residential development, forestry, mining, agriculture, water diversions, and illegal harvest.

At present, there are no known selenium toxicity studies for bull trout. There are toxicity tests for the taxonomically similar, dolly varden, both in the genus *Salvelinus*. Research shows dolly varden to be among the most tolerant fish species to Se. **Table 4-1** describes the whole body and egg ovary selenium toxicity thresholds for dolly varden being 34.9 (whole body) and 56.4 (egg ovary). DEQ modeled bull trout using the bioaccumulation modeling by Presser and Naftz (2020) by substituting the whole body guideline for the *Salvelinus* whole body toxicity threshold of 34.9 to determine that the proposed standards including the site specific water column standard of 0.8 μg/L is protective such that no individual bull trout will be harmed.

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Threatened Bull Trout (Salvelinus confluentus). U.S. Fish and Wildlife Service, Pacific Region, Portland, Oregon

APPENDICES

1. Appendix A – Selenium Technical Subcommittee written recommendations

28th August, 2020

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Sean Moore| Director, Watershed Science and Adaptation, Environmental Sustainability and Strategic Policy Direction, BC Ministry of Environment| LKMRC Co-Chair
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Dear LK MRC Co-Chairs and Members,

Please accept this recommendation on behalf of the Confederated Salish and Kootenai Tribes (CSKT) and the Kootenai Tribe of Idaho (KTOI), constituent governments of the transboundary Ktunaxa Nation. You will find herein our scientific justification and rationale, regarding the request to provide written recommendation on the inputs to the model developed by US Geological Survey (USGS), in support of a site-specific selenium criteria for Koocanusa Reservoir.[1]

The Transboundary Kootenai watershed sits entirely within the transboundary Ktunaxa Nation Territory and provides critical habitat for rare and threatened fish species including bull trout, burbot, westslope cutthroat trout, and endangered Kootenai River white sturgeon. Unabated selenium inputs from the Elk Valley mines into Koocanusa Reservoir demonstrate a clear, increasing trend dating back to 1984.[2] Selenium leaching from the Teck Ltd. mines in the Elk Valley of British Columbia is resulting in degradation of water quality and presenting unacceptable impairment and risks to Ktunaxa Territory resources. As noted in our previous

[1] Presser, T.S., Naftz, D.L. Naftz, 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A. and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020-1098, 40 p., https://doi.org/10.3133/ofr20201098.

[2] Unpublished data from 2019 collected by the U.S. Environmental Protection Agency, U.S. Geological Survey and Kootenai Tribe of Idaho for the Kootenai River and tributaries. 2019.

letters, we are specifically concerned about impacts on the water quality, fish and fish habitat, species at risk, impacts to other species and resources that depend on those waters and fish, and traditional cultural values, including human health impacts from consumption of contaminated fish, in the entire transboundary Kootenai watershed.

Based on historical and recent data for water quality and fish tissue, it is imperative that Montana work now to adopt a site-specific selenium criteria for the health and protection of all fish species in Koocanusa Reservoir and downstream in the Kootenai watershed. We recognize that existing data documents increasing selenium in several species of fish in Koocanusa Reservoir, including three species that exceed the 2016 EPA recommended criteria for selenium in fish tissue. Further, Koocanusa Reservoir is currently unprotected, given that Montana did not adopt the national recommended selenium criteria, as revised and released by EPA in 2016.[3] The best available science, including the 2020 USGS model and report, demonstrates that there are historical, on-going, and projected future inputs of selenium into Koocanusa Reservoir, and it is the responsibility of the State of Montana to adopt a selenium criteria that is sufficiently protective to ensure the immediate and long-term protection and restoration of Koocanusa Reservoir, and downstream uses in the Kootenai River, from the ecological impacts of selenium contamination. Given the current impacts and risk to Ktunaxa territory resources, the KTOI and CSKT are in full support of the commitment by the State of Montana to adopt a site-specific selenium criterion by December, 2020, including initiation of the formal rule-making process in September, 2020.

In addition, we support the scientifically defensible and peer-reviewed report and model developed by USGS in support of criteria development, including the approach of the USGS to base the model on a conservative and protective approach. The authors of the model are among the top selenium experts in North America, with decades of experience in the field of selenium toxicology, and the model they have developed is peer-reviewed and capable of generating a defensible, protective criterion for the reservoir, based on the factors that influence selenium in the reservoir.

Given that Koocanusa Reservoir is already degraded due to input of contaminants from mining in the Elk Valley of British Columbia, we support a criterion that manages the reservoir to improve and restore from the already degraded condition. Current levels of selenium contamination caused by Elk River coal mining above and below Libby Dam is with high probability already causing, and threatens to continue, negative physiological effects to organisms dependent on aquatic resources, including birds, and possibly humans. A conservative site-specific criterion is needed to support management that improves and restores the water quality and aquatic life in the reservoir.

[3] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

There is evidence of significant bioaccumulation of selenium already occurring across the Kootenai ecosystem, including the Idaho and BC portions of the Kootenai.[4] This bioaccumulation has been occurring and will continue even at current water column selenium concentrations that are below the current criteria/exceedance limits. Literature provides evidence that body burden concentrations found in Kootenai River white sturgeon, burbot, mountain whitefish, and freshwater mussels are likely already having significant physiological effects. This is a critical concern to the Ktunaxa Nation governments, given the cultural significance of these species, as well as the tremendous effort and resources dedicated to ecosystem restoration.

The selection of a conservative and protective site-specific selenium criterion is necessary to, at minimum; prevent further increases in selenium into the Kootenai ecosystem. Current data is showing increasing concentrations of selenium in larger portions of the reservoir, which in turn will increase selenium concentrations below Libby Dam.[5] This trend will continue until effective mine impact mitigation is implemented at an appropriate scale.

The overall selenium loading into the reservoir from the Elk River needs to be stabilized and reduced in order to prevent near-future partitioning and release of selenium into the reservoir and also the downstream Kootenai River.

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L. Therefore, based on the specific framework of the USGS model W6, Model run #2, the CSKT and KTOI are specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Based on the attached background, modeling recommendations and rationale, the KTOI and CSKT recommends using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates.

In summary, we are recommending a conservative site-specific criterion for selenium in Koocanusa Reservoir, based on the following uncertainties;

1. Koocanusa Reservoir currently demonstrates system degradation and impairment. This is demonstrated by the following:
    a. Fish tissue concentrations (muscle, whole body, and/or egg ovaries) at times exceed USEPA and B.C. recommend thresholds.

[4] https://governmentofbc.maps.arcgis.com/apps/webappviewer/index.html?id=0ecd608e27ec45cd923bdcfeefba00a7

[5] Presser, TS, and DL Naftz. 2020. Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, USA, and British Columbia, Canada: US Geological Survey Open-File Report 2020-1098, 40 p. https://doi.org/10.3133/ofr20201098.

b. The reservoir has increasing pollutant loads, as demonstrated by B.C. long-term monitoring station on the Elk River at HWY 93.
c. The reservoir has an increasing mass of selenium over an increasing reservoir area (Presser and Naftz, Figure 17).
d. The reservoir has declining burbot populations.
e. Fish populations demonstrate gonadal disfunction and dysfunctional selenium dietary bioaccumulation.

2. Water quality monitoring data indicate the Koocanusa Reservoir is a dynamic system and it is possible that current monitoring efforts have not defined nor captured critical time periods or critical portions of the reservoir.
3. A delay or lag in uptake of selenium into the food web, from the water column, is highly likely and at a magnitude that presents a significant risk. The outcome is increasing and perpetuated bioaccumulation of selenium in benthos and fish above elevated levels.
4. To return to a restored condition, MT DEQ must avoid normalizing current degraded conditions and strive for a condition that is improved from current conditions.
5. On-going revisions to the modeling in the Elk and Fording River, including the Implementation Plan Adjustment to the Elk Valley Water Quality Plan, that increases the observed and modeled future contaminant delivery into Koocanusa Reservoir from the Elk Valley Mines.[6]

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

Sincerely,

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[6] 2019 Implementation Plan Adjustment Annex B - Regional Water Quality Model Modifications
https://www.teck.com/media/Annex-B-Regional-Water-Quality-Model-Modifications.pdf

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August 28, 2020

Dear SeSTC Committee Members and Co-Chairs,

Selenium Technical Sub-Committee members were requested to submit written recommendations to the SeTSC Co-Chairs for the site-specific selenium criteria. Below you will find our recommendations, serving as a representatives of the Kootenai Tribe of Idaho (KTOI) and the Confederated Salish and Kootenai Tribes (CSKT). Please see below for background, recommendations and rationale for the site-specific criteria.

We based on our recommendation on a site-specific criterion that protects burbot (*Lota lota*), the fish species that are most sensitive to selenium bioaccumulation in Koocanusa Reservoir. Burbot have been functionally extirpated from the reservoir and are culturally important to the Ktunaxa Nation community. Burbot populations declined over two decades ago when the ambient reservoir Se concentrations were below what is currently seen today. In published literature, burbot have been shown to be particularly sensitive and susceptible to the bioaccumulation of selenium.[1] Muscatello and Janz observed significant bioaccumulation in burbot (10 ug/g dw WB) at low aqueous (<0.5 µg/L) and benthic invertebrate (0.5-3 µg/g) selenium concentrations.[2] This is reinforced with the general knowledge that the burbot population decline[3] and eventual functional-extirpation in Koocanusa Reservoir coincides with the Elk River Coal Mines operational history and subsequent water pollution caused by those coal mines; and severely complicates the restoration of burbot above Libby Dam.[4]

The burbot population declined when the ambient reservoir Se concentrations were below the aqueous concentrations that are currently seen today. Limited KTOI data is also showing that burbot in the mainstem Kootenai River are accumulating selenium at rates that are known to cause significant negative physiological effects on other fish species. Those effects include reproductive failure, reduced growth, and mortality (KTOI, unpublished data).
Further, mining contaminant inputs into Koocanusa Reservoir present a critical uncertainty in the Kootenai River Ecosystem Restoration program[5], and will continue to act in synergy with

[1] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[2] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[3] Dunnigan, J., J. DeShazer, T. Ostrowski, M. Benner, J. Lampton, L. Garrow, and M. Boyer. 2018. Mitigation for the Construction and Operation of Libby Dam, 1/1/2017 – 12/31/2017 Annual Report, 1995-004-00. 252 pp.
[4] Cope, A. 2018. Upper Kootenay River Burbot Conservation Strategy, Draft Report. 59 pp.
[5] www.http://restoringthekootenai.org

the habitat alterations perpetuating white sturgeon and burbot recruitment failure below Libby Dam.

In addition to burbot, it is critically important that the criterion is based on considerations for protection and restoration of the Kootenai River white sturgeon (*Acipenser transmontanus*) downstream of Libby Dam given their sensitivity to reproductive impacts from selenium toxicity. We note that white sturgeon are the most toxicologically sensitive fish as ranked by the US EPA in its national guidance.[6]

With respect to birds and wildlife, the Kootenai River Basin was once one of the more ecologically productive inter-montaine ecosystems, supporting resident and migratory bird populations; however, Koocanusa Reservoir currently does not support robust shorebird populations. Shorebirds are particularly vulnerable to selenium toxicity, as they are highly sensitive to selenium exposures.[7] Skorupa et al found reproductive failure in aquatic birds with 3.0 µg/g selenium concentrations in their eggs.[8] Birds have been shown to be particularly sensitive to selenium exposures due to their feeding habits that are linked to the aquatic environment.[9] Stanley et al found that a 7 mg Se/kg dietary exposure in mallard ducks caused a >30% embryo mortality.[10]

Hamilton reviewed approximately 40 different studies investigating selenium toxicity for fish, aquatic birds, phytoplankton, and zooplankton.[11] Several tables within this paper provided a comprehensive compilation of species tested, tissues sampled, selenium concentrations tested for effects, corresponding physiological effects, and study citations. The physiological effects concluded by the individual studies listed throughout the review tables are “Mortality”, “Reduced Growth”, “Reproductive Failure”, “Reduced Weight”, and “Reduced Cell Replication”.

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[6] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

[7] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[8] Skorupa, JP, HM Ohlendorf, and RL Hothem. In press. Interpretive guidelines for selenium-exposed waterbirds. J. Wildlife Management.

[9] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[10] Stanley, TR Jr, GJ Smith, DJ Hoffman, H Heinz, and R Rosscoe. 1996. Effects of boron and selenium on mallard reproduction and duckling growth and survival. Environ Toxicol Chem 15:1124-1132

[11] Hamilton, SJ. 2003. Review of residue-based selenium toxicity thresholds for freshwater fish. Ecotoxicology and Environmental Safety 56:201-210.

For several fish and aquatic bird studies listed, the selenium toxicity levels causing mortality, reduced growth, reproductive failure, and/or reduced weight were whole body tissue and/or egg concentrations as low as 1-4 ppm.

Thorley cites data collected from water and fish tissue (whole body and egg/ovary) Se concentrations for Koocanusa Reservoir.[12] Water concentrations ranged 0.5 -1.5 µg/L, and corresponding fish tissues from several fish species ranged from 1.0 – 6.0 ppm for whole body, and ~2.0 to 80.0 for egg/ovary. Even if the 80.0 µg/g observation is an outlier, results from peamouth chub (*Mylocheilus caurinus*), redside shiner (*Richardsonius balteatus*), and Northern pikeminnow (*Ptychocheilus oregonensis*) were predominantly 10.0 – 40.0 µg/g for egg/ovary samples. These are tissue concentrations at water concentrations of 0.5-1.5 µg/L.

Thorley also presents data collected from zooplankton and benthic macroinvertebrate Se concentrations for sample sites located within Koocanusa Reservoir.[13] Zooplankton selenium concentrations ranged between <1 to 5 µg/g, with some samples upwards of 14 µg/g Se. Benthic macroinvertebrate tissue concentrations ranged between <1 to 12.5 µg/g Se, with the mean Se concentration near 5 µg/g Se.

The EPA whole-body threshold of 8.5 mg/kg dw is based upon the known sensitivity of white sturgeon. This is scientifically defensible and appropriate on the national level. However, the 8.5 mg/kg dw whole-body criterion does not account for other potentially sensitive and susceptible fish species or protection of the most sensitive designated use, which includes tribal harvest treaty rights. Whitefish (*Prosopium williamsoni*) and burbot are culturally important fish species that are consumed by Ktunaxa citizens from all three Ktunaxa Nation governments. A minimum whole-body threshold of 5.6 mg/kg dw should be considered. Using the BC MOE egg/ovary guideline of 22 mg/kg dw, and factoring in the safety/assessment factor of 2, and using the EC10 egg/ovary to whole-body conversion for rainbow trout of 1.9, this leads to a more conservative 5.6 mg/kg dw whole-body recommendation. The KTOI and CSKT recommend using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates. The KTOI and CSKT recommend a conservative site-specific criterion for Koocanusa Reservoir until additional science and data collection demonstrate otherwise.

Current reservoir selenium outflows are approximately 1.0 µg/L (range between 0.8 and 1.2 µg/L, depending upon dam operations, time of year, and hydrologic conditions within the basin). Kootenai River white sturgeon egg selenium concentrations in the mainstem river

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[12] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

[13] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

below Libby Dam range between 3.0 and 6.0 mg/kg dw. Of the five whole-body burbot tissue samples collected by the KTOI, one was above the 8.5 mg/kg dw EPA threshold, and mountain whitefish egg concentrations exceed EPA's 15.1 mg/kg dw threshold, with some of these values almost double the EPA recommended criteria (KTOI 2020; unpublished data). These measurements indicate that, like Koocanusa Reservoir, the Kootenai River requires the development of a site-specific water column selenium criterion. KTOI and CSKT understand that this will likely require a multi-year effort to collect adequate data and develop a site-specific criterion for the Kootenai River, and we encourage DEQ to begin this effort immediately in collaboration with both Tribes. For now, KTOI and CSKT support MT DEQ setting an interim criterion for the Kootenai River that is equal to EPA's national recommended value for water column, fish tissue, and egg/ovaries. In summary, we support the adoption of a conservative site-specific criterion for Koocanusa Reservoir now, to reduce uncertainty and risk in the Kootenai River downstream, and the subsequent initiation of a rigorous, scientific process to develop a site-specific criterion for the Kootenai River.

After evaluating multiple scenarios using a reasonable range of variable values within the USGS models provided to the SeTSC, the KTOI and CSKT recommends using the 'W6. TFM with TL3 100% Aquatic Insects' model. This model is conservative and protective of the most selenium-susceptible trophic levels; and is also considered the most protective, as it incorporates whitefish and burbot.

We recognize the variability of TTF's, conversion factors, and K_d values. Given the uncertainty and wide fluctuations in K_d throughout the reservoir (values ranging between 400 and 7000), a conservative K_d should be used. In order to be protective of the reservoir ecosystem across time and location, the 90th percentile K_d should be used to capture the worst-case scenario. The use of the median K_d value is also supported in literature. The use of the 1.1 TTF is supported by literature and is scientifically defensible. To manage the uncertainty in the water concentration guideline, Jenni, Naftz, and Presser (2017) suggested triangular distributions with a TTF for invertebrates (aquatic insects and zooplankton combined) between 1 and 3.5 with a mode of 1.3, a TTF for fish between 0.6 and 1.6 with a mode of 1.1 and a Kd between 800 and 6,500 with a mode of 3,000.

Model Input Recommendations

With respect to the specific model inputs, we provide the following recommendations and rationale; Given the varying K_d values within the reservoir, and the two recommended TTF values for aquatic insects, we ran six variations of the W6 model that incorporate the different K_d and TTF values. Listed below are the outputs from the six model runs.

1. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.22 µg/L (given the model correction of 100% Se bioavailability) to 0.37 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

2. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.37 µg/L (given the model correction of 100% Se bioavailability) to 0.61 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

3. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.53 µg/L (given the model correction of 100% Se bioavailability) to 0.89 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

4. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.29 µg/L (given the model correction of 100% Se bioavailability) to 0.49 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

5. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.49 µg/L (given the model correction of 100% Se bioavailability) to 0.82 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

6. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.71 µg/L (given the model correction of 100% Se bioavailability) to 1.18 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L.

Based on the specific framework of the USGS model W6, Model run #2 as described above, the CSKT and KTOI is specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Current whole-body fish tissue samples from Northern pikeminnow, peamouth chub, redside shiner, and largescale sucker in Koocanusa Reservoir exceed, and in many individuals sampled, greatly exceed, the EPA whole-body criteria in the current aqueous conditions in the reservoir.[14] This clearly indicates to KTOI and CSKT that to be protective of all fish species in the reservoir, the site-specific criterion should be lower than the current selenium concentrations

[14] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

sampled in the reservoir. Also, as noted in Presser and Naftz, 2020, it is important to determine where Koocanusa Reservoir is in an impairment-restoration cycle so as not to base protection on survivor bias, the maintenance of a currently degraded ecosystem, or normalized toxicity. In a broader context, one of the overall consequences of revised selenium regulations is that their derivation is now dependent on being able to define and understand the status of the ecosystem on which protection is based. And, as described in Presser and Naftz, 2020, the Koocanusa Reservoir system demonstrates traits of a currently degraded system (see Table 1 in the report and subsequent discussions). This further illustrates to CSKT and KTOI that a protective site-specific water column selenium criterion should be lower than existing conditions in the reservoir.

Given that there may be a lag in the biological uptake and detection of selenium across the food web in the reservoir, it is important to adopt a more conservative criterion at this time, to ensure protection under unknown future selenium levels and the increasing contaminant trends. Any selenium concentrations above the background concentrations represent an increase from baseline conditions for the Kootenai Basin and are likely already having, and will perpetuate negative impacts upon the ecosystem. According to Chapman et al[15] in the Selenium Risk Characterization chapter 7, Lentic systems were identified to be at an increased risk of Se-caused adverse effects due to the maximized mobility of selenium into the food web, thereby increasing the chance for elevated exposures.

Continuing downriver into the altered lower-river ecosystem driven by Libby Dam operations, the food web in the mainstem Kootenai River is quite different than the reservoir; therefore the movement of selenium from Koocanusa Reservoir through Libby Dam and into the lower-river is relatively unknown. Water and tissue sampling in the Kootenai River below Libby Dam suggests the current selenium concentrations and loading into the river are already having negative impacts on the ecosystem.

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

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August 29, 2020

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RE: KNC Selenium Technical Sub-Committee recommendations on Presser and Naftz (2020) selenium bioaccumulation model for Koocanusa Reservoir

In response to the request from the co-chairs of the Selenium Technical Sub-Committee (SeTSC) of the Koocanusa Reservoir Monitoring and Research Working Group, the Ktunaxa Nation Council (KNC) technical representatives of the SeTSC are pleased to offer our recommendations for consideration in setting the water quality benchmark (also "criterion" or "objective") for the protection of designated uses in the Koocanusa Reservoir. Specifically, we are providing our recommendation on the requested topics, including model inputs, quantitative approaches to using the USGS model outputs (Presser and Naftz 2020), and the proposed range of water column concentrations that would be protective of designated uses.

We would highlight that the following technical recommendations were developed based on our current understanding of the Koocanusa Reservoir and the data and information available (e.g., Beaman 2020; DeForest 2020; Lotic Environmental 2018; Presser and Naftz 2020; Thorley 2020). KNC technical representatives are providing recommendations at this time to honour the Se TSC co-chair request in recognition of the timelines required by Montana Department of Environmental Quality (DEQ) and their process for rule-setting. We also must acknowledge that KNC is committed to working government to government with the Province of British Columbia in developing a water quality objective for Koocanusa Reservoir. The technical recommendations made in this memo will be provided to KNC's Lands and Resources Council in September 2020, after which KNC's formal recommendations for the water quality objective for Koocanusa Reservoir will be submitted.

Model Inputs

Target Tissue Concentration

The ecosystem-scale model ("USGS model") used by Presser and Naftz (2020) estimates the concentration of selenium in trophic levels within a food web beginning with particulate material (i.e., sediment, phytoplankton, and detritus) in the water column through primary consumers (e.g., benthic invertebrates and zooplankton) and into higher trophic levels including planktivorous and piscivorous fish. The objective of the USGS model is to derive a range of selenium concentrations in the water column that are predictive of whole-body concentrations in fish that meet the protection goal of preventing adverse effects on fish reproduction. Various tissue-based whole-body selenium guidelines have been proposed to meet this objective, including the USEPA whole-body guideline of 8.5 µg/g dry weight (dw).

KNC technical representatives, along with some other members of the SeTSC, have recommended the BCMOE (2014) tissue-based guideline of 11 µg/g dw in egg/ovary tissue be used to provide assurances that the most sensitive species at the most sensitive life-stage would be protected during indefinite exposure. To facilitate the use of the BCMOE (2014) tissue-based guideline in the USGS model, we have converted the egg/ovary tissue concentration to a whole-body tissue concentration. The BCMOE (2014) guideline is based on an egg hatchability/viability test with rainbow trout. USEPA (2016) provides an egg/ovary:whole-body conversion factor of 1.96 for rainbow trout. Applying the conversion factor results in a whole-body tissue guideline of 5.6 µg/g dw, which can be used directly in the USGS model as the target tissue concentration.

In previous communications, KNC technical representatives requested the evaluation of a tissue-based guideline applied to the diet of fish and other wildlife (i.e., birds and mammals). The threshold of 4 µg/g dw (BCMOE 2014) was recommended as a protective threshold in prey fish (e.g., redside shiner, peamouth chub). However, based on an evaluation of the data available in the reservoir, the trophic transfer factor (TTF) between prey fish (e.g., peamouth chub) and piscivorous fish (e.g., northern pikeminnow) is roughly 1, indicating that the 4 µg/g dw threshold may be lower than the site-specific data warrants.

When setting water quality objectives, it is important to understand whether the protection goal sought (i.e., fish reproduction) would be protective of other designated uses (e.g., wildlife, recreation, human health). The target tissue concentration can be compared to whole-body tissue-based guidelines for other designated uses, including wildlife consumers (e.g., birds) and human consumers. With respect to avian receptors, Ohlendorf and Heinz (2011) suggest diets greater than 5 µg/g dw could reduce egg hatchability in sensitive avian receptors, including the mallard duck (EC_{10} of 4.9 µg/g dw; Joe Skorupa, USFWS, SeTSC member; pers. comm.). J. Skorupa (pers. comm.) also stated that an

appropriate threshold for protection, considering whole-body concentrations, would likely be in the 4.5 to 6 µg/g dw range (pers. comm) when considering an egg/ovary:whole-body conversion factor for burbot (i.e., 2.3) and using either the BCMOE (2014) egg/ovary threshold of 11 µg/g dw or USEPA (2016) egg/ovary threshold of 15.1 µg/g dw, respectively.

Recognizing the importance of food security and the continuity of Ktunaxa practice including hunting, fishing, trapping, camping, ceremonial practices, and the transmission of knowledge and identity to future generations, we also evaluated whether tissue concentrations would be protective of Ktunaxa citizens at their preferred consumption rates. BCMOE (2014) provides human health screening values for low (i.e., 30 g/day) to high (i.e., 220 g/day) fish consumption. By using the approach in BCMOE (2014), we calculated a whole-body threshold that we consider to be protective of cultural practices such as harvesting and consuming fish at preferred rates. By applying the most current estimate of Ktunaxa preferred consumption rates of 245 g/day, and selecting mountain whitefish as a focal species due to our understanding of cultural practices, we estimate that a muscle concentration of 1.6 µg/g wet weight (ww) or 6.8 µg/g dw would be an appropriate screening value. Using the muscle:whole-body conversion factor for mountain whitefish of 1.27 (USEPA 2016), an appropriate whole-body tissue threshold of 5.3 µg/g dw would achieve the BCMOE (2014) screening values and protect Ktunaxa cultural practices at currently understood preferred rates. We note that the Ktunaxa preferred consumption rate is our current best-estimate based on diet surveys and likely underestimates the importance of fish in Ktunaxa diet resulting from the exclusion of anadromous salmon from Ktunaxa Territory (ʔamakʔis Ktunaxa).

Given the estimates of ecological and human health thresholds for whole-body selenium concentrations noted above, we offer the following recommendation.

> **Recommendation 1:** We recommend that the water quality objective for Koocanusa Reservoir protect ʔa·kxaṁʔis q̓api qapsin (All Living Things) and that the Kootenay ecosystem (including the reservoir) be managed in such a way that Ktunaxa rights, title, and practices are protected. Accordingly, a target tissue concentration of 5.3 ug/g dw has been identified as protective of cultural use and a target tissue concentration of 5.6 µg/g dw using the BCMOE tissue-based guideline for the protection of ecological receptors. Therefore, it is our recommendation that 5.3 µg/g dw be used as the target tissue concentration in the USGS model, as it is protective of all uses.

Food Web Model

To assist in the development of a range of selenium concentrations in the water column that are predictive of whole-body concentrations in a variety of fish species, Presser and Naftz (2020) developed a series of food web models accounting for variations in the source (e.g., aquatic insects, zooplankton, fish) and proportions of food sources in the diet of model species. From our perspective, the *TFM w TL3 100% AqIns* model, which reflects the food web of burbot, bull trout, and northern pikeminnow is considered appropriately conservative and protective of the culturally important burbot, which has shown significant population declines (Hardy and Paragamian 2013). This food web assumes a piscivorous feeder with a diet consisting of prey fish that feed on aquatic insects (e.g., juvenile rainbow trout, westslope cutthroat trout, redside shiner, and longnose sucker).

Given the protection goal of protecting all fish species in the reservoir, we offer the following recommendation.

> **Recommendation 2:** We recommend the use of *TFM w TL3 100% AqIns* to represent the food web in the USGS model.

Trophic Transfer Factors (TTFs)

Presser and Naftz (2020) rely on literature derived TTFs in the mechanistic model as presented in Presser and Luoma (2010). The TTFs used, include:

- $TTF_{Particulate\ to\ Insects}$: 2.8
- $TTF_{Particulate\ to\ Zooplankton}$: 1.5
- $TTF_{Prey\ to\ Fish}$: 1.1

To account for any site-specific differences in the TTFs resulting from differences in community structure (e.g., proportion of rotifers, Presser and Naftz 2020; or, proportion of sediment, detritus, and algae in the particulate material, Beaman 2020), Presser and Naftz (2020) applied a bioavailability factor of 60%. As an example, in a food web that includes a focal species feeding on 100% insects, the bioaccumulation factor from particulate material to fish would be 2.8 multiplied by 1.1 or 3.08. To account for site-specific bioavailability, the bioaccumulation factor of 3.08 would be adjusted downwards to 1.85, assuming 60% bioavailability. The bioavailability adjustment was derived through a validation exercise (Presser and Naftz 2020).

Members of the SeTSC and supporting consultants have proposed alternative TTFs. Beaman (2020) proposed to expand the original Presser and Luoma (2010) TTF dataset to include additional studies as presented in (USEPA 2016). Both Thorley (2020) and DeForest (2020) derived site-specific TTFs for aquatic insects/benthic invertebrates of 1.2

(Figure 1). Thorley (2020) and DeForest (2020) also derived site-specific TTFs for zooplankton. Thorley (2020) accounted for seasonality in the zooplankton selenium concentrations (Figure 2), while DeForest (2020) presented statistical estimates of the TTF based on pooled data (i.e., arithmetic mean of 0.53). When considering seasonality, the upper estimate of mean TTFs was 0.85, which corresponds to September. Conceptually, this relationship is supported with data from Woods (1982) where it is demonstrated that primary productivity in the reservoir typically peaks in August; it would therefore be expected, that peaks in primary consumers would follow soon after.

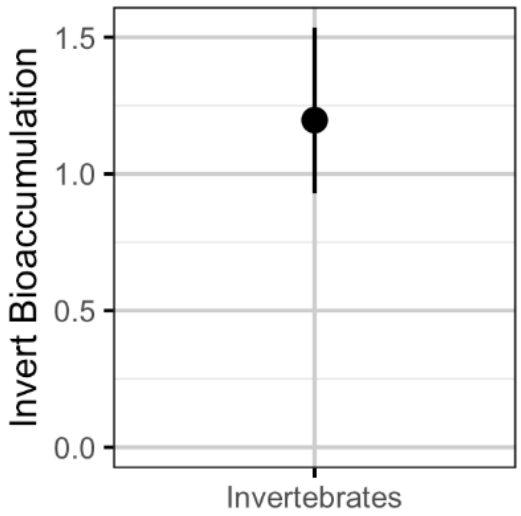

Figure 1. Estimated particulate to invertebrate selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

Presser and Naftz (2020) and Beaman (2020) acknowledged that there are sparse data available on aquatic/benthic insects and zooplankton, which results in greater uncertainty in site-specific TTFs. Secondly, an important assumption of lab- or field-derived bioaccumulation factors is that system is in steady-state with respect to exposure concentrations. Koocanusa Reservoir is not in steady state, driven largely by the dynamic nature of the operation of the Libby Dam and increasing selenium loads from the Elk River (Presser and Naftz 2020). Accordingly, Presser and Naftz (2020) and Beaman (2020) propose the use of literature-based TTFs. Despite relying on literature-based TTFs, Presser and Naftz (2020) apply a bioavailability factor of 60% to account for site-specific conditions via a validation exercise. Thorley (2020) estimated bioavailability factors of 43% and 57% for invertebrates and zooplankton, respectively, using data collected from the reservoir. KNC technical representatives also determined that the literature-based TTFs were likely too high when considering the concentrations observed in the invertebrate and zooplankton data.

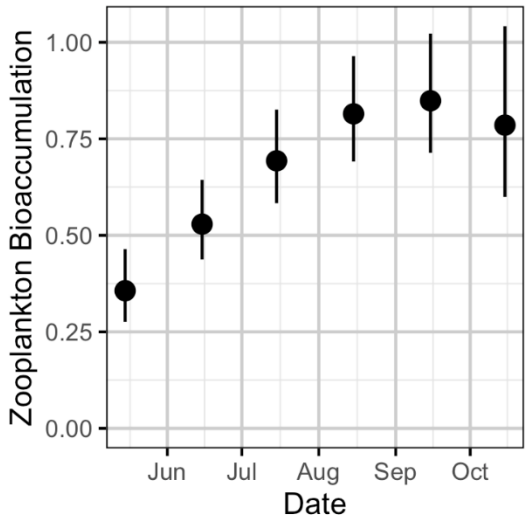

Figure 2. Estimated particulate to zooplankton selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

We consider both approaches to estimating TTFs as reasonable and defensible methods and acknowledge the uncertainties in these estimates due to the non-steady state of the system. The implications of a temporal lag in selenium inputs and biological uptake, as well as with respect to selenium retention within the reservoir, are currently unknown. Given that we do have some information on site-specific bioaccumulation within the system from the previous 5 to 10 years, we recommend relying on the measured site-specific TTFs. It must be acknowledged that the adjusted TTFs from Presser and Naftz (2020) and the site-specific TTFs developed by Thorley (2020) are relatively similar (Table 1).

> **Recommendation 3:** We recommend using the site-specific TTFs of 1.2 for invertebrates and 0.85 for zooplankton.

Kd Results

The USGS model determines an aqueous selenium concentration that would be considered ecologically protective for each of the measured Kd estimates (n = 87) for each food web model (e.g., *TFM w TL3 100% AqIns*). The Kd estimates ranged from 424 to 7,475 with a median Kd of 4,547 (Presser and Naftz 2020). The measured Kds were typically higher in the epilimnion of the reservoir compared to the hypolimnion (Figure 3; Thorley 2020). Given the slight differences in Kd estimates between the epilimnion and hypolimnion, and the greater degree of primary productivity in the epilimnion, the Kd estimates from the epilimnetic zone should be included for any quantitative approach to application of the

Table 1. Summary of trophic transfer factors and bioaccumulation factors evaluated for use in the USGS model.

	TTFs - Presser and Naftz (2020)	TTFs - Site-Specific (Thorley 2020)	Estimated Bioavailability Factors (Thorley 2020)	
Trophic Transfer Step				
TTF_{invert}	2.8	1.2	2.8	--
$TTF_{zooplankton}$	1.5	0.85	--	1.5
TTF_{fish}	1.1	1.1	1.1	1.1
Bioavailability Adjustment	0.6	--	0.43	0.57
Bioaccumulation Factor				
BAFinvert-fish	1.85	1.3	1.3	--
BAFzooplankton-fish	0.99	0.94	--	0.94

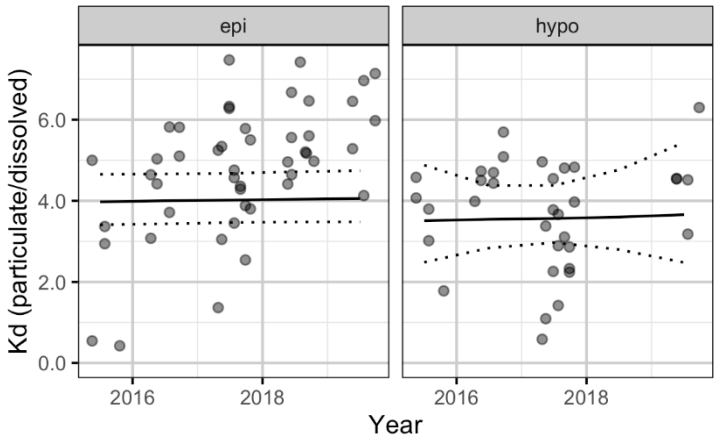

Figure 3. Estimated and observed ratio of particulate to water selenium (Kd = ratio x 1,000) in the epilimnion and hypolimnion with 95% confidence intervals (from Thorley 2020).

model results (see below). This would allow for a more conservative approach to quantifying a Kd for deriving the ecological benchmark considering the uncertainties in the degree of selenium assimilation in the water column of the hypolimnion and also considering that estimated Kd at the sediment water interface in the reservoir are more similar to the epilimnion than the hypolimnion (ranging between 2,641 and 5,812; median: 4,775; Table 2).

Recommendation #4: Kd estimates from the epilimnion (n = 50) should be carried forward into the quantitative assessment of model results below.

Table 2. Predicted Kd estimates in detritus/sediment of the reservoir proper using benthic tissue samples (collected with ponar; predominantly chironomids; Table T19 - Presser and Naftz 2020). Predicted particulate Se was calculated using the site-specific TTF for invertebrates (1.2; Thorley 2020) and associated water quality (mean concentrations by date and depth) in the reservoir (Presser and Naftz 2020).

Site Name	Sample ID	Invertebrate Se (µg/g dw)	Predicted Particulate Se (µg/g dw)	Associated Water Quality (µg/L)	Estimated Kd
Tenmile/Forebay	C3418	5.07	4.23	1.6	2,641
Tenmile/Forebay	C3421	8.37	6.98	1.2	5,812
Rexford	C345-BI-D1	4.50	3.75	1.0	3,750
Rexford	C345-BI-D2	5.73	4.78	1.0	4,775
Tenmile/Forebay	C3426-BI-D	6.43	5.36	0.93	5,761

Quantitative Approaches to Application

It is expected that some primary consumers and higher trophic level receptors (i.e., fish) would reflect reduced variability in selenium tissue burden compared to primary producers under varying selenium concentrations. Biokinetic modeling presented by DeForest *et al.* (2015) suggests that zooplankton would likely exhibit similar, but only slightly reduced variability, while benthic invertebrates and fish would have moderate and high reductions in variability, respectively when considering continuous exposure over varying Kd measurements. However, these higher trophic organisms would also be slower to respond to trends over time. Accordingly, it is reasonable to assume that fish are integrators of the varying selenium concentrations and that some measure of central tendency, with an appropriate level of conservatism to account for changes over time is reasonable.

Analysis of the Distribution

Beaman (2020) recommended a percentile, such as the median be used, to select the Kd for guideline derivation. Thorley (2020) estimated the expected Kd for the epilimnion of Koocanusa Reservoir at roughly 4,000 while the expected Kd in the hypolimnion is 3,500. The upper 95% upper confidence interval in the epilimnion is roughly 4,800 (Figure 3). The median Kd using all measurements is 4,547 while the median Kd in the epilimnetic measurements is 5,017.

> **Recommendation #5:** We recommend the range of Kd estimates for use in the model is between 4,547, the median of pooled Kd estimates (n = 87), and 5,017, the median of epilimnetic Kd estimates (n = 50).

Protective Concentrations

The USGS model was populated with the recommendations above to estimate the range of aqueous concentrations considered to be protective of all uses. It is estimated that the range of protective concentrations in Koocanusa Reservoir is 0.73 to 0.80 µg/L; model results are presented in Table 3. These results align well with the estimated 95% lower confidence interval of the mean dissolved selenium concentration (i.e., roughly 0.9 µg/L) associated with a whole-body concentration in burbot of 5.6 µg/L (Thorley 2020).

Table 3. Estimated range of ecologically protective concentrations using the recommendations for model inputs and Kd selection presented in this memorandum and the USGS model (Presser and Naftz 2020).

[1.] Kd scenario that most closely matches the pooled median of 4,574 and epilimnetic median of 5,071.

Tissue Target Concentration	Aquatic Insect TTF	Fish TTF	Bioavailability Factor	Kd[1]	Benchmark (µg/L)
5.3	1.2	1.1	1.0	5,000	0.73
5.3	1.2	1.1	1.0	4,579	0.80

Considerations for Future Activities/Monitoring

- We recommend that a mass balance analysis be conducted to quantify inputs from the Elk River (and other sources), exports (via Libby Dam) and the pool of selenium in the reservoir and the rate at which it is increasing over time.
- We recommend that fish tissue monitoring be conducted to get an accurate measure of ripe egg selenium concentrations (pre-spawn) to validate conversion factors.
- We recommend efforts focus on the estimation of assimilation efficiency factors (Kds) in periphyton (e.g., through the deployment of artificial substrates, seeded with periphyton)
- We recommend collection of additional benthic invertebrate samples for tissue analysis from the vicinity of the artificial substrates as well in the reservoir proper.
- We recommend collection of additional zooplankton samples in various seasons throughout the year at multiple locations throughout the reservoir.

Closure

The recommendations put forth in this letter provide a range of reasonable and defensible model parameters considering the data and information collected to date. Using the recommendations for model parameters in the USGS model, we estimate a range of ecologically protective benchmarks between 0.73 and 0.80 µg/L. This range is

corroborated by the work conducted by Poisson Consulting, which estimated a criterion for burbot, a high selenium accumulator, of roughly 0.9 µg/L (Thorley 2020). It is important to note that this range of estimates is lower than the current concentrations (i.e., 1 µg/L; Thorley 2020) in the reservoir. Accordingly, these analyses suggest that there is a need to stabilize and reduce loadings of selenium into Koocanusa Reservoir in order to meet protection goals as we do not have confidence that increasing selenium concentrations above these levels would be protective of aquatic life or Ktunaxa cultural practices.

We appreciate the opportunity to provide feedback and look forward to further engagement on this process.

Sincerely,

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Heather McMahon
Project Biologist
Ktunaxa Nation Council

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The Selenium Technical Subcommittee (SeTSC), was established by the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), at the direction of the Steering Committee and is comprised of selenium experts from both the US and Canada and supported by the Montana Department of Environmental Quality and BC Ministry of Environment.

The overall objectives for the Se TSC are to develop selenium criteria/objectives for Lake Koocanusa that are protective of the uses of lake including, but not limited to, aquatic life, human health, recreation, wildlife, and agriculture, with the specific goal of answering the questions:

- Are the current Canadian (selenium target of 2 µg/L, as set out in the BC Water Quality Guideline) or Montana (WQS = 5 ug/L), protective of the uses in Lake Koocanusa in their respective jurisdictions?
- If not, what is an appropriate target value for selenium in Lake Koocanusa that can be adopted and implemented by Montana and British Columbia?

The SeTSC has been meeting regularly for four years through conference calls and in-person workshops with the primary goal of evaluating available data facilitating the development of a site-specific selenium criterion for Lake Koocanusa. More specifically, the process has involved the following tasks:

- Collection and analysis of existing Lake Koocanusa information to identify gaps in scientific understanding of the lake chemistry and ecology that are relevant to the stated research objectives.
- Prioritize monitoring and research activities and tasks
- Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- Definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa.

Seminal products developed during this process include the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019). These products provided critical information enhancing the SeTSC

understanding of the available data and the mechanistic model proposed for use in development of a site-specific water quality threshold protective of the designated uses of Lake Koocanusa.

The SeTSC in consideration of jurisdictional requirements, and with input from stakeholders, developed a set of constraints and considerations critical to the modeling efforts (*Proposed Workplan for Developing a Site-Specific Selenium Water-Column Criterion for Lake Koocanusa*; "the workplan") and resulting outputs including:

- The site-specific criterion will meet the regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.
- The site-specific criterion will also consider ecologically significant species and those important to stakeholders
- The definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir
- The site-specific criterion will provide long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all selenium loading profiles, and all water years (precipitation/runoff scenarios).
- The Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- site-specific criterion also will protect downstream uses including protection of the endangered Kootenai River white sturgeon

Using the requirements and constraints set forth above, four main alternative levels of protection were proposed for model runs for Lake Koocanusa:

- Two scenarios based on the BCMoE egg-ovary selenium guideline of 11.0 mg/kg dw that consider individuals and populations of fish species explicitly.
- Two scenarios based on the USEPA national 304(a) egg-ovary selenium criterion of 15.1 mg/kg dw that consider individuals and populations of fish species explicitly.

An additional scenario was proposed by stakeholders as a potential no effect threshold (BCMoE tissue guideline of 4.0 mg/kg dw) protective of sensitive wildlife receptors. This value is like a "no-effect" threshold of 5.5 mg/kg dw proposed to EPA by USFWS in 2005 (J. Skorupa personal communication).

SeTSC members were charged with providing comments on the USGS modeling report "*Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada*" Open-File Report 2020–1098.

Committee members were asked to comment on:

- Model Inputs (used to confirm or run model scenarios):

- Tissue threshold (use of whole body 8.5 ug/g, 4 ug/g, or other?)
- Model(s) to run, i.e. Invertebrate to fish model and/or Trophic level fish model and species-specific model (Table 10.)
- Food Web / diet fraction(s), e.g. %aquatic insect / %zooplankton: 100/0, 75/25, 50/50
- TTFs (invert to fish): generic option (1.1) or other?
- Bioavailability (100%, 60% or other percentage)
- Kd data set (Specific sub-sets?)

The following comments reflect my personal scientific views on the USGS model assumptions and outputs from the report. The focus of these comments is based on regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

1. Model Inputs - Tissue threshold

The USGS selected the USEPA whole-body (wb) selenium criterion element 8.5 mg/kg dw, a value translated from the egg-ovary criterion of 15.1 mg/kg dw. In EPA, 2016, Species-specific whole-body values were calculated directly from whole body tissue [Se] concentrations measured in reproductive toxicity studies, or more commonly by applying an egg-ovary (EO) to whole-body (WB) conversion factor (CF) based on taxonomic relatedness. The lowest WB concentration in the available data is from the white sturgeon (9.2 mg/kg dw), the most sensitive species in the EPA national toxicity dataset. The criterion (8.5 mg/kg dw) is based on an OLS regression-based projection using an n = 15 taxa. The EPA used a generic (median of all fish [1.78]) conversion factor (CF) to convert the egg-ovary criterion to a whole-body threshold. EPA derived CFs for matched pairs of field-collected egg or ovary and whole body samples and used medians based on the available species-specific distributions of whole body and reproductive tissue data. Concern has been expressed over the EPA's use of the median, as it may not be appropriate to a specific site (i.e., Lake Koocanusa). There is a paucity of field data of sufficient quality to calculate field-based EO-WB conversion factors for resident species. Ideally, laboratory data directly measure reproductive tissues (mature eggs) and whole-body measurements would be more readily available. There are two studies available bluegill (Hermanutz, 1993, 1996) and brown trout (Formation 2009) with directly measured tissues resulting in EO-WB conversion factors of 1.38 and 1.59 respectively, providing support for the EO-WB CF of 1.78 used in the national freshwater selenium criterion EPA, 2016)

National criteria are limited to predicting sensitivity to a given contaminant based on the range of toxicity data available, and the national data set may be modified by applying the Recalculation Procedure (40CFR131.11(b)ii) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while including tested surrogates for untested resident species. As discussed previously (Beaman presentation to SeTSC, October 2019), the fish assemblage in Lake Koocanusa is well comprehensively represented by the EPA selenium toxicity database (EPA, 2016) augmented by other data (DeForest 2012, Teck, draft redside shiner toxicity report 2020). This database provides precise quantitative reproductive toxicity values for 5/13 resident species, as well as qualitative species or genus surrogate level tissue values for an additional 4 species (mountain whitefish, largescale and longnose sucker, and redside shiner), leaving 4/13 species unrepresented in the site-specific toxicity database. Early in the process, the SeTSC agreed that all fish species without data would be deemed equally sensitive to the white sturgeon, the most sensitive species in EPA's toxicity dataset. Therefore, the

white sturgeon whole body value of 9.2 mg/kg dw would be applied to the burbot, northern pikeminnow, peamouth chub, and yellow perch. When these 4 values are added to the censored site database (removing non-resident species), the resulting criterion is 9.2 mg/kg since the 5 most sensitive taxa are all equally sensitive. This provides valuable information demonstrating that the EPA tissue threshold of 8.5 mg/kg dw is likely sufficiently protective of the assemblage of fish species in Lake Koocanusa, including those with no toxicity data.

Recommendation: use the EPA whole body criterion element of 8.5 mg/kg dw, as it provides a protective goal for the species assemblage in Lake Koocanusa.

2. Bioavailability of selenium in Lake Koocanusa & Bioaccumulation Potential of Resident Fish Species
Selenium bioavailability at the base of the food web is impacted by the form of inorganic selenium (inorganic selenate and selenite) and its interaction with different types of particles in the aquatic environment. The relationship between the [Se] in suspended particulate matter (SPM) and the [Se] in the invertebrate (TTF) is a function of the type of particulate that the invertebrate encounters (sediment, detritus, phytoplankton) and the assimilation efficiency (AE) of the organism based on the form of selenium encountered adsorbed on (elemental Se or inorganic Se) or contained in (organic Se) the particulate.

Because selenium speciation data for SPM was not available for Lake Koocanusa, USGS addressed this uncertainty by using AEs of various species from other studies (e.g., Presser and Luoma 2010a) and using different types of particulate matter to account for the site- or species-specific bioavailability of foods likely to be consumed by invertebrates.

Two SPM bioavailability factors (100 percent and 60 percent) are used within each food-web scenario to quantify the efficiency of assimilation of SPM by invertebrates. USGS cited AEs varying from 55 to 86 percent among various invertebrate species, with smaller differences among living food types such as different species of algae. In my review of EPA 2016, I note that the saltwater copepods have AEs of ~50 – 55% whereas other invertebrates, particularly saltwater mollusks have median AEs ranging 61 – 96% (EPA 2016 Appendix B). Conversely, laboratory studies using freshwater species (including surrogates for resident macroinvertebrates:

Species	Median AE	Range AE	Taxonomic surrogacy
Water flea (D. magna)	40.6%	24.9 -57.9%	zooplankton
Blackworm (L. variegatus)	16.5%	9 – 24%	chironomid (benthic detritivore)
Mayfly	39%	38 – 40%	resident aquatic insect

Even available data for mollusks, a taxon known to have high selenium AE, are notably lower in FW (asiatic clam – 55%; zebra mussel – 26% [18-46%]) indicate that lower AE may be more appropriate.

Given the paucity of resident invertebrate data, USGS used [Se] ranges to examine the impact of bioavailability assumptions on model validation. For macroinvertebrates collected in Montana waters, the AE = 100% resulted in significant overpredictions (2X upper limit of 18.8 for predicted vs 9.1 for observed, 2018), whereas the AE = 60% resulted in more comparable results (observed [Se], 0.4–9.1 µg/g dw vs predicted 0.7–11.3 µg/g dw). Similar observations were made for validation comparisons of macroinvertebrates collected in the lake south of the Elk River, as well as zooplankton collected in US and Canadian waters. The table shows a comparison of water values calculated using the TFM or IFM model using assumed bioavailability of 60% or 100%

Table 1. Influence of Bioavailability of water column values in IFM and TFM models

Centile	TFM (100%)	IFM (100%)	TFM (60%)	IFM (60%)
median	0.55	0.61	**0.92**	**1.01**
40th centile	0.52	0.58	**0.87**	**0.96**
30th centile	0.49	0.54	**0.82**	**0.90**
20th centile	0.45	0.50	**0.75**	**0.83**
10th centile	0.40	0.44	**0.66**	**0.73**
5th centile	0.38	0.42	**0.63**	**0.70**

Recommendation: use the bioavailability of 60% based on model validation results and literature values (EPA 2016) that support freshwater AEs ≤ 60%.

Bioaccumulation Potential (BAP)
USGS developed the following scenarios for the insectivorous fish model:

- 100-percent aquatic insect (rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker),
- 50-percent aquatic insect and 50-percent zooplankton (peamouth chub, largescale sucker, mountain whitefish),
- 75-percent zooplankton and 25-percent aquatic insect (rainbow trout December–March), and
- 100-percent zooplankton (kokanee).

The following scenarios were used for piscivores. The scenarios for bull trout, burbot (winter and summer), and northern pikeminnow are as follows:

- 100-percent insectivores,
- 50-percent aquatic insect and 50-percent zooplankton, and
- 100-percent planktivores.

Given the paucity of stomach content data available to confirm older food web studies illustrated in Lotic Environmental 2017, the USGS developed insectivorous and piscivorous food webs using conservative assumptions regarding the weighting of dietary components. This is consistent with the principles discussed in the USGS workplan, that "definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir".

Recommendation: although some refinement of food webs would be more consistent with previous food web studies documented in the Lotic Environmental report, the use of "model food webs" are consistent with the modeling principle that assumes maximum dietary exposure.

3. Water -Particulate Partitioning Coefficient (Kd)
Kds were collected by different agencies at different times during the years 2015 -2019. Kds were not spatially and temporally comparable on a year over year basis. Due to the density, spatial and temporal inconsistencies in Kd sampling between 2015 and 2019, it is difficult to partition a subset of Kds for examination of its influence on modeled water concentrations for Lake Koocanusa. Therefore, USGS used the entire Kd datasets allowing for 87 independent scenarios within each (IFM and TFM) model. The full distribution of scenarios can be assessed statistically, with selection of a protective water value

expressed as a percent of scenarios where maximally exposed fish are protected (based on attainment of the whole-body value of ≤ 8.5 mg/kg dw using conservative food web assumptions.

Table 2. Centile Distribution of Kds and Water Values (ug/L) generated by IFM and TFM model scenarios

			Model	
Centile	Kd	Centile	IFM	TFM
median	4547	median	1.01	0.92
60th centile	4788	40th centile	0.96	0.87
70th centile	5090	30th centile	0.90	0.82
80th centile	5569	20th centile	0.83	0.75
90th centile	6311	10th centile	0.73	0.66
95th centile	6611	5th centile	0.70	0.63

This table displays the mean epilimnetic and hypolimnetic Kds by year and location.

Table 3. Annual Average Kd by Site, Sampling Year, and Layer

Location	Layer	Sampling Year				
		2015	2016	2017	2018	2019
South of Elk River	epi			4525	5482	
	hypo			2722		
US/Can Border	epi	3971	4256	3748	5745	6573
	hypo	2956	4625	3662		5012
Tenmile	epi				5222	
Forebay	epi	1446	5150	5458	5480	5518
	hypo	4188	4878	3150		3864

Kds collected in the hypolimnion were typically substantially lower than those collected in the epilimnion, however this is not unexpected. Particulate in the hypoliminion is typically composed of detritus (scenescing phytoplankton from the epilimnion or other particulates). In reducing environments such as the epilimnion, selenium released from detritus is recycled as selenite or organoselenium, but is not typically re-incorporated in particulate, resulting in lower Kds. These Kds should not be discounted, since detritivores that tolerate hypoxic conditions such as chironomids are an important macroinvertebrate food source to benthic invertivores and omnivores. These fish species are then preyed upon by demersal predators like the burbot.

Kds in the epilimnion of the lakes forebay were consistent between 2015 and 2019. Epilimnetic Kds were more variable at the international border and appear to be increasing from 2017 – 2019. This is uncertain due to the presence of an extreme value (Kd = 7139) in a small dataset (n = 3) in 2019.

A more refined species-specific approach applying more toxicological knowledge about the resident fish community could allow for examination of individual Kds using Table 10. Because we know that applying the 8.5 mg/kg dw threshold is overprotective for a number of species, I substituted the actual available toxicity data for resident species and their surrogates using data and information from EPA, 2016, DeForest, 2012, and Teck, personal communication (See attached tables, Table 10 SSC Comparison). For the species with no toxicity data (pikeminnow, yellow perch, burbot, and peamouth),

the whole-body threshold of 8.5 mg/kg dw was used as a default. The default TTF of 2.8 was used for invertebrates, and assumed TTFs were used for each fish species, however a TTF of 1.7 was used for burbot. Then, centiles of the Kd distribution (median, 60th, 70th, 80th, and 90th) were selected to represent a "steady state" exposure at the base of the food web throughout the lake. This provided a species-specific water threshold corresponding to each whole-body value from the toxicity database (n = 12 species). The median, 40th, 30th, 20th, and 10th centile values of the distributions are displayed below.

Table 4. Site Specific Water Thresholds (ug/L) for Fish Species Assemblage in Lake Koocanusa

	Lake Koocanusa Kd				
Centile	**Median**	**60th**	**70th**	**80th**	**90th**
	4547	**4788**	**5090**	**5569**	**6311**
Median	1.32	1.25	1.18	1.08	0.95
40th centile	1.16	1.11	1.04	0.95	0.84
30th centile	1.03	0.97	0.92	0.84	0.74
20th centile	0.83	0.78	0.74	0.67	0.59
10th centile	0.76	0.73	0.68	0.62	0.55

Observations:

- The maximum possible water value that might be considered protective of the fish assemblage at Lake Koocanusa is 1.32 ug/L, lower than the EPA national default lentic concentration of 1.5 ug/L.
- Approximately 50% of the water values are above 0.9 ug/L, including a water value representing a 90th centile of potential exposure based on Kd (0.95 ug/L).
- Based on the overall distribution of Kds in the dataset, the selection of a Kd between the 60th and 80th centile would provide robust temporal and spatial coverage for of the Kd distribution collected from the lake between 2015 and 2019

Sources of uncertainty related to various aspects of the toxicity dataset:

- The default WB EC10s for four species with no toxicity data.
- The median CF (EPA 2016) applied to species vs site specific CFs

The sensitivity of the untested species will likely have the largest impact on the range of protective values for species in Lake Koocanusa, so this could influence the selection of protective water value. If one or more of the untested species are tested and more sensitive, selection of a lower water value from the distribution may be more defensible particularly if this species is highly exposed to a food web with high bioaccumulation potential.

Recommendation: Selection of water values should be based on Kd, (either selection of a protective centile of the range of water threshold outputs in the IFM/TFM models or consideration of a conservative Kd based and considering the sensitivity of the species assemblage.

4. Trophic Transfer Factor (TTF)

Due to the paucity of insect data collected, and the lack of concurrently collected particulate data, USGS selected TTFs derived and used in previous studies (Presser & Luoma 2010).

The mean "all insect" TTF (2.8) that USGS is using to model Lake Koocanusa is composed of:

mayfly, caddisfly, cranefly, stonefly, damselfly, corixid (waterboatmen), and chironomid (midge)
The median "all insect" TTF used in EPA 2016 is composed of:
mayfly (match), Diptera (cranefly surrogate – match?), damselfly (match) dragonfly, waterboatmen (match), and chironomid (midge) - match

Both the P&L 2010 and EPA 2016 datasets have significant (4/7) taxonomic overlap.

The USGS model uses the mean as its central tendency estimate, whereas the EPA 2016 derivation uses the median ratio. The median ratio is not influenced by extreme values. For example, the median TTF for the waterboatmen dataset (n = 29) is 1.48, whereas the mean is 57% higher (2.32). The range of the distribution of 28 TTFs values is 0.139 – 5.62, with one extreme value, 21.0. If this extreme value was excluded, then the mean of the distribution would be 1.65, similar to the median.

Another uncertainty is the [Se] and TTFs associated with terrestrial insects. The Lotic Environmental food web report indicated that insectivorous fish species and lifestages consume a substantial proportion of terrestrial insects. For example, trout consumed between 40-50% of terrestrial insects between April and November (Table A.3.5 from Dalbey, 1996). These insect's exposure to Se is uncertain and is likely a product of local site-specific terrestrial conditions due to most insects small home range. For example, terrestrial insects from riparian habitats near the Elk River likely have higher [Se] than terrestrial insects inhabiting nearshore areas of the forebay. This makes prediction of TTFs for insectivores more complex.

Recommendation: Combine the P&L 2010, and the EPA 2016 TTF datasets to maximize the available TTF data, producing a more robust dataset from which to derive a central tendency estimate. Examine TTF distributions and select the most appropriate central tendency distribution on a species-specific basis. I Use of the median (vs the mean) of the more robust combined datasets avoids conducting additional analyses to determine censorship of extreme values (outliers).
The EPA TTF dataset (EPA 2016) has been provided to the SeTSC co-chairs upon their request.

5. Protection of Downstream Uses:
A key principle of the modeling effort is to ensure that the site-specific criterion adopted for Lake Koocanusa will protect downstream uses including protection of the endangered Kootenai River white sturgeon. This is consistent with the regulatory requirements of U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

The available data for eggs collected from white sturgeon in the Kootenai River from 2015 – 2019 indicate moderate elevation of [Se]. The maximum [Se] observed in the dataset was 5.76 mg/kg dw roughly 63% lower than the white sturgeon EC10 from Linville, 2006 (EPA, 2016).

Table 5. Selenium from white sturgeon eggs sampled in Kootenai River.

	2015	2016	2017	2018	2019
average	4.2	3.3	4.1	3.9	4.2
75th centile	4.7	3.4	4.7	4.2	4.5
95th centile	5.3	3.4	5.6	4.8	5.3

Based on current conditions, selenium concentrations in sturgeon eggs do not show an increasing trend between 2015 and 2019, however water thresholds adopted in Lake Koocanusa should ensure that this

trend does not increase over time. An important data gap is the loading of selenium (both particulate and total, and its fate in the downstream Kootenai River. Continued monitoring of sturgeon should continue to enable early detection of increasing selenium in mature eggs to insure protection of this important species. Future understanding of selenium loading may allow for refinement of the Lake Koocanusa SS water threshold, as well as water quality standards proposed for the Kootenai River by Montana.

Overall Recommendation for adoption of a water column threshold for Lake Koocanusa:
I believe that the SSC water value should strike a balance between protection of the fish assemblage in Lake Koocanusa and downstream uses (protection of white sturgeon) based on current conditions, and the opportunity to refine regulatory thresholds in the future based on future monitoring actions targeted on refining the USGS model.

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MEMORANDUM

To: Lauren Sullivan (MT DEQ) and Sheldon Reddekopp (BC ENV)

From: David DeForest

Subject: Comments on Koocanusa Reservoir Selenium Modeling and Recommendations for Site-specific Selenium Criteria

Date: August 28, 2020

This memorandum provides my comments on the Koocanusa Reservoir selenium model developed by the U.S. Geological Survey (Presser and Naftz 2020) and my recommendations for site-specific selenium criteria. Based on review of the U.S. Geological Survey (USGS) model and other lines of evidence, including empirical monitoring data for the reservoir, it is my opinion that the U.S. Environmental Protection Agency's (USEPA's) surface water quality criterion of 1.5 µg/L for lentic water bodies is protective of fish and the aquatic community in Koocanusa Reservoir. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessment. The following summarizes the lines of evidence that support this conclusion.

1 Selenium - Fate and Effects in Freshwater Aquatic Systems

There is scientific consensus that selenium concentrations in fish tissue, and particularly in fish eggs, are the strongest indicator of potential selenium toxicity to fish (Chapman et al. 2010). Fish primarily bioaccumulate selenium from their diet in the form of organic selenium. Selenium bioaccumulated by females is maternally transferred to ovaries and eggs. Adult fish are insensitive to selenium, but if concentrations are sufficiently high in the eggs, selenium may cause mortality, deformities, or edema in developing larvae as the yolk sac is absorbed (Janz et al. 2010).

Selenium is typically mobilized or released into surface waters, from both natural and anthropogenic sources, as inorganic selenium (typically selenate or selenite) (Maher et al. 2010). Inorganic selenium species are taken up at the base of the food web (e.g., algae), and transformed to organic selenium species. Site-specific surface water characteristics have a substantial influence on selenium speciation and bioaccumulation potential (Stewart et al. 2010). For example, a lentic (standing) water body with high biological

productivity, a long retention time, and strong reducing conditions has a greater selenium bioaccumulation potential then a lotic (flowing) water body with low biological productivity, a short retention time, and oxic conditions. This means that surface water selenium concentrations that may reach a toxic concentration in fish eggs may range by two orders of magnitude or more among sites.

1.1 USEPA Selenium Criteria

Based on selenium's fate and effects in aquatic systems, the USEPA derived ambient water quality criteria for selenium with the following hierarchy (USEPA 2016):

1. Fish egg selenium concentration of 15.1 mg/kg dry weight (dw)
2. Adult fish muscle selenium concentration of 11.3 mg/kg dw or adult fish whole body selenium concentration of 8.5 mg/kg dw
3. Surface water selenium concentration of 1.5 µg/L for lentic waters or 3.1 µg/L for lotic waters

The fish egg (or ripe ovary) selenium criterion of 15.1 mg/kg dw supersedes (is given priority over) the selenium criteria for muscle or whole body tissue and for surface water concentrations.

1.1.1 Fish Egg Selenium Criterion

The USEPA fish egg selenium criterion is based on maternal transfer studies in which parent females were exposed to diet-borne organic selenium in the laboratory or naturally exposed to diet-borne organic selenium in the field. Larval survival and development were assessed in the offspring of exposed parent females and selenium EC10s (10% effect concentrations) calculated based on the egg selenium concentration.

The USEPA's egg selenium criterion is based on genus mean EC10s for eight different genera. White sturgeon (*Acipenser transmontanus*) has the lowest EC10, which is 15.6 mg/kg dw. The EC10s for the remaining seven genera used to derive the USEPA's egg selenium criterion range from 20.6 mg/kg dw for bluegill (*Lepomis macrochirus*) to 56.2 mg/kg dw for Dolly Varden (*Salvelinus malma*). The fish egg selenium criterion of 15.1 mg/kg dw is based on the 5th percentile of the genus sensitivity distribution, which is extrapolated to a concentration less than the white sturgeon EC10 (Figure 1).

1.1.2 Fish Muscle and Whole Body Selenium Criteria

Muscle and whole body selenium criteria were developed using the same approach as for the egg selenium criterion. The USEPA compiled EC10s based on muscle or whole body selenium concentrations in parent females from maternal transfer studies. Direct measures of muscle and whole body selenium concentrations were used to calculate EC10s when reported. When necessary, egg-to-muscle or egg-to-whole body conversion factors were applied to estimate muscle or whole body selenium EC10s. As for the egg selenium criterion, the muscle and the whole body selenium criteria were calculated

based on the 5th percentile genus mean EC10s, and both criteria were extrapolated to concentrations less than the EC10 of white sturgeon (Figures 2 and 3).

1.1.3 Surface Water Selenium Criterion

The USEPA surface water criterion was developed to ensure protection of fish and the aquatic community for sites with high selenium bioaccumulation potential. This means that the surface water criterion may be conservative for sites with moderate to low selenium bioaccumulation potential. Simply put, the surface water selenium criterion was back-calculated from the egg criterion, which is driven by the sensitivity of white sturgeon, based on sites with high selenium bioaccumulation potential.

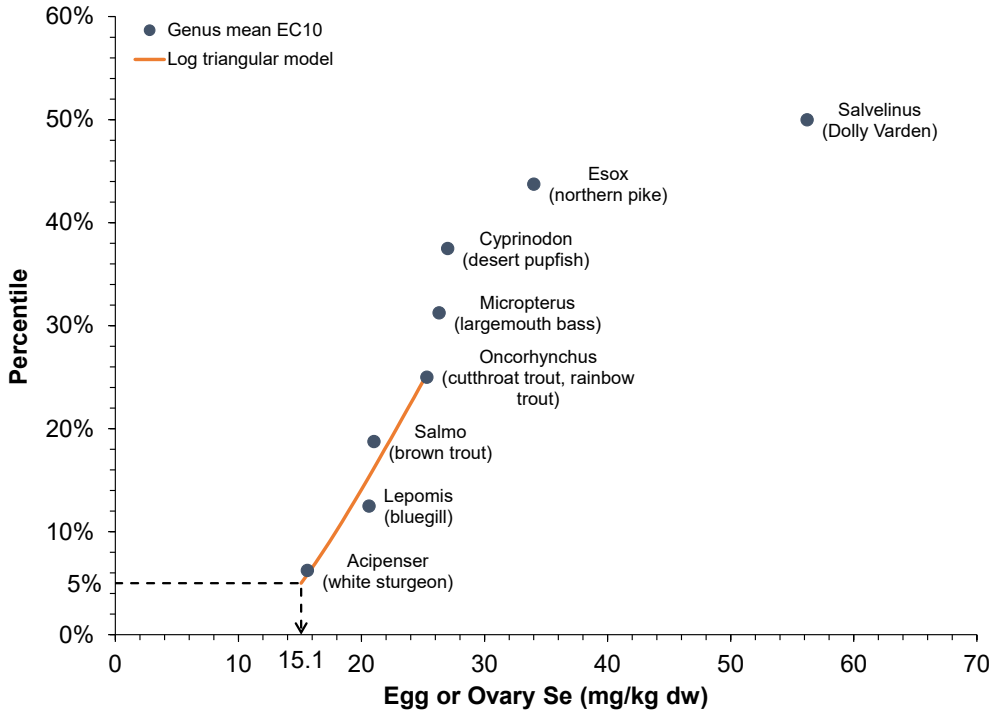

Figure 1. Derivation of the USEPA's egg/ovary selenium criterion of 15.1 mg/kg dw.
Note: Blue symbols are genus mean EC10s with the labels identifying the genus and, in parentheses, the species that comprise each genus mean EC10 (with the exception of two trout species comprising *Oncorhynchus*, all other genus mean EC10s are comprised of a single fish species). The orange curve is the log triangular distribution model that the USEPA traditionally uses to calculate the 5th percentile of the genus sensitivity distribution for criteria development. This model is fit to only the four most sensitive genera. Although the total number of genus mean EC10s is eight, the total sample size is based on an n of 15, which is why the percentiles on the y-axis do not extend to 100%. The sample size of 15 accounts for less sensitive fish genera for which definitive EC10s could not be calculated and less sensitive invertebrates.

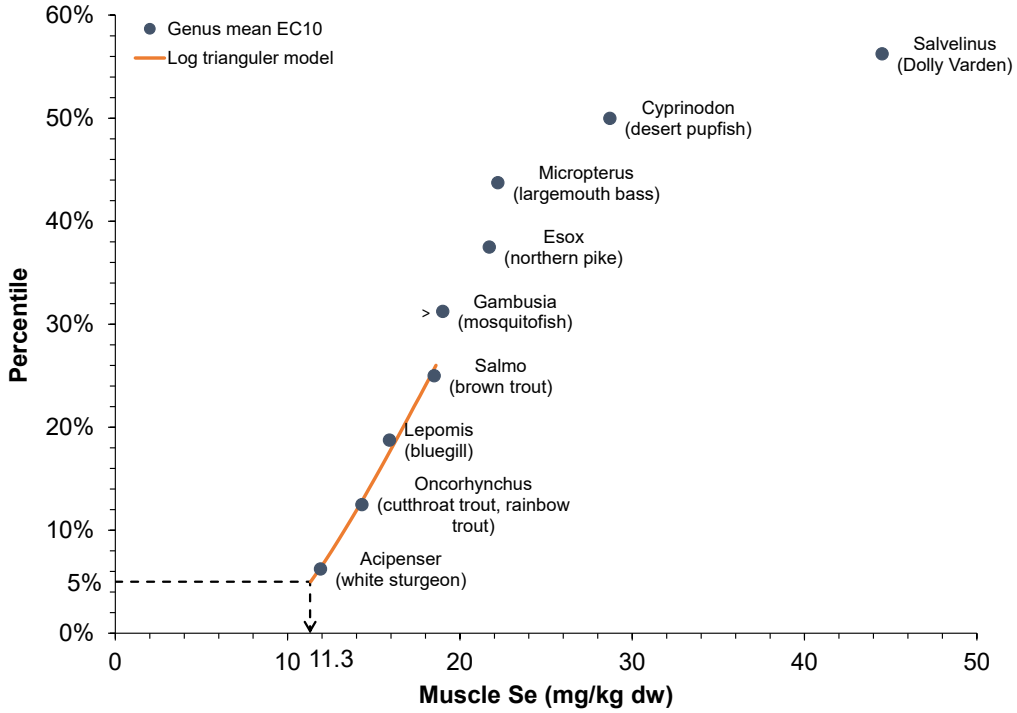

Figure 2. Derivation of the USEPA's muscle selenium criterion of 11.3 mg/kg dw.
Note: See Figure 1 for description.

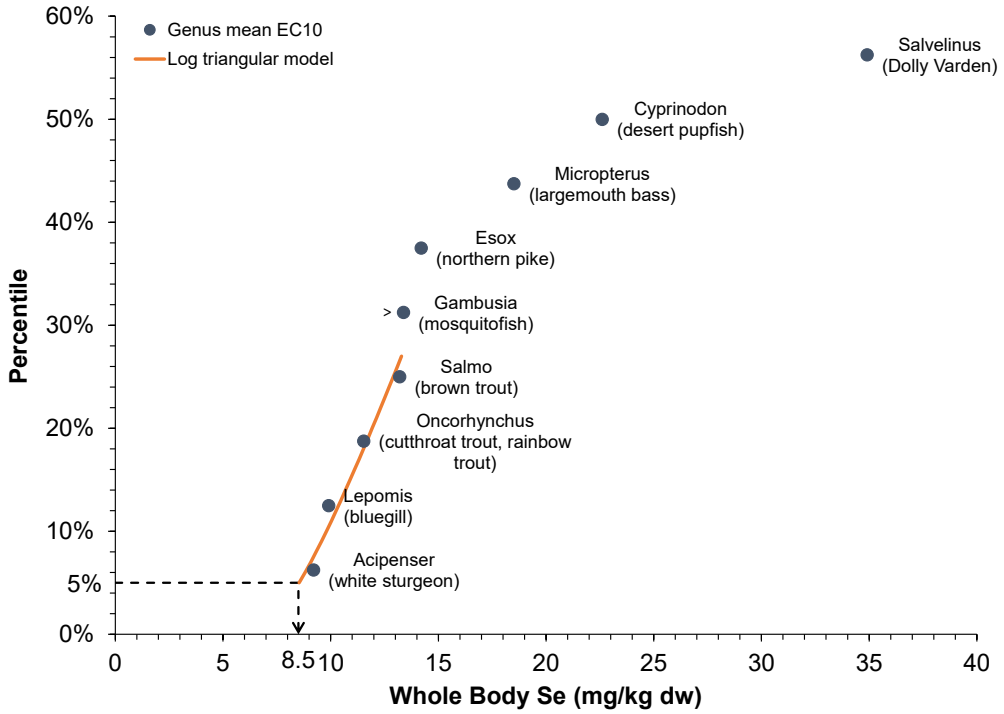

Figure 3. Derivation of the USEPA's whole body selenium criterion of 8.5 mg/kg dw.
Note: See Figure 1 for description.

1.2 BC Environment Selenium Guidelines

1.2.1 Fish Egg Selenium Guideline

BC Environment compiled selenium toxicity data for a variety of fish species, including many of the same species considered by the USEPA in deriving its selenium guideline (BCMOE 2014). As outlined within the guideline, it was determined that toxicity data for the genus *Oncorhynchus* would be a sensitive surrogate for fish in BC. Egg selenium EC10s of 22.05 and 21.97 mg/kg dw for rainbow trout and westslope cutthroat trout were compiled. An uncertainty factor of 2 was applied to each of these, which resulted in a mean of 11 mg/kg dw. The basis for the uncertainty factor of 2 was described as "a value that meets the balance between adequacy and protection, while addressing the inherent uncertainties in published toxicity threshold estimates." However, the basis for determining that it meets this balance was not described. Selenium data for comparison to the guideline should be based on the mean concentration of at least eight samples (eggs or ripe ovary from 8 individual females) (BCMOE 2014).

1.2.2 Fish Muscle and Whole Body Guideline

BC Environment derived a whole body fish selenium guideline of 4 mg/kg dw, which was developed by weighting literature-based evidence and the mean of published effects data for multiple species with an uncertainty factor of 2 (BCMOE 2014). BC Environment similarly derived an interim muscle selenium guideline of 4 mg/kg dw, which was based on low effect concentrations for rainbow trout, brown trout, and bluegill. However, the guideline was defined as interim due to uncertainty and limited primary toxicity data in their review. As for egg selenium, collection of selenium data for comparison to the whole body and muscle guidelines should be based on the mean concentration of at least eight whole body or muscle samples (BCMOE 2014).

1.2.3 Surface Water Selenium Guideline

The BC surface water selenium guideline of 2 µg/L was based on several studies that had lowest observed effect concentrations that converged around a water selenium concentration of 10 µg/L—this concentration was divided by an uncertainty factor of 5 to derive the guideline of 2 µg/L (BCMOE 2014). An alert guideline of 1 µg/L was also derived based on evidence from some studies that concentrations above this could pose a risk to aquatic life (BCMOE 2014). The water selenium concentration measured for comparison to the alert concentration and the guideline should be the mean of five evenly spaced samples over 30 days (BCMOE 2014).

1.3 USEPA Selenium Criteria are Protective of Fish

The USEPA's egg selenium criterion of 15.1 mg/kg dw is 1.4-times greater than the BC egg selenium guideline of 11 mg/kg dw, and the USEPA's muscle and whole body selenium criteria of 11.3 and 8.5 mg/kg dw are 2.8-times and 2.1-times greater, respectively, than BC's muscle and whole body guidelines of 4 mg/kg dw. The USEPA's lentic water criterion of 1.5 µg/L falls between the BC alert concentration of 1 µg/L and

guideline of 2 µg/L. The BC fish tissue guidelines are less than the USEPA's fish tissue criteria, but this difference is driven by the applied uncertainty factor of 2. In my opinion, the uncertainty factor of 2 is overly conservative and not supported by the science. As such, USEPA's fish tissue-based selenium criteria are protective of fish.

As previously noted, USEPA's fish tissue-based selenium criteria are based on the 5th percentile of genus mean EC10s (which are less than the EC10 for the most sensitive species, white sturgeon). This is a conservative approach for selenium criteria development for several reasons:

- First, EC10s cannot literally be considered 10% effect concentrations. This is because EC10s often fall within the statistical "noise" of selenium toxic effect thresholds. Most concentration-response data are inadequate to calculate concentrations associated with extremely low effects (e.g., EC0 or EC1) without having extremely high uncertainty (USEPA 2015). More importantly, very low effect concentrations are often indistinguishable from natural biological variability in data used to develop the concentration-response relationship. Concentration-response data for sensitive coldwater fish species are provided in Figure 4, which show that the USEPA's ovary selenium criterion of 15.1 mg/kg dw falls within the variability of responses at the egg selenium concentrations associated with the "flat" region of the concentration-response curve. As such, the USEPA's egg selenium criterion of 15.1 mg/kg dw and BC egg guideline of 11 mg/kg dw are equally protective.

  Another way to demonstrate this is to plot the concentration-response data for the most sensitive fish species in the USEPA's ambient water quality criteria document (USEPA 2016). Concentration-response models were fit using USEPA's Toxicity Relationship Analysis Program (TRAP) (USEPA 2015), which the USEPA used in developing its selenium criteria. TRAP defines the "control value" as Y0, which is the plateau in the concentration-response relationship before an inflection point indicating an adverse response is detected and then increasing levels of effect are fit by the model. Concentration-response data are normalized for their respective Y0 value so that data from multiple species and tests can be plotted together, as shown in Figure 5. Individual data points are given open symbols to denote those treatments that comprised Y0 (i.e., considered part of the control population) and filled symbols denote those treatments not considered part of the control population. As shown in Figure 5, the USEPA's egg selenium criterion of 15.1 mg/kg dw (i.e., the 5th percentile of genus mean EC10s) falls within the grouping of open symbols, which shows that use of EC10s to derive criteria are within the "noise" of no-effect concentrations.

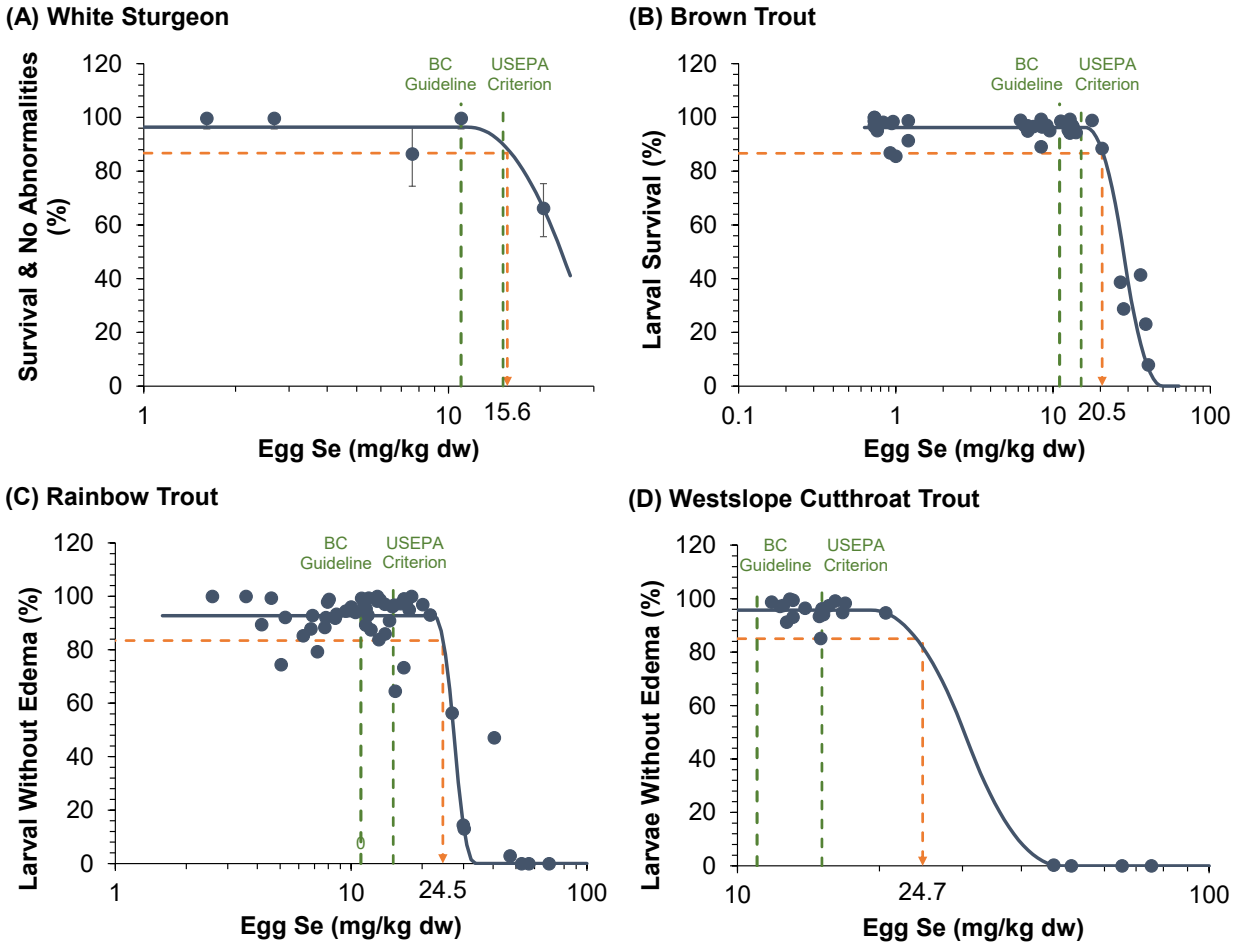

Figure 4. Examples of concentration-response relationships for (A) white sturgeon; (B) brown trout; (C) rainbow trout; and (D) westslope cutthroat trout.
Note: Orange line denotes the EC10, which falls within the variability of the biological response at low egg selenium concentrations. Green dashed lines denote the USEPA's chronic egg/ovary selenium criterion of 15.1 mg/kg dw and the BC chronic egg/ovary selenium guideline of 11 mg/kg dw.

- Second, with the exception of white sturgeon, all egg selenium EC10s compiled in the USEPA's selenium criteria document are ≥20.6 mg/kg dw. The USEPA (2016) egg selenium toxicity data for criteria development, along with data for additional species, are plotted in Figure 6 (sources of data are provided in Table 1). As shown, the first fish species for which egg selenium thresholds could be calculated were fathead minnow and bluegill in the early 1990s. As additional species have been tested, only one (white sturgeon) has been identified as being more sensitive than bluegill. In fact, there tends to be an increasing pattern of less sensitive species being tested with time. This suggests the likelihood of identifying species that are more sensitive than the most sensitive species tested to date is low.

Based on the above, the remainder of my comments focus on evaluations relative to the USEPA's fish selenium criteria.

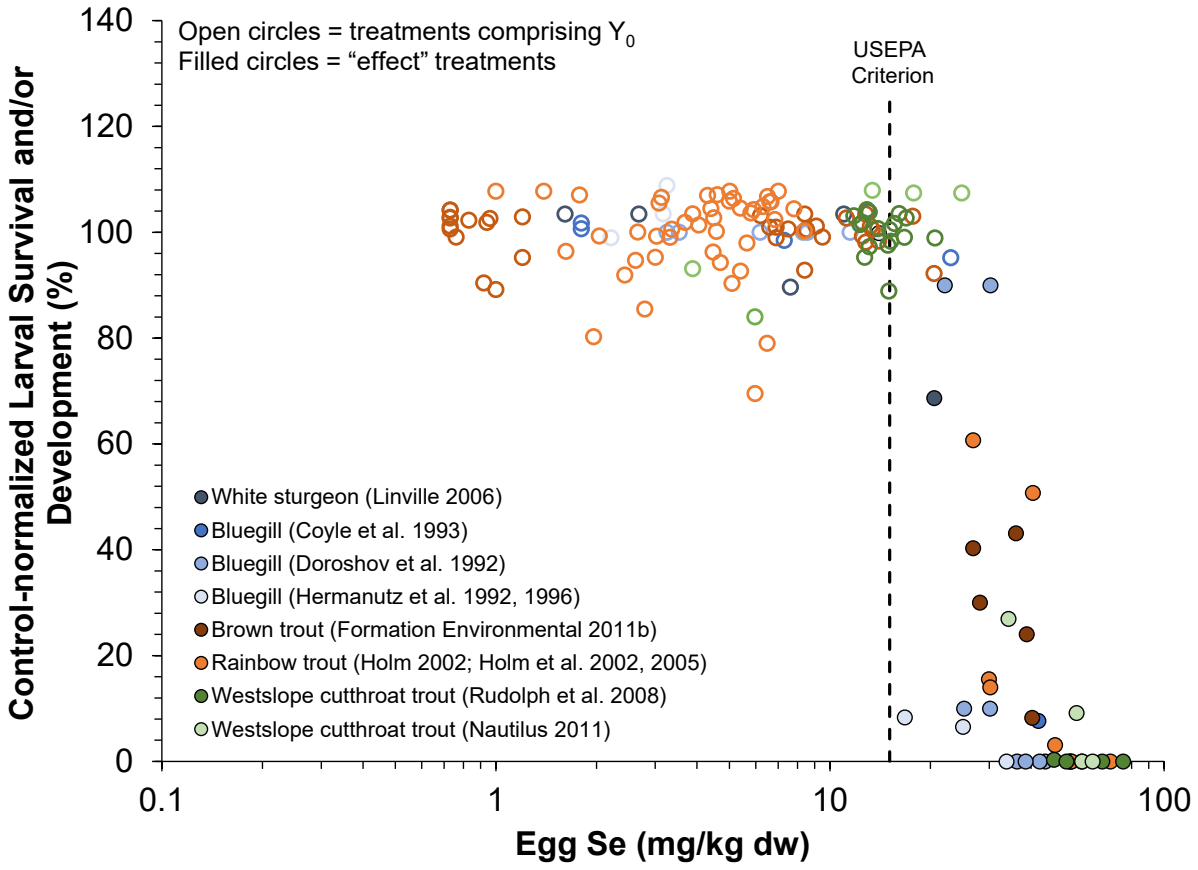

Figure 5. Concentration-response data for the four most sensitive genera in the USEPA's ambient water quality criteria document for selenium (USEPA 2016): (1) *Acipenser* (white sturgeon); (2) *Lepomis* (bluegill); (3) *Salmo* (brown trout); and (4) *Oncorhynchus* (rainbow trout, westslope cutthroat trout).
Note: Open symbols denote treatments that comprised Y0 (i.e., considered part of the control or reference population) and filled symbols denote those treatments that do not comprise Y0.

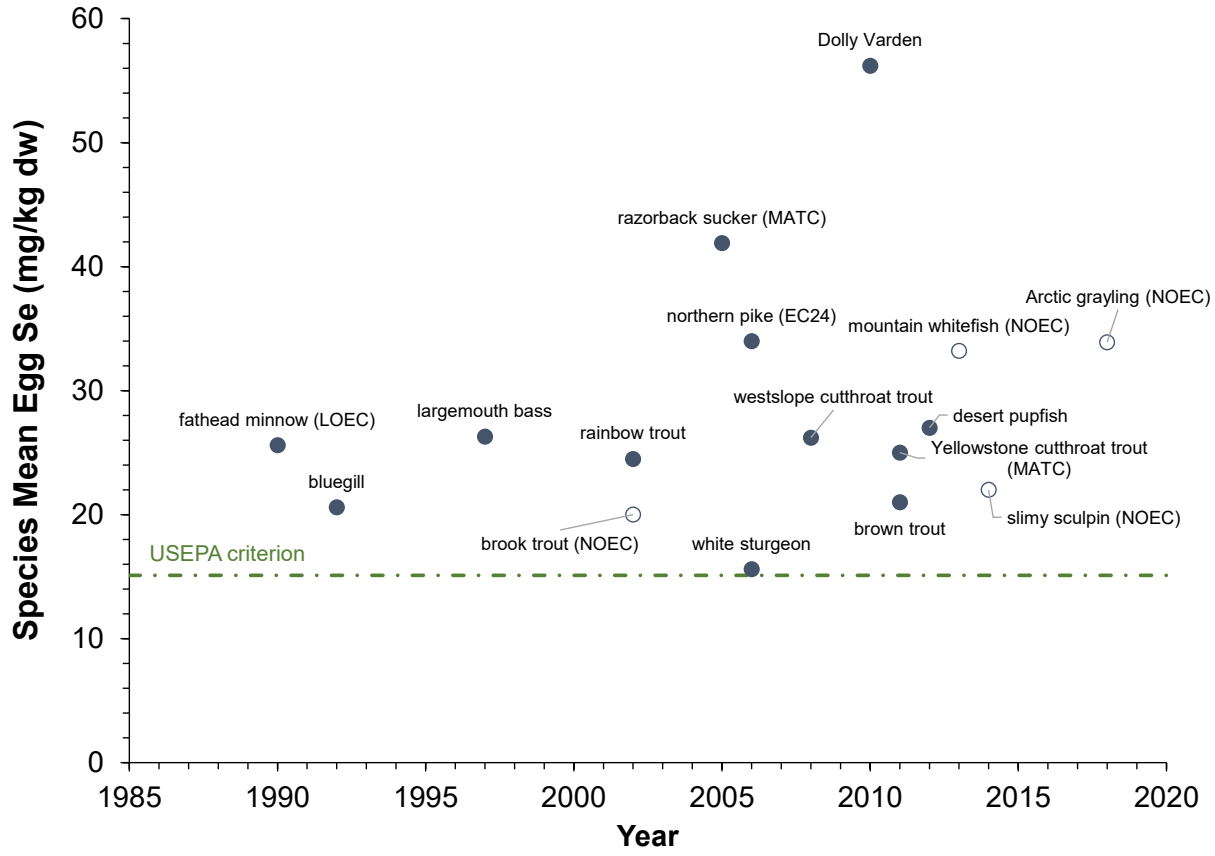

Figure 6. Relationship between fish egg selenium EC10s (and other toxicity thresholds) as a function of the year published or reported.
Note: Open symbols denote no-observed-effect concentrations (NOECs) from tests in which EC10s could not be estimated.

Table 1. Egg/ripe ovary selenium toxicity thresholds for North American freshwater fish.

Species	Statistical Endpoint	Species Mean Egg or Ovary Se (mg/kg dw)	Genus Mean Egg or Ovary Se (mg/kg dw)	Original Study Reference	Source of Toxicity Value Selected[1]
Salvelinus malma (Dolly Varden)	EC10	56.2	56.2[a]	McDonald et al. 2010	USEPA 2016
Salvelinus fontinalis (brook trout)	NOEC	>20		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Xyrauchen texanus (razorback sucker)	MATC	41.9	41.9	Hamilton et al. 2005a,b	DeForest et al. 2012
Esox lucius (northern pike)	EC24	34	34	Muscatello et al. 2006	USEPA 2016
Thymallus arcticus (Arctic grayling)	NOEC	>33.9	>33.9	Windward et al. 2018	Windward et al. 2018
Prosopium williamsoni (mountain whitefish)	NOEC	>33.2	>33.2	Nautilus 2013	Nautilus 2013
Cyprinodon macularius (desert pupfish)	EC10	27	27	Besser et al. 2012	USEPA 2016
Micropterus salmoides (largemouth bass)	EC10	26.3	26.3	Carolina Light and Power 1997	USEPA 2016
Pimephales promelas (fathead minnow)	LOEC	<25.6	<25.6	Schultz and Hermanutz 1990	USEPA 2016
Oncorhynchus clarkii lewisi (westslope cutthroat trout)	EC10	26.2	25.2[b]	Rudolph et al. 2008; Nautilus 2011	USEPA 2016
Oncorhynchus clarkii bouvieri (Yellowstone cutthroat trout)	MATC	25		Formation Environmental 2011a	DeForest et al. 2012
Oncorhynchus mykiss (rainbow trout)	EC10	24.5		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Cottus cognatus (slimy sculpin)	NOEC	>22	>22	Lo et al. 2014	Lo et al. 2014
Salmo trutta (brown trout)	EC10	21	21	Formation Environmental 2011b	USEPA 2016
Lepomis macrochirus (bluegill)	EC10	20.6	20.6	Doroshov et al. 1992; Coyle et al. 1993; Hermanutz et al. 1992, 1996	USEPA 2016
Acipenser transmontanus (white sturgeon)	EC10	15.6	15.6	Linville 2006	USEPA 2016

[1] EC10 values (or alternative statistical endpoints) were not always provided in the original study source, so source of value is provided.
[a] The genus mean value for *Salvelinus* was set equal to the EC10 for Dolly Varden, as no effects were observed in brook trout at the highest concentration tested.
[b] Although the statistical endpoint for Yellowstone cutthroat trout was an MATC, it was geometrically averaged with the EC10 values for westslope cutthroat trout and rainbow trout because values for all three species were similar.
EC10 = 10% effect concentration
EC24 = 24% effect concentration
NOEC = no-observed-effect concentration
LOEC = lowest-observed-effect concentration
MATC = maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC)

2 Empirical Fish Tissue Selenium Data for Koocanusa Reservoir

This section compares empirical fish selenium concentrations to the USEPA's egg, muscle, and whole body selenium criteria. Data for non-Cyprinids and Cyprinids are discussed separately in Sections 2.1 and 2.2, respectively.

2.1 Non-Cyprinids

Out of more than 1,200 individual samples of non-cyprinid fish species in the reservoir, there have been only three measurements (0.2%) that exceeded criteria. These include two ovary selenium measurements (Figure 7) and one muscle selenium measurement (Figure 8). No exceedances of the whole body selenium criterion have been observed (Figure 9). For each of the aforementioned individual selenium criteria exceedances, mean selenium concentrations from other samples of the same species, from the same location and time did not exceed criteria:

- Rainbow trout ovary Se concentration of 19.8 mg/kg dw
  - Mean of 14.0 mg/kg dw for sampling location and time (n = 2) and less than the rainbow trout-specific EC10 of 24.5 mg/kg dw
- Longnose sucker ovary Se concentration of 21 mg/kg dw
  - Mean of 12.8 mg/kg dw for sampling location and time (n = 3)
- Yellow perch muscle Se concentration of 15 mg/kg dw
  - Mean of 4.6 mg/kg dw for sampling location and time (n = 10)

Consequently, empirical monitoring of selenium concentrations in non-cyprinids supports that these species are not adversely impacted by selenium in the reservoir.

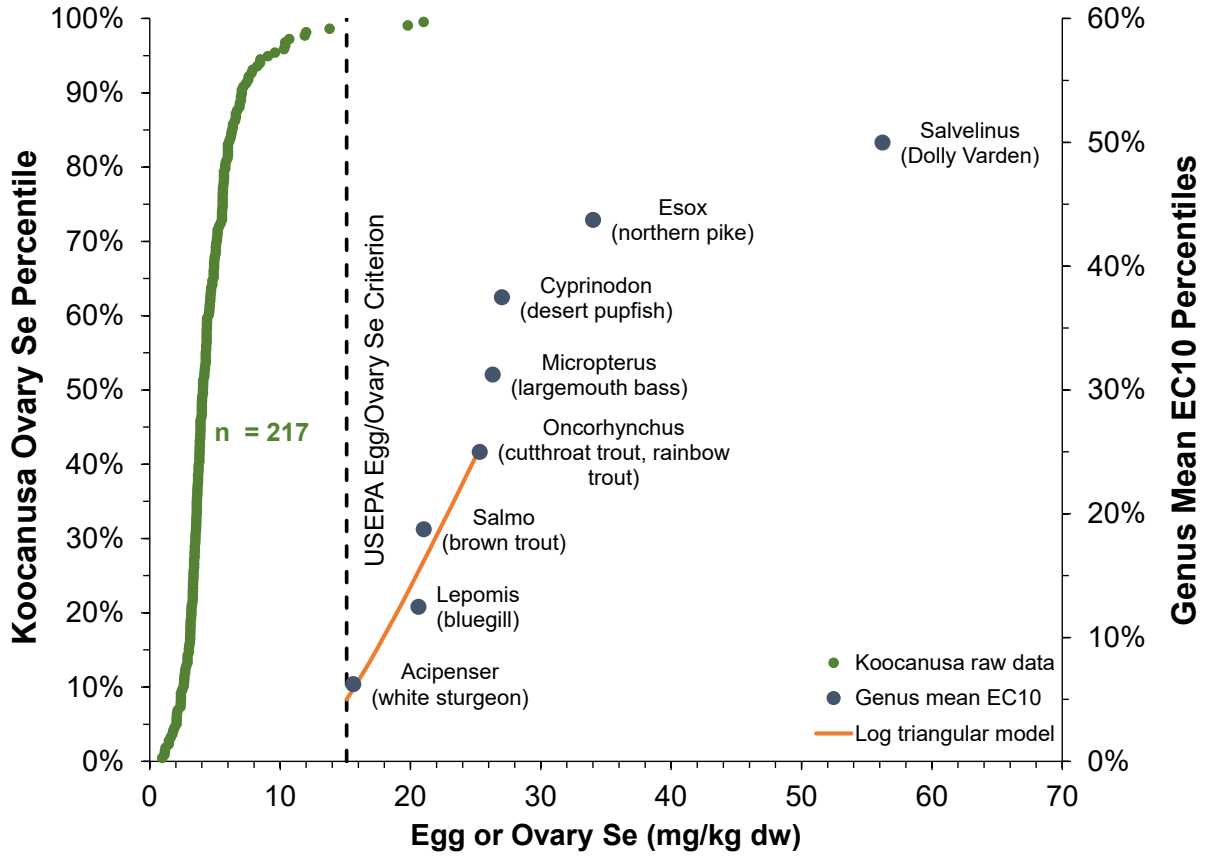

Figure 7. Cumulative distribution of empirical selenium concentrations in fish ovary samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of egg/ovary selenium concentrations used to derive the USEPA egg/ovary selenium criterion.

Note: Ovary selenium data for northern pikeminnow, peamouth chub, and redside shiner are excluded (see Section 2.2 for discussion of these cyprinids).

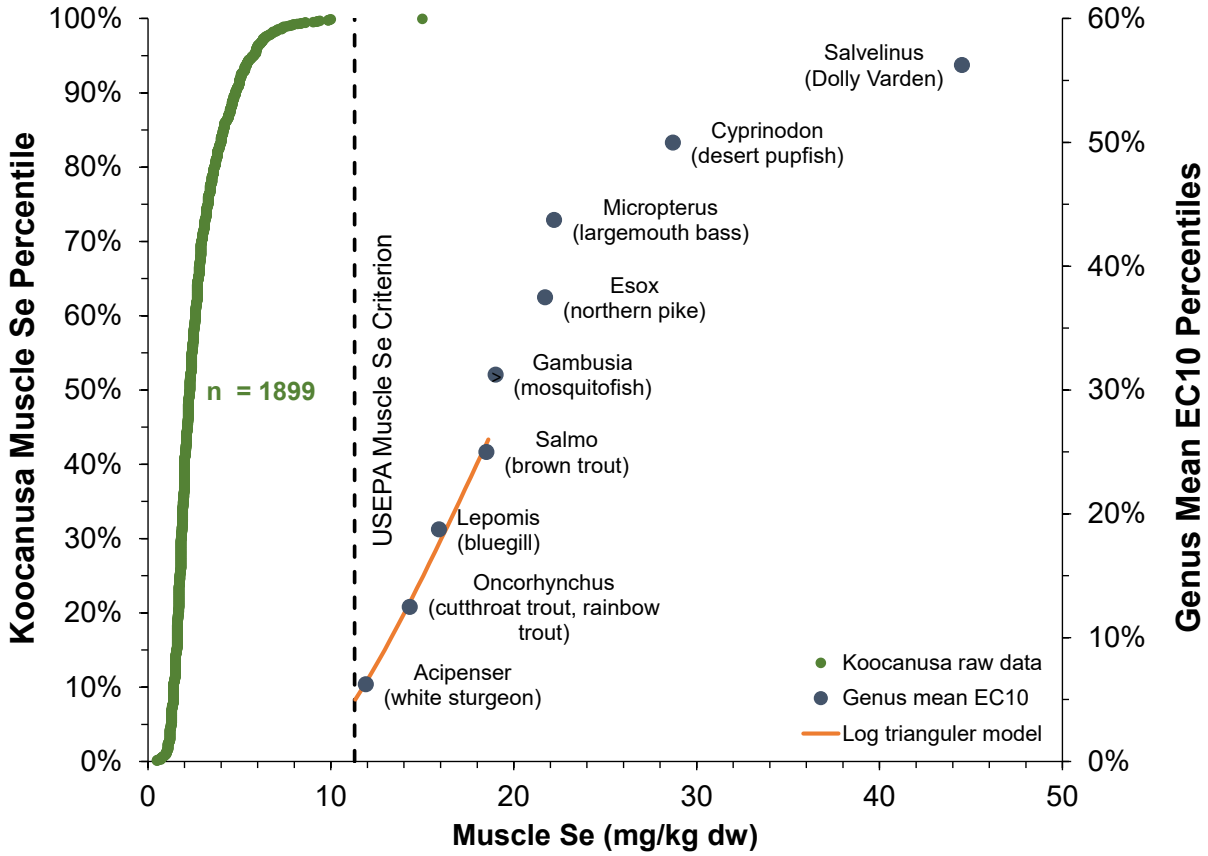

Figure 8. Cumulative distribution of empirical selenium concentrations in fish muscle samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of muscle selenium concentrations used to derive the USEPA muscle selenium criterion.

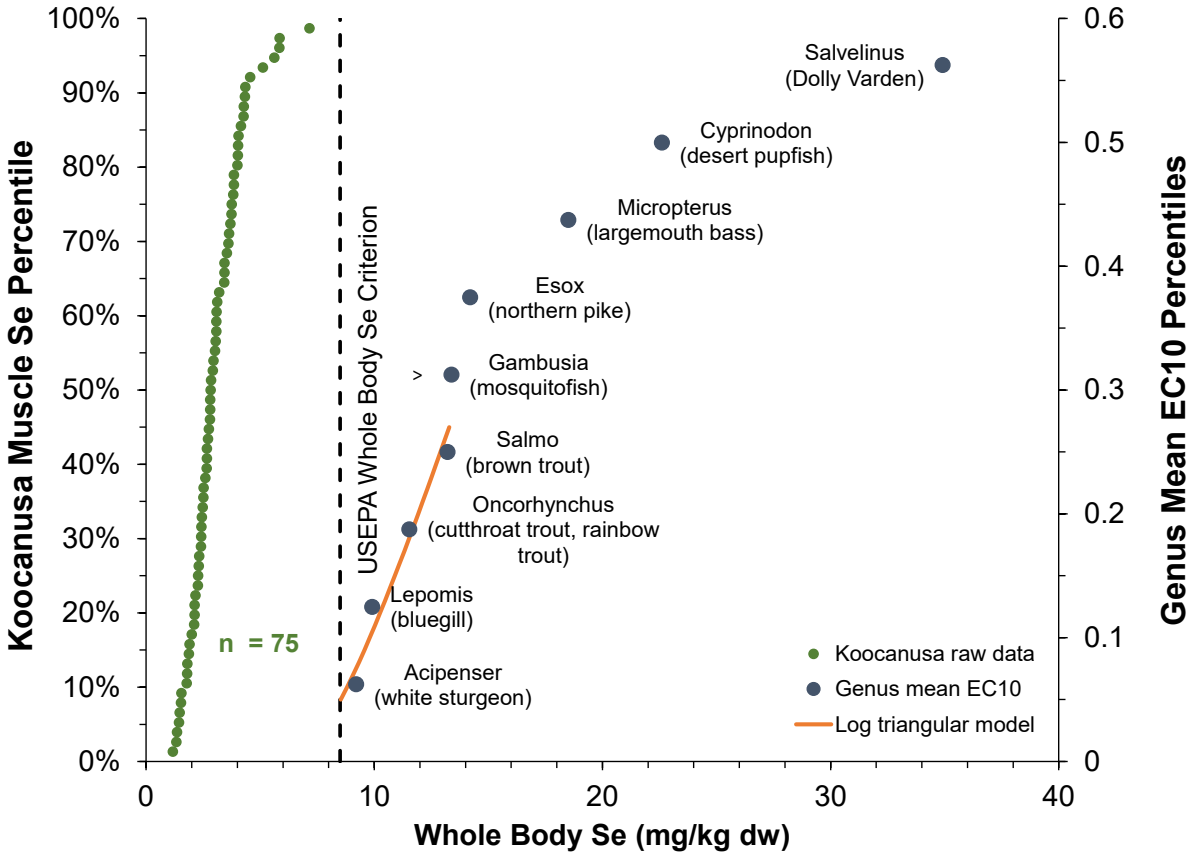

Figure 9. Cumulative distribution of empirical selenium concentrations in whole body fish samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of whole body selenium concentrations used to derive the USEPA whole body selenium criterion.

2.2 Cyprinids

The three cyprinids in Koocanusa Reservoir are northern pikeminnow, peamouth chub, and redside shiner. Muscle and whole body selenium concentrations in these three species have never exceeded USEPA criteria. Ovary selenium concentrations for these species have exceeded the USEPA's egg selenium criterion but, as discussed further below, most of the ovary selenium data were not collected during spawning and concentrations are likely overestimated and should not be used. Further, the USEPA has compiled evidence that cyprinids are not uniquely sensitive to selenium.

2.2.1 Ovary Selenium Concentrations and Maturity

Based on routine monitoring of northern pikeminnow ovary selenium concentrations in Koocanusa Reservoir, it was previously observed that concentrations were higher and more variable in the BC portion of the reservoir, and lower and less variable on the Montana side. Further evaluation of these data revealed that fish collected in BC were

smaller than those collected in Montana, reflective of different sampling techniques. In addition, there was an indication that ovary selenium concentrations and the gonado-somatic index (GSI) were inversely related in northern pikeminnow collected in BC (GSI had not been measured in the northern pikeminnow collected in Montana). The GSI is a measure of ovary maturity, so ideally selenium would be analyzed in ovaries of fish with a GSI that is reflective of the fish's condition at the time of spawning.

These observations led to the northern pikeminnow study conducted in 2019, which included additional sampling at different times during the spawning period to collect fish with a range of sizes and GSI values. This report concluded that ovary selenium concentrations should not be used for assessing selenium risks to northern pikeminnow when the GSI is <5%, as selenium concentrations in the ovaries of these fish overestimate selenium concentrations that are relevant to the time of spawning (EcoTox et al. 2020). The study report was provided to the SeTSC and the study presented to the SeTSC by Dr. Brix in June 2020 (for completeness a copy the report is provided in Attachment 1).

A preliminary evaluation of ovary selenium concentrations and GSI for peamouth chub in Koocanusa Reservoir likewise indicates that there is a minimum GSI below which ovary selenium concentrations should not be used for assessing selenium risks. Based on a study of peamouth chub in the Columbia River (WA, USA), females had a mean GSI of about 8% during the period of spawning (Figure 10; Gray and Dauble 2001). Of the 153 peamouth chub ovary selenium concentrations measured in Koocanusa Reservoir, 29 (19%) exceed the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 11). Of those, the GSI was >8% in just three of the samples. Although this evaluation is not as robust or definitive as the northern pikeminnow study, it similarly highlights that future monitoring of selenium concentrations in peamouth chub should target spawning periods.

Although not a cyprinid, a recent evaluation of mountain whitefish data similarly found that ovary selenium concentrations are inversely related to GSI (Brix et al. 2020). Thus, there is an increasing body of information highlighting the importance of measuring selenium concentrations in ripe ovaries during spawning, as measurement of selenium in immature ovaries may overestimate selenium concentrations at spawning. The mountain whitefish evaluation is included as Attachment 2.

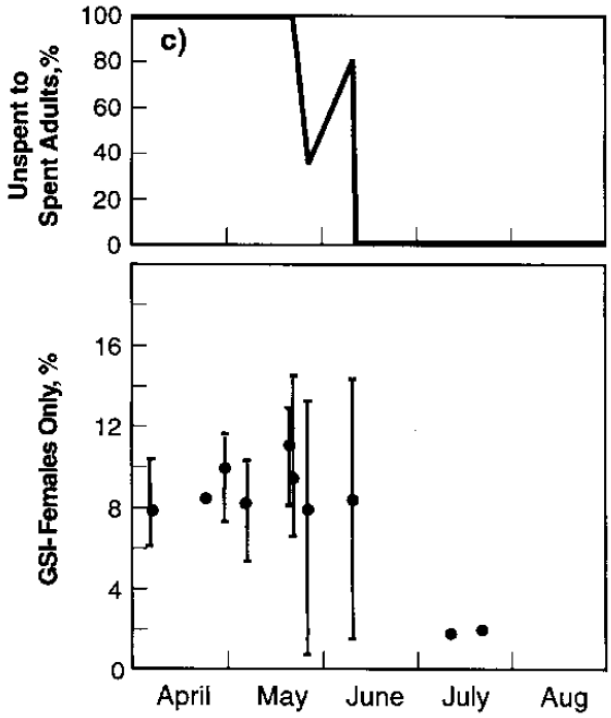

Figure 10. Spawning periods (top panel) and female GSI for peamouth chub in the Columbia River (WA, USA).
Note: Figure 3 in Gray and Dauble (2001).

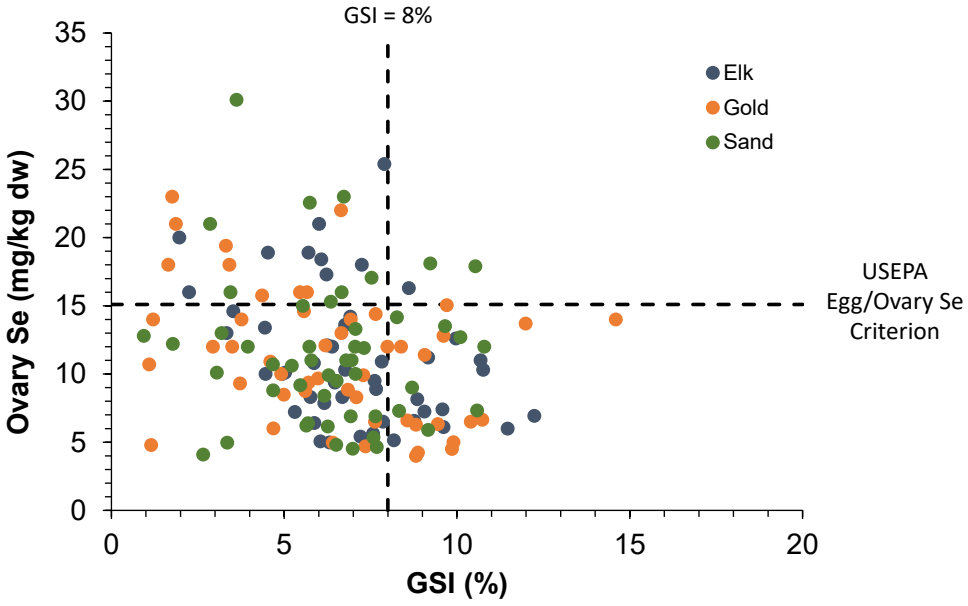

Figure 11. Relationship between ovary selenium concentrations in peamouth chub and GSI.

2.2.2 Conversion Factors for Ripe Ovaries

As noted in Presser and Naftz (2020), egg-ovary tissue can be substituted in the selenium model if the appropriate tissue-to-tissue conversion factors are available. Muscle-to-egg/ovary and whole body-to-egg/ovary conversion factors from USEPA (2016), for example, may be obtained for species of interest. Ideally, site-specific tissue-to-tissue conversion factors may be calculated. If site-specific muscle-to-ovary or whole body-to-ovary conversion factors are derived from Koocanusa Reservoir data, caution must be used to ensure that the ovary data used to calculate the conversion factors are based on ripe ovaries during the fish's spawning period. The following provides an example based on northern pikeminnow.

The northern pikeminnow study resulted in the conclusion that ovary selenium concentrations should not be used to assess selenium risk if the GSI is <5%. Based on this, I derived a site-specific northern pikeminnow muscle-to-ovary conversion factor from those muscle and ovary pairs from fish with a GSI >5% (Figure 12). Following USEPA (2016) methods, the conversion factor is calculated as the median, which is 2.5 for this dataset. This median muscle-to-ovary conversion factor of 2.5 was then applied to northern pikeminnow muscle data to estimate selenium concentrations in ripe ovaries representative of spawning conditions. Reservoir-wide annual mean estimates of selenium concentrations in ripe ovaries range from 3.3 to 7.7 mg/kg dw (Figure 13). Mean estimated selenium concentrations in ripe ovaries as a function of sampling location and sampling time range from 3.0 to 13.0 mg/kg dw, which are all still lower than the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 14). This northern pikeminnow example demonstrates that evaluation of selenium concentrations in ripe ovaries has an important influence in evaluating potential selenium-related impacts.

2.2.3 Cyprinid Sensitivity to Selenium

In developing its ambient water quality criteria for selenium, the USEPA conducted a review of cyprinid sensitivity based on a review of field and laboratory data (Appendix E in USEPA [2016]). Based on field studies of regions in the United States with elevated selenium concentrations, there was no evidence of selenium-related impacts and the USEPA concluded that "native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species." Based on these conclusions, the USEPA's egg selenium criterion, driven by the sensitivity of white sturgeon, is conservative for cyprinids. Current studies on the sensitivity of redside shiner to selenium, and planned studies on the sensitivity of northern pikeminnow to selenium,[1] will provide additional information on the sensitivity of cyprinids.

[1] The 2019 northern pikeminnow study was intended to also test its sensitivity to selenium, but an insufficient number of ripe females were captured from which eggs could be manually expressed.

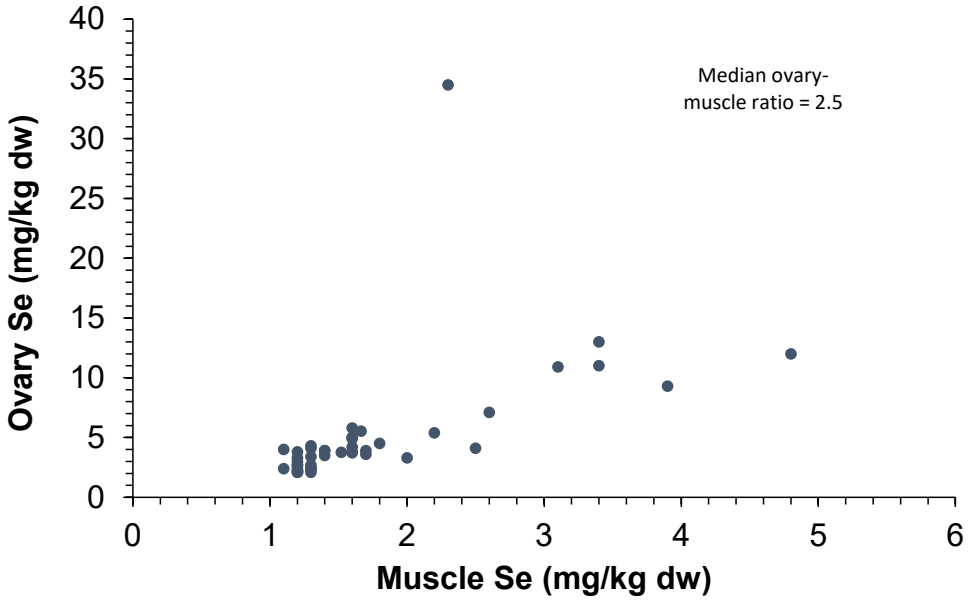

Figure 12. Site-specific relationship between ovary and muscle selenium in northern pikeminnow with a GSI >5%.
Note: The median ovary-muscle ratio is 2.5 with and without the apparent high outlier.

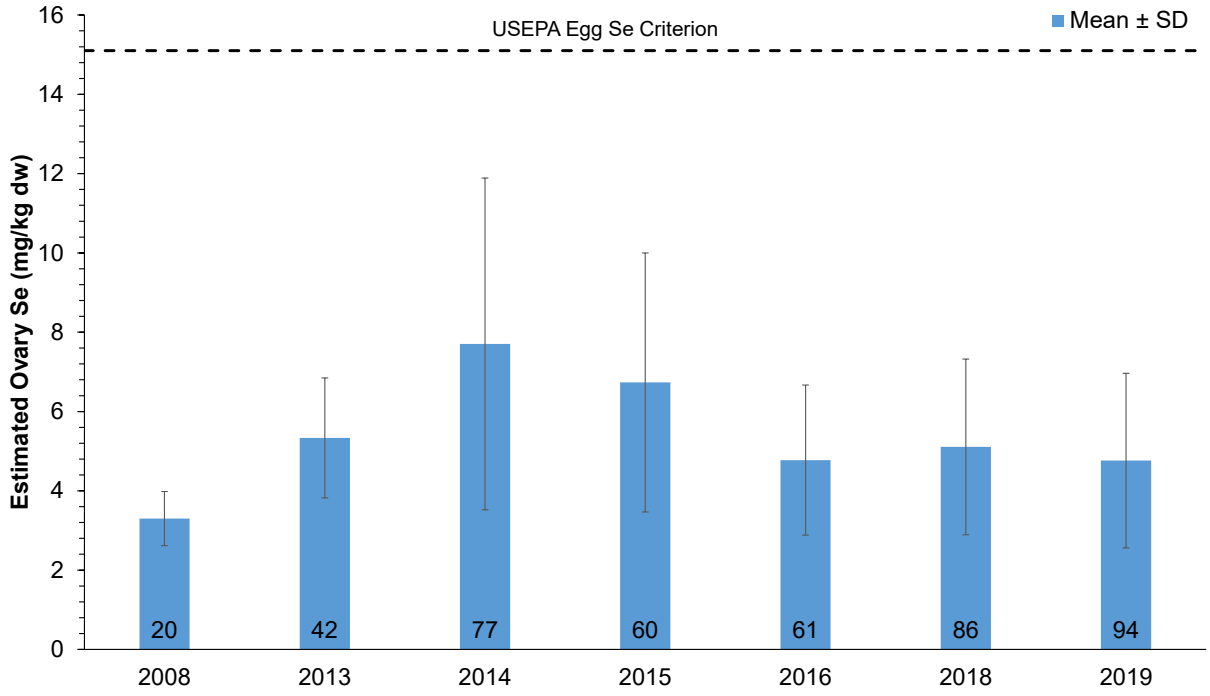

Figure 13. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow based on all samples in Koocanusa Reservoir by year.
Note: Median ovary-muscle ratio is 2.5. Numbers within base of column denote sample sizes.

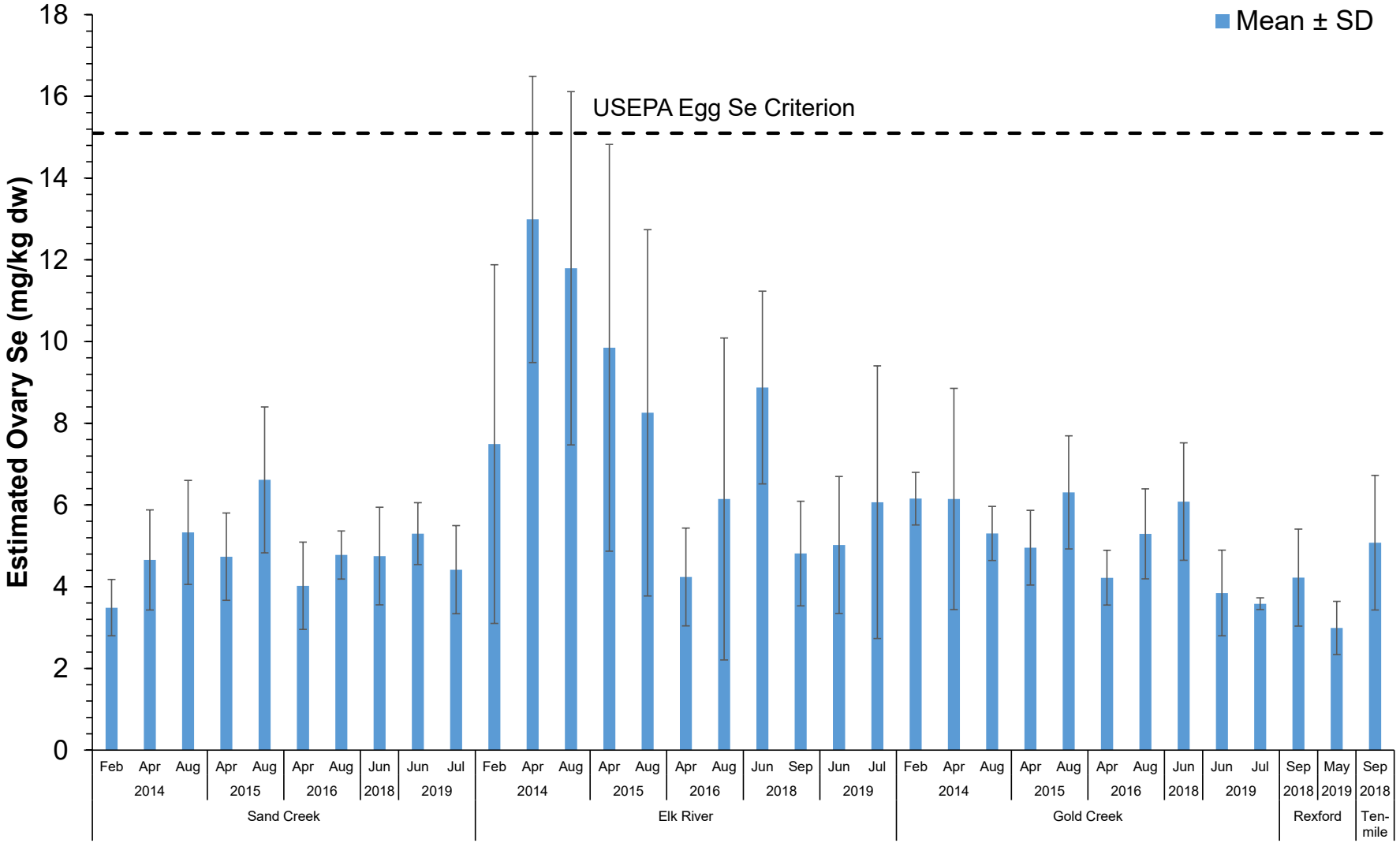

Figure 14. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow by location and sampling time within Koocanusa Reservoir.
Note: Median ovary-muscle ratio is 2.5.

3 Review and Validation of Koocanusa Reservoir Selenium Bioaccumulation Models

Section 3.1 first discusses the USGS selenium model (Presser and Naftz 2020) and modifications to model inputs based on consideration of site-specific data for invertebrates are provided in Section 3.2. Validation of model outputs based on site-specific fish selenium data is then provided in Section 3.3.**USGS Model**

The USGS selenium model was developed to support derivation of a site-specific water selenium criterion for Koocanusa Reservoir (Presser and Naftz 2020). This model was based on the framework for Koocanusa Reservoir previously presented in Jenni et al. (2017), which was developed following the ecosystem-scale selenium modeling methodology described in Presser and Luoma (2010). This model uses k_d values to describe partitioning of selenium from water to particulates at the base of the food web and trophic transfer factors (TTFs) to describe partitioning of selenium between food web components (e.g., from particulates to invertebrates and from invertebrates to fish). For example:

$$C_{fish} = C_{water} \times k_d \times TTF_{invert} \times TTF_{fish} \times 0.001 \quad (1)$$

Where: C_{water} = Se concentration in water (µg/L)
C_{fish} = Se concentration in fish tissue (mg/kg dw)
k_d = ratio of Se concentration in particulates and water (L/kg dw)
TTF_{invert} = ratio of Se concentration in invertebrates and particulates
TTF_{fish} = ratio of Se concentration in fish and invertebrates
0.001 = conversion factor to convert from µg to mg

Based on k_d and TTF data or assumptions, and a target fish selenium concentration of interest (e.g., a criterion), Equation 1 can be rearranged to solve for the water selenium concentration predicted to result in the target fish selenium concentration:

$$C_{water} = \frac{C_{fish}}{k_d + TTF_{invert} + TTF_{fish}} \quad (2)$$

Equations 1 and 2 can be modified to include additional trophic level steps, such as a prey fish consumed by piscivorous fish. The equations can also be modified to include relative dietary fractions of invertebrates, such as 50% benthic invertebrates and 50% zooplankton. The following summarizes model inputs used in Presser and Naftz (2020):

- **k_d:** Selenium k_d values were calculated from 87 paired particulate and surface water selenium concentrations[2] measured in large volume suspended sediment (LVSS) samples. These samples were collected at the epilimnion and hypolimnion

[2] At the time of writing, these complete data have not been made available by the USGS and were requested on August 18, 2020.

from four locations in the reservoir: (1) south of Elk River; (2) the international boundary; (3) Tenmile (hypolimnion only); and (4) the forebay.

- **TTF_{invert}:** TTFs of 2.8 and 1.5 were assumed for benthic invertebrates and zooplankton, respectively. These TTFs were derived and reported in Presser and Luoma (2010) based on data from other studies and are not specific to Koocanusa Reservoir. Presser and Naftz (2020) incorporated a bioavailability fraction of 0.6 (60%), which effectively reduce the benthic invertebrate TTF from 2.8 to 1.7 and the zooplankton TTF from 1.5 to 0.9.

- **TTF_{fish}:** A whole body selenium TTF of 1.1 was assumed in the presentation of the model in the report, which is the mean TTF from 25 fish species as reported in Presser and Luoma (2010). It was noted, however, that species-specific TTFs could be considered and examples were provided.

- **C_{fish}:** The USEPA's whole body selenium guideline of 8.5 mg/kg dw was used in the report, but it was noted that other fish tissue selenium concentrations of interest could be considered.

For presentation and discussion of the model herein, water selenium concentrations are back-calculated from the whole body criterion of 8.5 mg/kg dw based on a k_d of 5,000 L/kg dw and benthic invertebrate, zooplankton, and fish TTFs of 2.8, 1.5, and 1.1, respectively (with and without the bioavailability factor of 0.6 for invertebrates). Water concentrations were back-calculated for four different food chain scenarios to bracket the range of exposure conditions: (1) 100% benthic invertebrates; (2) 100% zooplankton; (3) 50% benthic invertebrates and 50% zooplankton; and (4) 100% fish. These example calculations are similar to those provided in Table 10 of Presser and Naftz (2020). Presser and Naftz (2020) did not recommend selection of a single representative k_d for the reservoir, but a k_d of 5,000 L/kg dw was selected because this value was used in their Table 10 and it is also approximately the median k_d for the reservoir and thus a reasonable estimate of central tendency.

Based on the above model input assumptions and food chain scenarios, the water selenium concentrations projected to result in the whole body selenium criterion of 8.5 mg/kg dw range from 0.5 µg/L (100% fish diet) to 1.0 µg/L (100% fish diet) with a bioavailability factor of 100% (first set of green-highlighted rows in Table 2). If the bioavailability factor of 0.6 is assumed, the range in back-calculated selenium concentrations increases to 0.84 to 1.7 µg/L (second set of green-highlighted rows in Table 2).

Table 2. Examples of water selenium concentrations projected to result in whole body selenium criterion of 8.5 mg/kg dw based on different k_d, TTF, and food chain scenarios.

Scenario	Diet	TTF			SPM Bioavail-ability Fraction	k_d (L/kg dw)	WB Criterion (mg/kg dw)	Water Se (µg/L)	Mean Water Se (µg/L)
		Insects	Zoo-plankton	Fish					
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1	100% Insects	2.8	1.5	3.1*	1	5000	8.5	**0.55**	**0.70**
	100% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**1.0**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**0.72**	
	100% Fish	2.8	1.5	3.1*	1	5000	8.5	**0.50**	
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	100% Insects	2.8	1.5	3.1*	0.6	5000	8.5	**0.92**	**1.2**
	100% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.7**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.2**	
	100% Fish	2.8	1.5	3.1*	0.6	5000	8.5	**0.84**	
Alternative Assumptions									
Alternative Assumptions: Median k_d from USGS (2020) and median site-specific TTFs for inverts	100% Insects	1.1	0.52	1.2*	1	4547	8.5	**1.5**	**2.1**
	100% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**3.3**	
	50% Insects / 50% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**2.1**	
	100% Fish	1.1	0.52	1.2*	1	4547	8.5	**1.4**	
Alternative Assumptions: 75th percentile k_d from USGS (2020) and 75th percentile site-specific TTFs for inverts	100% Insects	1.2	0.65	1.3*	1	5268	8.5	**1.2**	**1.5**
	100% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**2.3**	
	50% Insects / 50% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**1.6**	
	100% Fish	1.2	0.65	1.3*	1	5268	8.5	**1.1**	

SPM = suspended particulate matter
TTF = trophic transfer factor
WB = whole body
*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

3.2 Alternative Inputs for Selenium Model

As discussed above, TTFs for benthic invertebrates and zooplankton in the USGS model are based on data compiled in Presser and Luoma (2010) and are not specific to Koocanusa Reservoir. As a point of comparison to those invertebrate TTFs, I developed zooplankton and benthic invertebrate TTFs based on site-specific data.

Site-specific selenium TTFs for zooplankton were developed by co-locating zooplankton and surface water selenium samples. In this paired dataset, zooplankton selenium concentrations ranged from 0.34 to 4.21 mg/kg dw and particulate selenium concentrations ranged from 2.0 to 6.6 mg/kg dw (Figure 15A). With one exception, resulting zooplankton TTFs were less than 1 and the median TTF was 0.52 (Figure 15B). The one exception was a TTF of 1.9, was calculated from the lowest particulate selenium concentration in the paired dataset. This observation is consistent with patterns in other studies, in which TTFs are greater at low selenium exposure concentrations (DeForest et al. 2007). **The median TTF of 0.52 based on site-specific data is less than the mean zooplankton TTF of 1.5 in the USGS model, as well as the TTF of 0.9 in the USGS model if the 60% bioavailability factor is applied.** As such and consistent with comments from other SeTSC members on August 25, 2020, TTFs for zooplankton as proposed by the USGS are overly conservative.

For benthic invertebrates, co-located selenium concentrations in particulates are not available. As such, a two-step approach was used to estimate site-specific selenium TTFs for benthic invertebrates. First, benthic invertebrate and surface water selenium samples were co-located. Benthic invertebrate selenium concentrations in these co-located samples range from 0.38 to 9.1 mg/kg dw and surface water selenium concentrations range from 0.15 to 2.4 µg/L (Figure 16A). These co-located data were used to develop bioaccumulation factors (BAFs), which are calculated as the benthic invertebrate selenium concentration divided by the surface water selenium concentration (multiplied by 1,000 to convert to units of L/kg dw). As observed in other selenium BAF datasets (DeForest et al. 2007), there is an inverse relationship between BAFs and water selenium concentration (Figure 16B). This relationship is described by a linear regression model in log-log scale (Figure 16C).

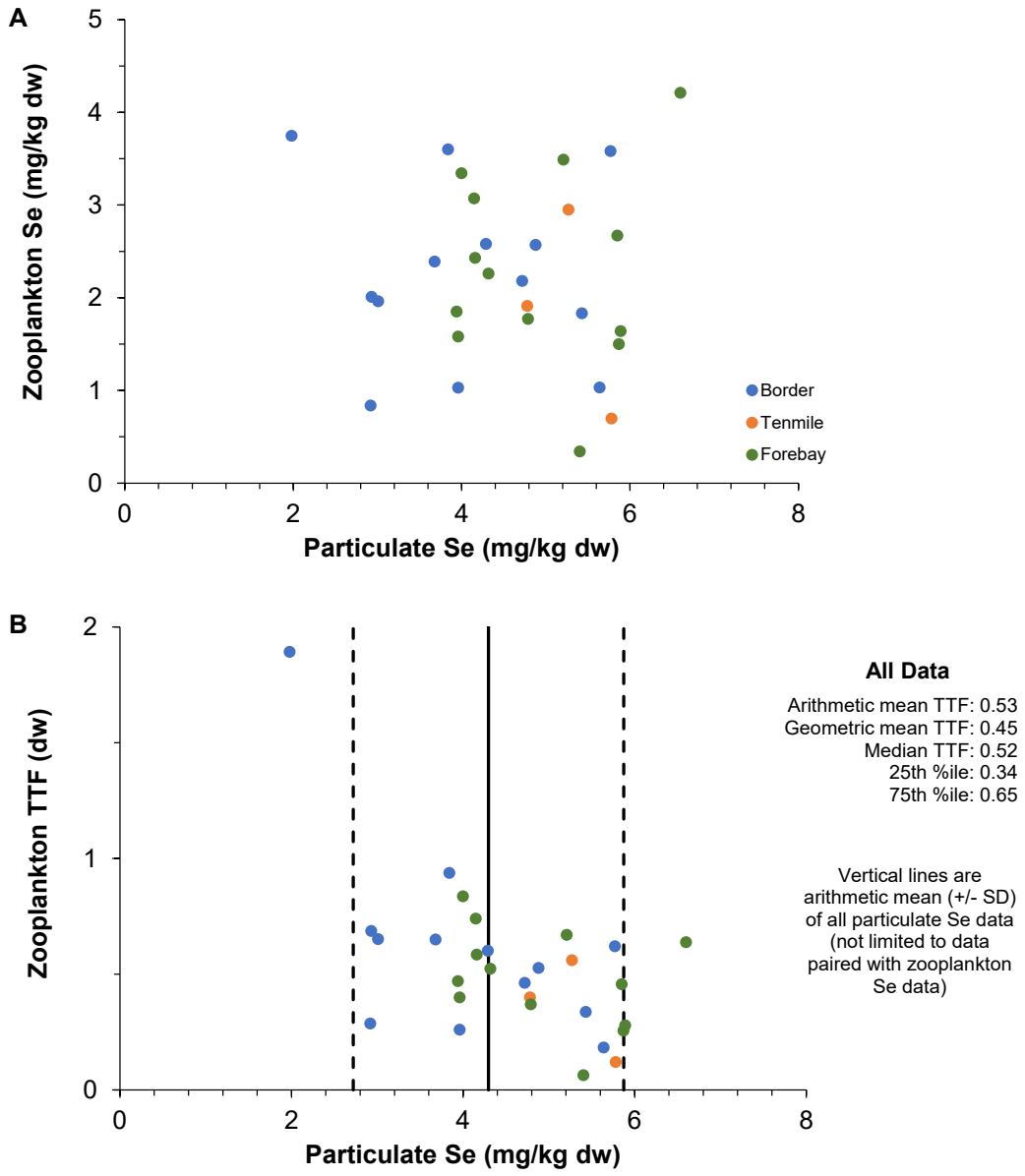

Figure 15. (A) Relationship between selenium concentrations in co-located zooplankton and particulate samples; and (B) relationship between zooplankton TTFs and particulate selenium concentrations.

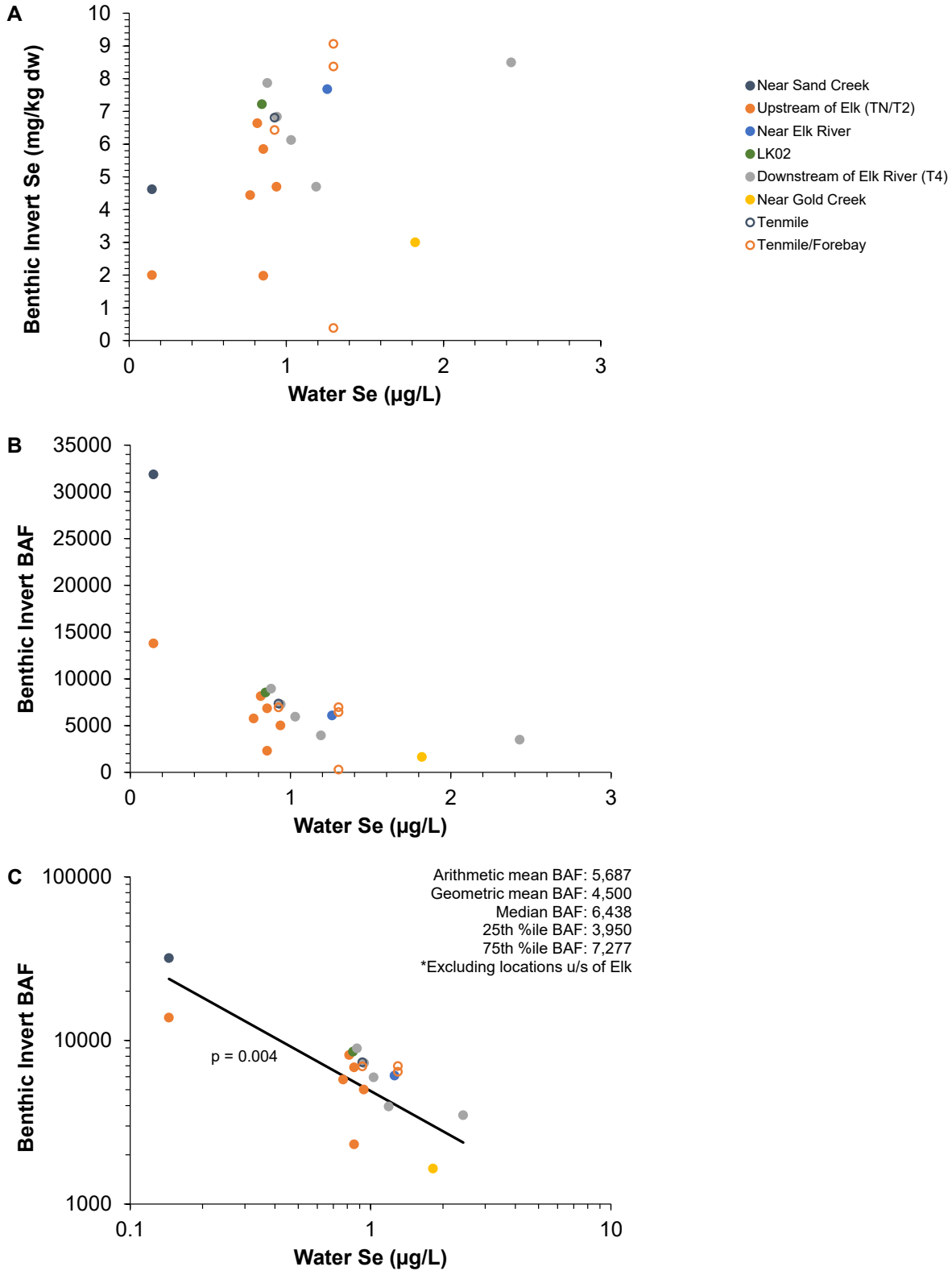

Figure 16. Relationships between (A) co-located benthic invertebrate and water selenium concentrations; and benthic invertebrate BAFs and water selenium concentrations in (B) normal scale and (C) log-normal scale.

The second step was to apply the benthic invertebrate BAF model from Figure 16C to surface water selenium concentrations used to develop the selenium k_d values. Thus, for each water selenium concentration in the k_d dataset, there is a co-located measure of particulate selenium and a co-located estimate of benthic invertebrate selenium (Figure 17A). Benthic invertebrate TTFs were then calculated based on the paired measured particulate selenium concentrations and estimated benthic invertebrate selenium concentrations. Based on the inverse relationship between benthic invertebrate BAFs and surface water, there is also an inverse relationship between estimated benthic invertebrate TTFs and particulate selenium concentrations (Figure 17B). To avoid undue influence of high TTFs at low selenium exposure concentrations and low TTFs at high selenium exposure concentrations, the benthic invertebrate TTF summary statistics were calculated over the range of the mean measured selenium concentrations plus and minus one standard deviation (Figure 17B). The median TTF within this particulate selenium range is 1.1. **The estimated TTF from site-specific data is less than the benthic invertebrate TTF of 2.8 in the USGS model, as well as the TTF of 1.7 in the USGS model if the 60% bioavailability factor is applied.**

Two additional model scenarios were then evaluated using the above-mentioned site-specific data. One model scenario considered the 50th percentile k_d and the 50th percentile TTFs of 1.1 and 0.52 for benthic invertebrates and zooplankton, respectively. The second scenario was more conservative by considering the 75th percentile k_d and 75th percentile TTFs of 1.2 and 0.65 for benthic invertebrates and zooplankton, respectively. Based on the 50th percentile assumption, the water selenium concentrations predicted to result in a whole body selenium criterion of 8.5 mg/kg dw range from 1.4 µg/L (100% fish diet) to 3.3 µg/L (100% zooplankton diet) (first set of blue-highlighted rows in Table 2). Based on the 75th percentile assumption, water selenium concentrations predicted to result in the whole body selenium criterion of 8.5 mg/kg dw range from 1.1 µg/L (100% fish diet) to 2.3 µg/L (100% zooplankton diet) (second set of blue-highlighted rows in Table 2).

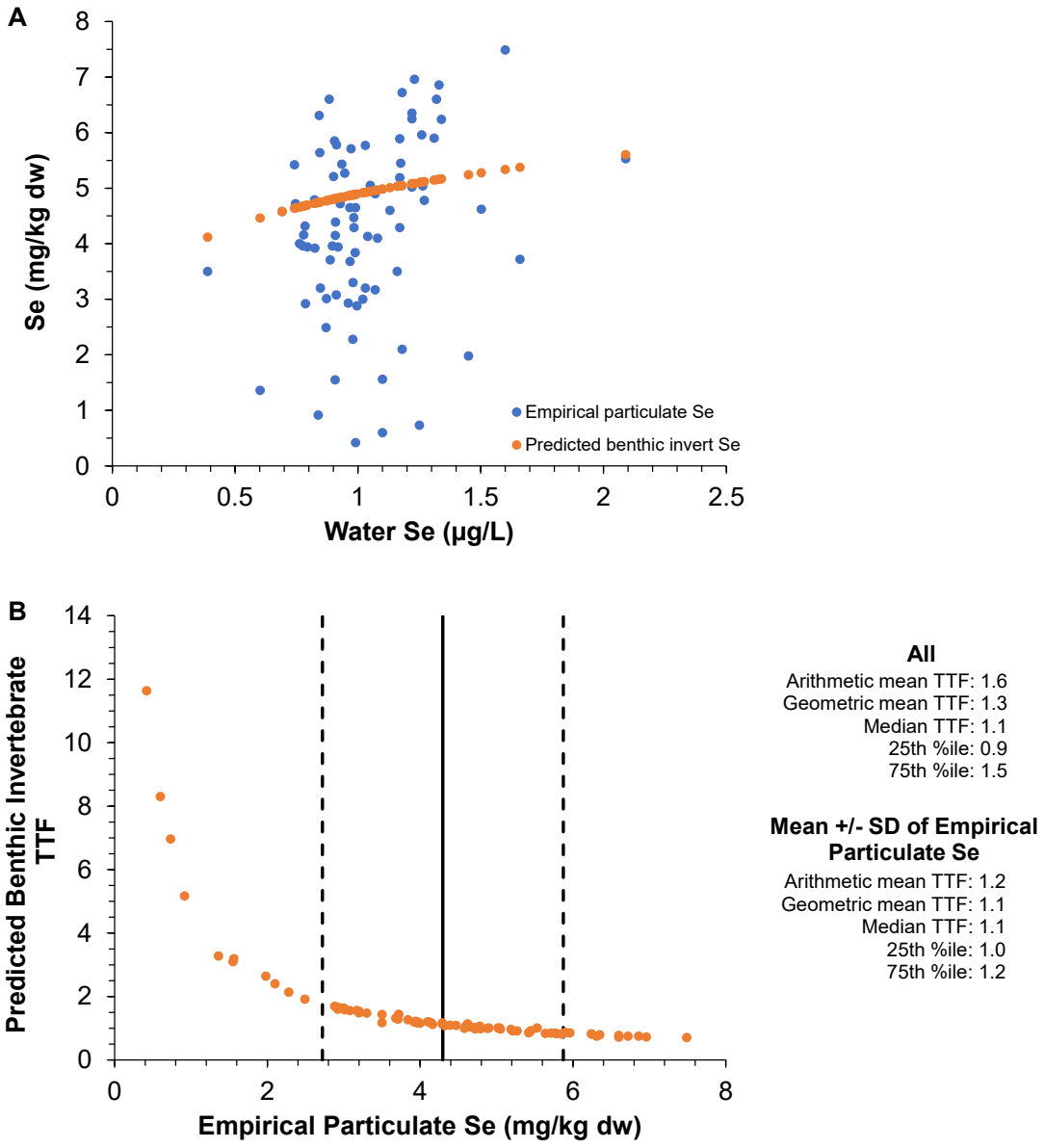

Figure 17. Relationships between (A) empirical particulate Se concentrations and predicted benthic invertebrate Se concentrations; and (B) between predicted benthic invertebrate TTFs and particulate Se concentrations.

3.3 Validation of Models

Empirical fish tissue selenium data for Koocanusa Reservoir were not considered in the development or validation of the selenium model described in Presser and Naftz (2020). It appears that empirical fish selenium data were not considered because it was assumed that the reservoir was currently degraded from the cumulative effects of various stressors and that, by inference, the suggestion appears to be that selenium concentrations in fish in the reservoir are not representative of what would be expected in an unstressed

system. A reservoir is of course a managed system and issues related to drawdown and flow-regime changes, even in the absence of any other stressors, can have a substantial influence on habitat and aquatic communities. In my opinion, consideration of empirical fish selenium data is a critical piece of evidence for evaluating selenium bioaccumulation in the reservoir. In addition, fish selenium data in the reservoir will presumably be important for determining compliance with selenium criteria in the future.

I conducted a validation of the USGS model and alternative input assumptions based on the invertebrate TTF evaluation described in Section 3.2. The first step of the validation process was to first identify paired fish tissue and water selenium concentrations in the reservoir. Fish selenium concentrations were then predicted from water selenium concentrations based on various k_d and TTF assumptions. The selection of TTFs was based on the dietary assumption for the fish species being evaluated in the validation. For example, peamouth chub are assumed to have a diet comprised of 50% benthic invertebrates and 50% zooplankton, so for this fish species the benthic invertebrate and zooplankton TTFs were each given 50% weight.

Validation of four different modeling scenarios (consistent with those described above) was evaluated:

1. USGS model with suspended particulate matter bioavailability fraction of 100%. A k_d of 5,000 L/kg was used for consistency with the model examples provided in Table 10 of Presser and Naftz (2020) (Table 3).

2. USGS model with suspended particulate matter bioavailability fraction reduced to 60% (Table 3).

3. Alternative model inputs based on the 50th percentile k_d from the USGS model and the 50th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

4. A more conservative alternative model with inputs based on the 75th percentile k_d from the USGS model and the 75th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

Table 3. Model scenarios included in the validation.

Scenario	k_d (L/kg dw)	SPM Bioavail-ability Fraction	TTF		
			Insects	Zoo-plankton	Fish
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1.0	5000	1	2.8	1.5	3.1*
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	5000	0.6	2.8	1.5	3.1*
50th %ile site-specific k_d and invert TTFs	4547	1	1.1	0.52	1.2*
75th %ile site-specific k_d and invert TTFs	5268	1	1.2	0.65	1.3*

*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

Predicted fish selenium concentrations were then compared to observed fish selenium concentrations. Comparisons were made using a standard selenium bioconcentration and trophic transfer figure, similar to that provided in Figure 18. This figure format visually shows the transfer of selenium from surface water to particulates, invertebrates, and fish as a function of assumed k_d values and TTFs.

Validation evaluations presented here are based on selenium concentrations in fish muscle and whole body tissue. The validation is focused on these two tissue types because of uncertainties in the ovary selenium data due to the timing of ovary sampling relative to spawning periods and ovary maturity. Further discussion of ovary selenium concentrations and maturity is provided in Sections 2 and 4.

Over the validation scenarios evaluated, measured muscle and whole body selenium concentrations were consistently over-predicted by the USGS model regardless of model assumptions (Figure 19). Based on the USGS model with the 60% bioavailability assumption, predicted fish selenium concentrations were over-predicted, on average, by a factor of 5.0 (Table 4). In most of the scenarios evaluated, this resulted in muscle and whole body selenium concentrations that were predicted to exceed the muscle selenium criterion of 11.3 mg/kg dw and whole body selenium concentration of 8.5 mg/kg dw, while measured fish selenium concentrations were generally less than 50% of criteria concentrations. Even when considering site-specific k_d summary statistics and site-specific invertebrate TTFs the USGS model predicts muscle and whole body selenium concentrations that, on average, are a factor of 2.9 greater than observed (Table 4).

In my opinion, the consistent over-prediction of selenium concentrations in fish tissue is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.

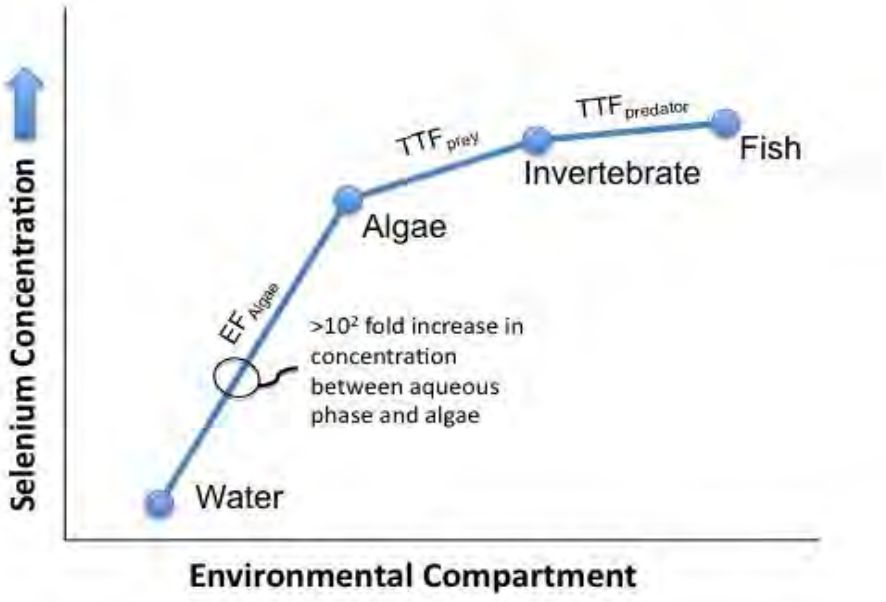

Figure 18. Example of multi-step selenium bioconcentration and trophic transfer plots in a fish food chain.
Source: Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Bioaccumulation and trophic transfer of selenium. In: Ecological assessment of selenium in the aquatic environment. Pensacola, FL:SETAC Press, 93-139.

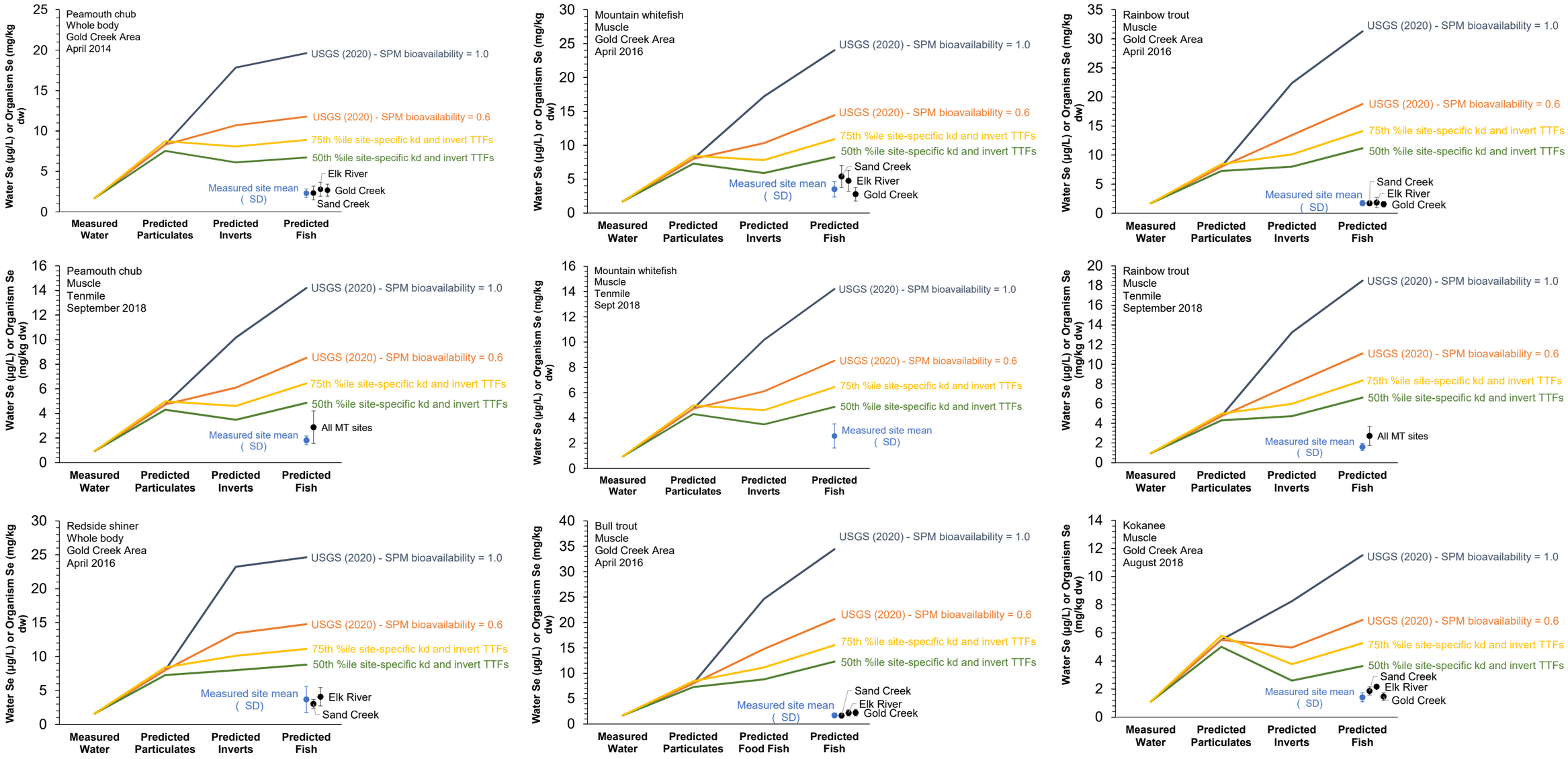

Figure 19. Comparisons of predicted and observed whole body selenium concentrations in Koocanusa Reservoir fish.
Note: See text boxes within each panel for the species, tissue type, sample location, and sampling data.

Table 4. Examples of observed and predicted fish selenium concentrations in Koocanusa Reservoir based on different modeling scenarios.

Site	Scenario	Water Se (µg/L)	kd (L/kg dw)	Predicted Particulate Se (mg/kg dw)	Bioavailability Fraction	TTF			Food Chain Se (mg/kg dw)			Fraction Diet			TTF Target Fish	Predicted Fish Se (mg/kg dw)	Mean Measured Fish Se (mg/kg dw)	SD Measured Fish Se (mg/kg dw)	Predicted-to-Observed Ratio
						Insects	Zoo-plankton	Fish	Insects	Zoo-plankton	Insect-ivorous Fish	Insects	Zoo-plankton	Fish					
Peamouth Chub - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	1	2.8	1.5	1.1	23.2	12.5	25.6	0.5	0.5	0	1.1	19.6	2.3	0.54	8.5
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	0.6	2.8	1.5	1.1	13.9	7.5	15.3	0.5	0.5	0	1.1	11.8	2.3	0.54	5.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.7	4547	7.5	1	1.1	0.52	1.1	8.3	3.9	9.1	0.5	0.5	0	1.1	6.7	2.3	0.54	2.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.7	5268	8.7	1	1.2	0.65	1.1	10.5	5.7	11.5	0.5	0.5	0	1.1	8.9	2.3	0.54	3.9
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	1	2.8	1.5	1.1	3.6	2.0	4.0	0.5	0.5	0	1.1	3.1	1.4	0.30	2.2
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	0.6	2.8	1.5	1.1	2.2	1.2	2.4	0.5	0.5	0	1.1	1.8	1.4	0.30	1.3
North of Elk (Kikomun Area)	50th %ile site-specific k_d & invert TTFs	0.26	4547	1.2	1	1.1	0.52	1.1	1.3	0.6	1.4	0.5	0.5	0	1.1	1.1	1.4	0.30	0.8
North of Elk (Kikomun Area)	75th %ile site-specific k_d & invert TTFs	0.26	5268	1.4	1	1.2	0.65	1.1	3.8	0.6	4.2	0.5	0.5	0	1.1	2.5	1.4	0.30	1.8
Peamouth Chub - Muscle																			
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	1.8	0.36	7.8
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	1.8	0.36	4.7
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	1.8	0.36	2.7
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	1.8	0.36	3.6
Mountain Whitefish – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0.5	0.5	0	1.4	24.0	3.5	1.1	6.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0.5	0.5	0	1.4	14.4	3.5	1.1	4.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0.5	0.5	0	1.4	8.2	3.5	1.1	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0.5	0.5	0	1.4	10.9	3.5	1.1	3.1
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	2.6	0.96	5.5
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	2.6	0.96	3.3
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	2.6	0.96	1.9
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	2.6	0.96	2.5
Rainbow Trout – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.4	31.3	3.5	1.12	8.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.4	18.8	3.5	1.12	5.4
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.4	11.2	3.5	1.12	3.2
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.4	14.1	3.5	1.12	4.0
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	1	0	0	1.4	18.5	1.6	0.35	11.6
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	1	0	0	1.4	11.1	1.6	0.35	6.9
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	1	0	0	1.4	6.6	1.6	0.35	4.1
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	1	0	0	1.4	8.4	1.6	0.35	5.2
Bull Trout - Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0	0	1	1.4	34.4	2.5	0.91	13.8
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0	0	1	1.4	20.7	2.5	0.91	8.3
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0	0	1	1.4	12.3	2.5	0.91	4.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0	0	1	1.4	15.5	2.5	0.91	6.2
Redside Shiner - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.1	24.6	3.7	1.9	6.7
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.1	14.8	3.7	1.9	4.0
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.1	8.8	3.7	1.9	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.1	11.1	3.7	1.9	3.0
Kokanee – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	1	2.8	1.5	1.1	15.4	8.3	16.9	0	1	0	1.4	11.5	1.4	0.32	8.2
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	0.6	2.8	1.5	1.1	9.2	5.0	10.2	0	1	0	1.4	6.9	1.4	0.32	4.9
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.1	4547	5.0	1	1.1	0.52	1.1	5.5	2.6	6.1	0	1	0	1.4	3.6	1.4	0.32	2.6
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.1	5268	5.8	1	1.2	0.65	1.1	7.0	3.8	7.6	0	1	0	1.4	5.3	1.4	0.32	3.7

4 Summary and Recommendations

- The USEPA's fish tissue selenium criteria are conservative and protective of fish in Koocanusa Reservoir. These criteria are based on the 5th percentile of genus mean EC10s, which is extrapolated to a concentration that is less than the white sturgeon EC10. There is less uncertainty in the egg selenium criterion (which supersedes the muscle and whole body selenium criteria) due to differences in muscle-to-egg and whole body-to-egg relationships among species, but conservatism in the criteria calculation method ensures that the muscle and whole body selenium criteria are still protective.
- With the exception of cyprinids (which are discussed in the next bullet), there have been just three exceedances of a fish tissue criterion based on over 2,800 measurements in individual fish within Koocanusa Reservoir. These include a rainbow trout and a longnose sucker ovary sample (see Figure 7), and a yellow perch muscle sample (see Figure 8). No whole body selenium concentrations have exceeded the USEPA's whole body selenium criterion (Figure 9). Based on evaluations of ovary selenium concentrations and ovary maturity discussed for cyprinids (as well as mountain whitefish), there is uncertainty in some of the ovary selenium data for other fish species, specifically the potential to over-estimate exposure due to immature ovaries. Despite this uncertainty, most ovary selenium concentration fall well below the USEPA's egg selenium criterion so this uncertainty is unimportant for most of the cases.
- Three cyprinids—northern pikeminnow, peamouth chub, and redside shiner—have had the highest ovary selenium concentrations in Koocanusa Reservoir and these concentration have exceeded the USEPA's egg selenium criterion. As shown in the northern pikeminnow study, however, the elevated ovary selenium concentrations are associated with immature ovaries as samples were not collected at time of spawning. A similar pattern appears to be observed in peamouth chub, while recent studies with redside shiner will soon provide more information on selenium bioaccumulation in redside shiner eggs and effects. These data on cyprinids in Koocanusa Reservoir, coupled with the USEPA's conclusion that cyprinids are not uniquely sensitivity based on evaluations of data from sites in the U.S. with high selenium concentrations, indicates it is unlikely that cyprinids in the reservoir are uniquely sensitive to selenium, and in fact may be relatively insensitive.
- Based on more than 2,800 empirical selenium concentrations in non-cyprinids that exceeded criteria in just three samples, along with an increasing level of understanding of selenium concentrations in cyprinids, there are currently no data to indicate that fish in Koocanusa Reservoir are at risk from selenium under current conditions. Surface water selenium concentrations in the reservoir over the period of fish selenium monitoring have ranged between 1 and 2 µg/L in the BC

portion of the reservoir downstream from the Elk River. Based on consideration of both empirical fish and surface water selenium concentrations, **it is my opinion that the USEPA's lentic criterion of 1.5 µg/L is protective of fish in Koocanusa Reservoir**. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessments consistent with the USEPA criterion.

- Additional support that the USEPA's lentic criterion of 1.5 µg/L is provided by the evaluation of alternative model inputs and the model validation relative to empirical fish selenium data. As shown in Figures 19 and in Table 4, even the least conservative model scenario evaluated (use of the 50th percentile k_d and site-specific invertebrate TTFs) consistently over-predicted measured fish selenium concentrations. This conservative model translated the whole body fish selenium criterion of 8.5 mg/kg dw to surface water selenium concentrations of:
    - 1.4 µg/L for piscivorous fish (100% fish diet),
    - 1.5 µg/L for fish with a 100% insect diet,
    - 2.1 µg/L for fish with a 50% insect and 50% zooplankton diet, and
    - 3.3 µg/L for fish with a 100% zooplankton diet.

  In my opinion, the consistent over-prediction of selenium concentrations in fish tissue by the USGS model is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.
- The USEPA's lentic criterion of 1.5 µg/L would also be protective of white sturgeon in the Kootenai River downstream of the Libby Dam. Current surface water monitoring data for the forebay and in the river downstream of the dam indicate that the selenium concentrations are similar. Egg selenium concentrations in white sturgeon collected from the Kootenai River from 2015 to 2019 have ranged from 3.0 to 5.7 mg/kg dw with an average of 4.1 mg/kg dw (n = 38). Thus, even the maximum egg selenium concentration is just 38% of the USEPA's egg selenium criterion of 15.1 mg/kg dw.

5 CLOSURE

Thank you for the opportunity to provide these comments. Please do not hesitate to contact me (DavidD@windwardenv.com; 206-812-5426) if you have any questions or if there is additional information I can provide.

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ATTACHMENT 1

Evaluation of Selenium Concentrations in Ovary of Northern Pikeminnow (*Ptychocheilus oregonensis*)

FINAL REPORT

EVALUATION OF SELENIUM CONCENTRATIONS IN OVARY OF NORTHERN PIKEMINNOW (*Ptychocheilus oregonensis*)

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Table of Contents

1. INTRODUCTION

Ongoing monitoring of Koocanusa Reservoir indicates ovary Se concentrations from some northern pikeminnow (NPM; *Ptychocheilus oregonensis*) collected from the Canadian side of the Reservoir were above both the 11 mg kg^{-1} dry weight (dw) egg Se guideline established by the British Columbia Ministry of the Environment and Climate Change Strategy (BC ENV, 2014) and the 15.1 mg kg^{-1} dw egg Se criteria established by the U.S. Environmental Protection Agency (USEPA, 2016). However, the sensitivity of NPM to Se is currently unknown and so the ecological risk posed by observed egg Se concentrations is uncertain. Further, historical ovary Se concentrations were collected from unripe fish (i.e., not in spawning condition) and the influence of gonadal maturation stage on egg Se concentrations is uncertain. The following presents results from a study to characterize the influence of gonadal maturation stage, fish size, and fish sampling location on ovary Se concentrations in NPM collected from Koocanusa Reservoir.

Efforts to also conduct a toxicity test evaluating the effects of maternally transferred Se on NPM embryo-larval development were unsuccessful in 2019 due to the inability to collect a sufficient number of female fish in spawning condition. As such, this test is not discussed further in this report.

2. BACKGROUND

Northern pikeminnow are distributed throughout the Pacific drainages as far north as the Nass River drainage in BC, Canada to the Columbia River drainage in the U.S. They are most common along sandy, cobble, gravel, boulder or bedrock shorelines during summer and deeper waters during winter (Scott and Crossman 1973, Coker et al. 2001). Northern pikeminnow are late spring-summer spawners, typically spawning when water reaches 14-18 °C with males generally present in larger congregations on breeding grounds over gravel and cobble shallows (Gadomski et al., 2001). Females may have multiple spawning bouts with more than one male throughout the season. Eggs hatch after 8-10 days at 15-17 °C (Coker et al., 2001; Gadomski et al., 2001; Scott and Crossman, 1973).

Koocanusa Reservoir was formed by Libby Dam, located 30 km northeast of Libby, Montana at river mile 221.9 of the Kootenai River[1]. The reservoir is 145 km long, of which 68 km are in BC, Canada. The predominant source of water to the reservoir is the Kootenay River, of which the Elk River is a tributary. Northern pikeminnow are resident to Koocanusa Reservoir and have been sampled for ovary and muscle Se in BC, Canada and Montana (MT), U.S. over the last 11 years.

1. The Kootenay River is referred to as the 'Kootenai River' in the U.S.

Monitoring data indicate NPM ovary Se concentrations on the MT side of the reservoir have low variability within and across sampling years compared to fish collected from the BC side of the reservoir (Figure 1). Some fish collected on the BC side of the reservoir are above both the BC ENV guideline and the USEPA fish egg Se criteria of 11 and 15.1 mg kg^{-1} dw, respectively. These data also indicate ovary Se concentrations in fish collected from the BC side of Koocanusa Reservoir appear to be significantly ($p<0.05$) higher than those collected from the U.S. side.

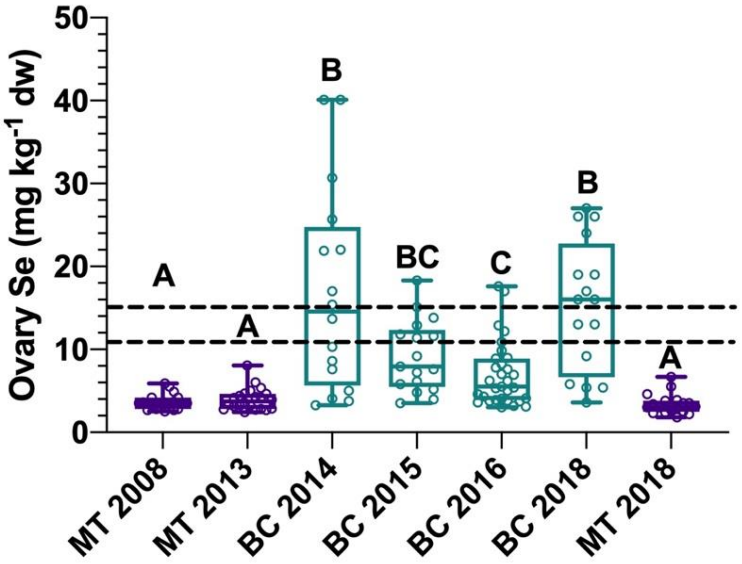

Figure 1. Ovary Se concentrations on the Montana (MT) and British Columbia (BC) sides of Koocanusa Reservoir (2008-2018). Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines. Different letters indicate significant differences ($p<0.05$).

There are several potential biases in the data collected to date that complicate the interpretation of differences in NPM ovary Se data. First, NPM typically reach spawning condition when they have a gonado-somatic index (GSI) of 8-12% (Gray and Dauble, 2001; Petersen and Ward, 1999). While GSI data are not available for fish caught in MT, only a single female on the BC side of the reservoir has been collected with a GSI in this range. The impact of collecting unripe ovaries on observed ovary Se concentrations is unknown, but much of the variability in ovary Se concentrations in the existing BC data is associated with a GSI <2%. Further 55% of ovaries collected from fish with a GSI <2% are above the BC ENV egg Se guideline of 11 mg kg^{-1} dw, while only 7% of ovaries collected from fish with a GSI >2% are above this guideline (Figure 2).

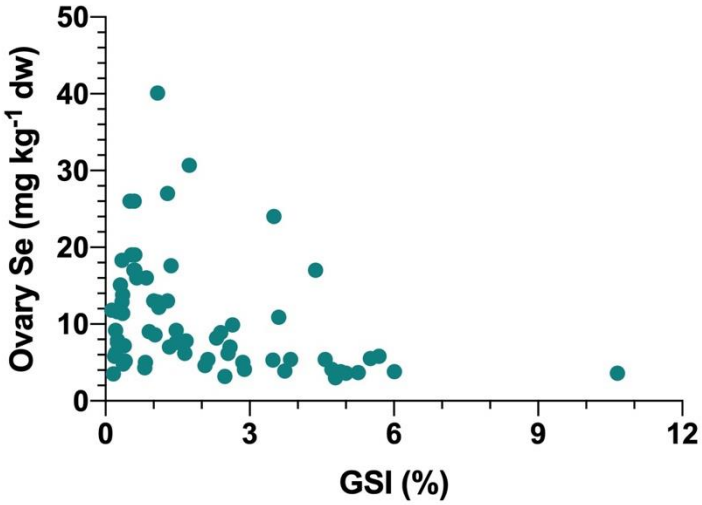

Figure 2. Relationship between ovary Se and GSI for northern pikeminnow collected on the BC side of Koocanusa Reservoir.

Second, there is a significant relationship between fish size and ovary Se concentrations (Figure 3A), and fish collected on the BC side of the reservoir tend to be smaller than those collected in MT (see Figure 3B). Collection of smaller fish on the Canadian side of the reservoir may be the result of sampling bias as fish collection has been restricted to angling, while on the MT side of the reservoir fish are collected using gill nets.

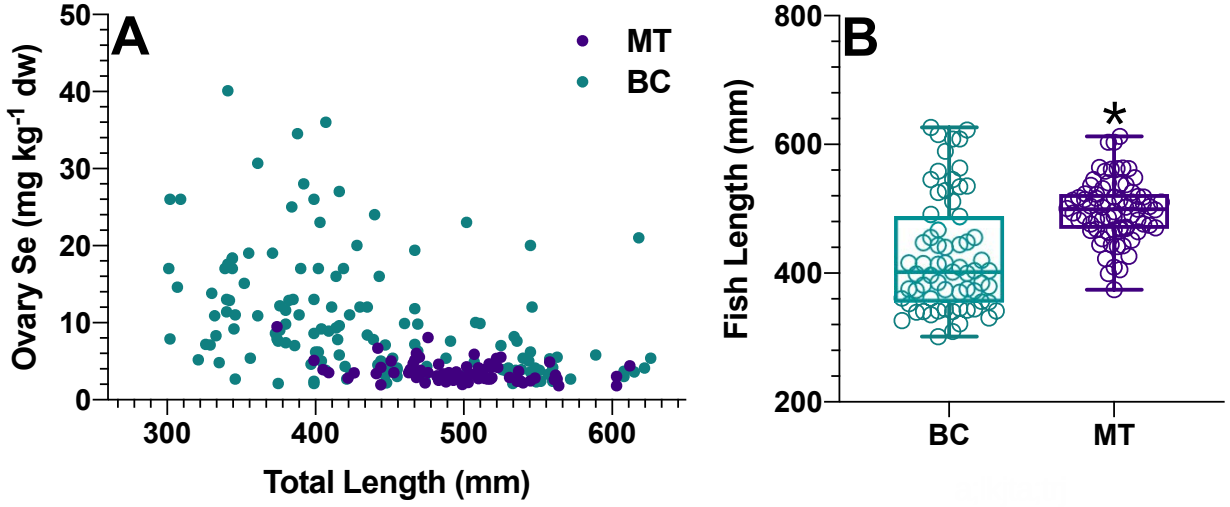

Figure 3. Relationship between ovary Se and fish length (A) and fish length distributions (B) for northern pikeminnow collected from Koocanusa Reservoir. Box plots represent mean, quartiles, maximum and minimum values. * = significant difference in the MT fish length compared to the BC fish lengths ($p<0.001$).

Overall, these observations suggest data collected to date on NPM ovary Se concentrations may be biased. However, this conclusion is uncertain due to the lack of ovary Se data in ripe fish, along with associated size and GSI data. Regardless of potential biases in historical ovary

Se sampling, the sensitivity of NPM to maternally transferred Se is not known. The original objectives of this study were to address both of these uncertainties.

3. OBJECTIVES AND STUDY DESIGN OVERVIEW

This study originally had two objectives:

1) To determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations; and
2) To evaluate the relationship between ovary Se concentrations and ovary development, fish size, and sampling location.

As discussed above, the inability to collect a sufficient number of female fish in spawning condition resulted in the first objective not being achieved. However, the extended effort to collect female fish in spawning condition lead to the collection of a large number (n=79 on the BC side of the reservoir) of samples for ovary Se analysis in support of the second objective. To achieve the second objective, the study had the following key elements:

1) Prior to NPM reaching spawning condition, unripe ovaries/eggs and muscle were collected and GSI measured in sexually mature females (30-60+ cm) to provide information on the relationships between ovary Se, GSI, fish size, and sampling location. As described earlier, historical data indicated fish size and sampling might influence egg Se concentrations, though these potential relationships may be confounded by other variables. The home range of NPM within the reservoir is unknown and so the extent to which ovary Se may reflect exposure to local Se sources (e.g., the Elk River) is also unknown. The developmental stage of a subset of ovaries were also characterized using histological techniques.
2) Attempts were made to collect a gradient of egg Se concentrations from ripe fish by collecting adult NPM of varying size (30-60+ cm) from several locations in Koocanusa Reservoir. This was ultimately unsuccessful but led to the collection of an increased number of ovary samples for Se analysis.

4. FIELD SAMPLING

Details of the field sampling strategy and methods employed are provided in the NPM Study Plan (EcoTox et al., 2019) and summarized here.

4.1 Sampling Strategy

There were originally two phases to the NPM field sampling program. In Phase 1 (beginning June 14, 2019), female NPM were collected from the BC side of the reservoir prior to reaching spawning condition to further characterize the effects of GSI, fish size, and sampling location on ovary Se concentrations as well as monitor spawning condition of the fish. Phase 2 sampling was

intended for once NPM reached spawning condition, and would involve collecting both male and female fish for the Se toxicity study. As only a few ripe fish were collected, Phase 2 sampling was never realized.

Although not explicitly part of this study, there was also an additional NPM sampling effort on the Montana side of the reservoir. In this effort (May 15, 2019), 15 female NPM were collected from Rexford in collaboration with personnel from Montana DEQ. This effort was made to ensure GSI data were collected and they represent the only fish from the Montana side of the reservoir for which GSI data are available.

Mature NPM were collected from various locations in Koocanusa Reservoir (BC side) using multiple sampling methods, consistent with scientific fish collection permit conditions and detailed in the NPM Study Plan (EcoTox et al., 2019). Six locations in Koocanusa Reservoir were initially identified in the study plan, but ultimately 10 locations were sampled in an attempt to collect additional females in spawning condition for the Se toxicity study (Figure 4). Sampling in these areas focused on inlets based on the assumption that NPM would congregate in these areas prior to moving upstream to spawn.

4.2 Sampling Methods

Northern pikeminnow were captured using multiple methods subject to and consistent with fish collection permit conditions. Short-set gill nets (starting with a maximum set time of 20 minutes) were used to reduce fish mortalities (Buchanan et al., 2002). Gill netting was anticipated to be the most efficient capture method and both cotton and monofilament 3-5” mesh nets were deployed. Short set times were used to avoid stress to both NPM and by-catch, particularly as species of concern, bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Onchorhynchus clarki lewisi*) are present in the reservoir. Three foot-diameter hoop nets were deployed and left to fish overnight (i.e., approximately 24 h). Cod pot traps were an additional capture method used, but not originally considered in the study plan. They function similarly to a minnow trap but on a larger scale (65” long x 40” wide, with 4” opening). These traps sit on the bottom substrate similar to the hoop net but sample a smaller area. Cod traps are quicker to deploy and pull; but are more difficult to transport as the metal frame cannot be collapsed. Similar to hoop nets, these traps were deployed and left to fish overnight (approximately 24 h). Angling was conducted from sampling boats. Angling was mainly employed between gill net sets as it has much lower catch per unit effort (CPUE) and often targets smaller individuals. Angling was also employed to scout the lower Elk River below the Elk River bridge at Kootenay Hwy 93. All fish captured were identified to species, enumerated, and all non-target individuals were released alive at the point of capture.

Northern pikeminnow sampled during Phase 1 were sacrificed by a decisive blow to the head. Fish processing and handling for tissue sampling was consistent with provincial guidelines (BC ENV, 2016).

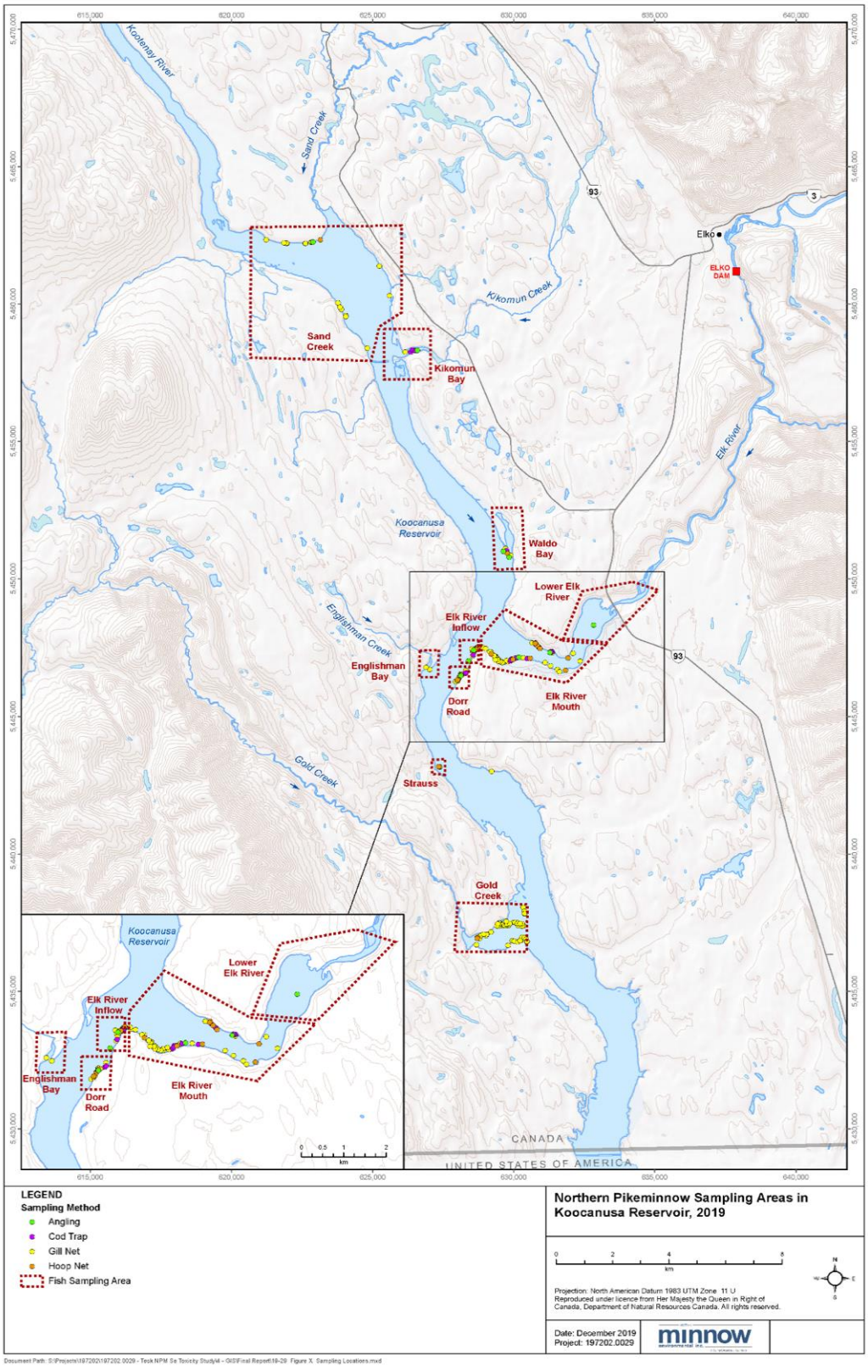

Figure 4. Northern Pikeminnow Sampling Areas on the Canadian Side of Koocanusa Reservoir.

Fish were kept on ice in coolers and transported to a dedicated field laboratory for processing as soon as possible following capture (i.e., within hours). Fork and total lengths and body weights were measured. Each fish was opened and the sex and/or sexual maturity recorded. Whole gonads and livers were removed from each fish and weighed to the nearest milligram using an analytical balance to allow for calculation of gonado-somatic indices. Whole ovaries were collected from each female and placed in separately labelled, polyethylene (Whirl-Pak®) bags. A skinless, boneless muscle fillet sample was also collected from each fish to provide supplemental data on muscle Se concentrations. Following these measures, age structures (i.e., otoliths) were removed from each fish. Each age structure was wrapped separately in waxed paper and placed inside a labelled envelope and archived for analysis. Internal or external deformities, erosions (fin and gill), lesions, or tumours (DELT) observed during processing (Sanders et al., 1999) and parasites were recorded. Tissue samples (ovaries, muscle, and age structures) were stored frozen pending shipment to the respective laboratory for analysis.

Mature female NPM retained for gonad and muscle collection were weighed and measured for total and fork length. Obvious external deformities, erosions (fin and gill), lesions, and tumours (i.e., DELT survey) and parasites observed during processing were recorded.

Ovary and muscle samples were all sent to Saskatchewan Research Council (SRC) in Saskatoon, SK for chemical analysis.

4.3 Permits

A permit for fish collection was obtained from the BC Ministry of the Environment and Climate Change Strategy (BC ENV *Application to Collect Fish for a Scientific Purpose*) and an additional permit was obtained for transport of eggs to the University of Saskatchewan (UoS) facility in Saskatoon, SK from Fisheries and Oceans Canada (License #119412), BC ENV (License #119412) and the Government of Saskatchewan (SK Import #2019-16).

5. LABORATORY METHODS

5.1 Ovarian Histology

All methods for histology preparation followed the UoS Toxicology Centre's draft standard operating procedure for histology (Appendix A). Field-collected NPM were dissected at the field laboratory and gonads excised, weighed and then immediately preserved in 10% buffered formalin. After 24 hours samples were transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming

table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

As, to the best of our knowledge, no previous studies have characterized NPM gonads histologically, oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish (OECD, 2009). Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

5.2 Analytical Chemistry

Ovary and muscle samples collected in the field were submitted for chemical analysis at SRC in Saskatoon, SK. In addition to Se, ovaries, eggs, and muscle were analyzed for 24 other elements (listed in Table 5 of the Study Plan). Results for these other elements are provided in Appendix C but are not discussed further in this report. Samples were analyzed using high resolution inductively coupled plasma mass spectrometry (HR-ICPMS). The detection limit for Se was 0.01 $\mu g\ g^{-1}$ dw. Moisture content was measured by freeze drying and results were reported on a dry weight basis along with moisture content to allow conversion to wet-weight values.

Standard quality control procedures for sample analysis were included as detailed in the Study Plan (EcoTox et al., 2019).

6. RESULTS

6.1 Fish Sampling

Four different methods were employed to capture NPM during the study: hoop nets, cod pot traps, gill nets and angling. The fish sampling was separated into 2 phases. The goal of Phase 1 was to sample approximately 36 sexually mature females of varying sizes and ranges of gonadosomatic index (GSI) values, with half being from sites directly influenced by the Elk River. This phase also allowed tracking of spawning condition within the population. When ripe fish were initially collected, Phase 2 sampling was initiated to focus on collecting fish for fertilization and assessment of larval deformities as a function of egg Se concentrations.

Different sampling methods had varying degrees of success in catching mature females and CPUE changed through the sampling period (Table 1). Monofilament gill nets were more successful than cotton mesh gill nets so after approximately two weeks remaining gill net sampling only used 3” and 4” monofilament nets. Overall, gill nets were the most successful capture method for mature females over the longest sampling period. Though gill nets had high

incidence of bycatch, survival rates were high (4 mortalities over 92 hours of effort in 9 sample areas) due to short set times. Elk Mouth, Elk Inflow and Gold Creek sites were sampled with greatest effort over the longest periods of time in response to capture success rates. Gold Creek and Elk Mouth, both at locations of tributary inflow, had the highest CPUE through the last weeks of June and tended to decrease through July. Elk Inflow area, where the Elk tributary inflow opens into the reservoir, had peak CPUE through mid-July then drastically declined moving into the last two weeks of July (Table 2).

Table 1. Female northern pikeminnow CPUE and total effort (hours) by gear type and sampling area.

CPUE	Dorr Rd.	Elk Inflow	Elk Mouth	Lower Elk	Sand Cr.	Gold Cr.	Englishman Bay	Strauss	Waldo Bay	Kikomun Bay	Total Gear CPUE	Total Gear Effort
Gill Net	5.24	60.00	55.34	-	7.78	136.61	0.00	8.11	0.00	-	273.08	
Gill Effort	7.68	18.00	19.47	-	10.85	31.45	1.70	1.50	1.20	-		91.85
Hoop Net	20.20	0.04	1.47	-	0.17	0.04	-	0.04	0.34	-	22.30	
Hoop Effort	596.25	67.68	492.08	-	46.43	93.50	-	95.18	117.37	-		1508.50
Cod Trap	0.43	0.19	0.25	-	-	-	-	-	0.00	0.04	0.91	
Cod Effort	238.43	237.76	420.62	-	-	-	-	-	24.00	99.42		1020.23
Angling	0.25	4.00	0.00	0.00	0.67	-	-	-	0.00	0.00	4.92	
Angling Effort	4.00	9.83	0.07	6.00	1.50	-	-	-	0.66	1.00		23.06
Total CPUE	26.12	64.23	57.06	0.00	8.62	136.65	0.00	8.15	0.34	0.04	**301.21**	
Total Effort	846.36	333.27	932.24	6.00	58.78	124.95	1.70	96.86	143.23	100.42		**2643.64**

Table 2. Gill net CPUE at most successful sample areas through the sampling period.

CPUE	Elk Inflow	Elk Mouth	Gold Creek
June	18.00	35.20	62.71
July 1-7	Not sampled	15.00	57.40
July 8-14	128.96	5.14	Not sampled
July 15-21	9.50	0.00	8.05
July 22-26	0.00	0.00	8.44

When sampling commenced many mature females had higher GSI than anticipated and there was difficulty capturing low GSI/large individuals and high GSI/small individuals. More fish were processed in an effort to capture the desired range of GSI and size. As the field season progressed and few ripe fish were captured, more fish were processed than originally anticipated with a total of 79 fish processed by end of the study (Table 3). This allowed inclusion of a greater GSI and size range as well as a range of egg development for histology analysis (15 fish). The high GSI/small size categories were eventually captured at Elk influenced sites but not at other sites (Table 3 and Figure 5).

Table 3. Fish GSI and total length for Rexford, Elk River and all other sites combined.

GSI/SIZE	300-400 mm	401-500 mm	501-600+ mm
Rexford, MT (n=15)			
<2%	2	6	0
2-4%	0	0	7
4-7%	0	0	0
7+%	0	0	0
Elk River, BC (n=49)			
<2%	3	6	1
2-4%	3	3	2
4-7%	2	3	3
7+%	3	11	9
Other Sites, BC (n=30)			
<2%	9	6	1
2-4%	0	2	1
4-7%	1	2	1
7+%	0	3	4

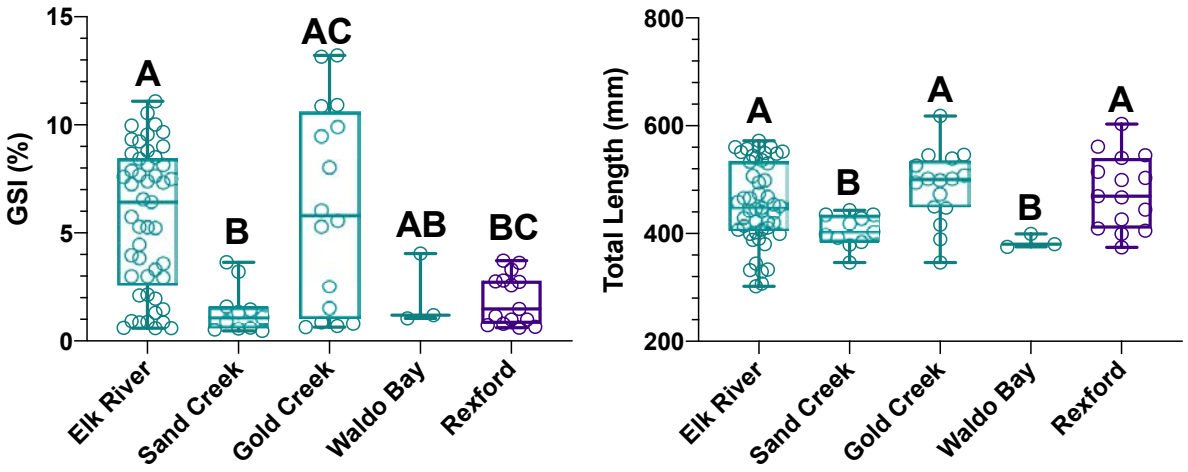

Figure 5. Northern pikeminnow GSI and total length by location for samples collected in 2019. Different letters indicate statistically significant differences ($p<0.05$) as determined by ANOVA.

6.2 Ovarian Histology

Between July 8 and 19, 2019, ovaries from 15 NPM were collected for histological analysis of maturation stages of oocytes across fish of different sizes and reproductive development (weight: 250 – 1800 g; fork length: 33.2 – 61.8 cm) and GSI (range: 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (Figure 6 and Table 4-1 in Appendix A). While there was no obvious relationship between the size of fish and GSI, there was a clear correlation between GSI and ovarian maturation stage (Figure 7) with fish having GSI >5% all being at oocyte maturation stage 3, with one exception. Similarly, there was a significant linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($r^2 = 0.81$), revealing that ovaries of mature fish with a GSI > 5% consisted of over 50% LVOs (Figure 6).

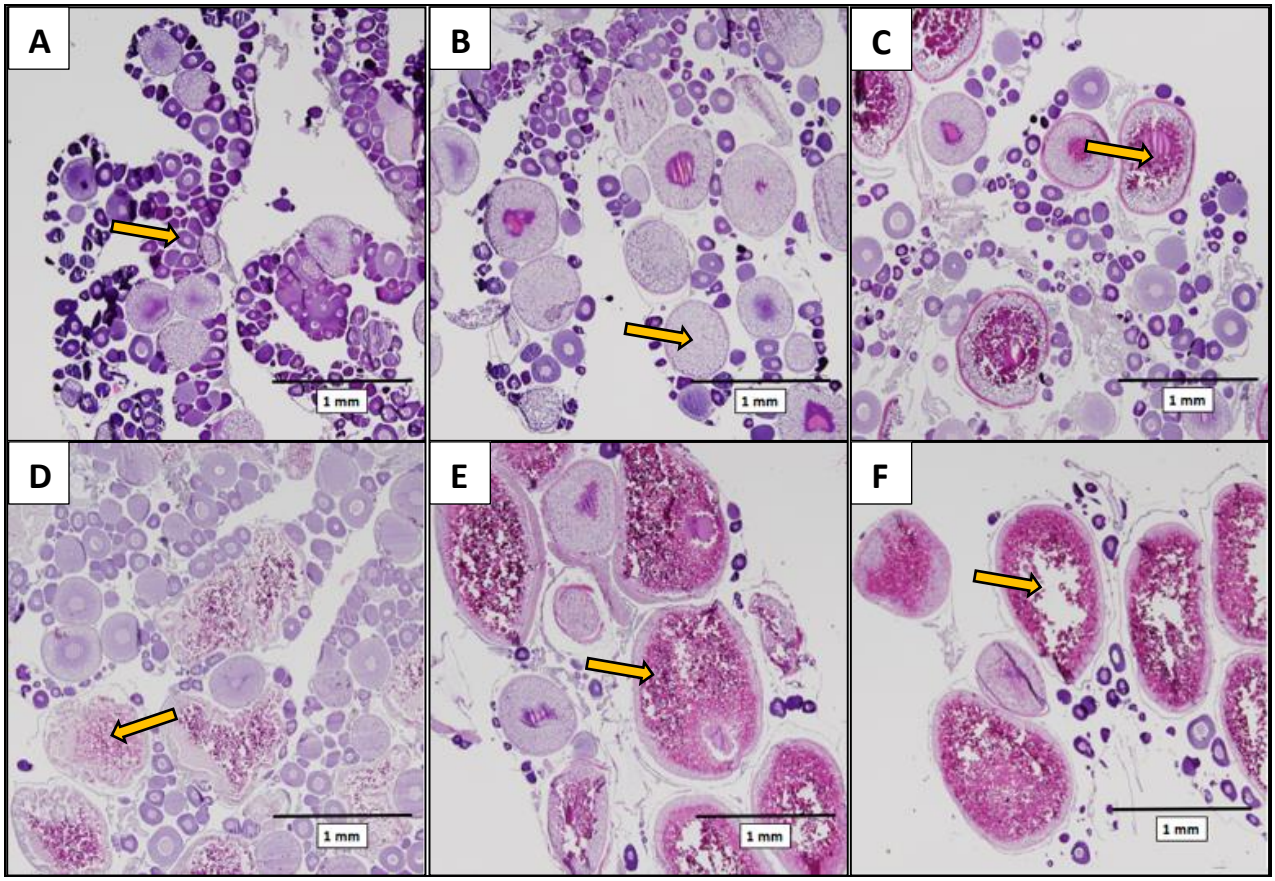

Figure 6. Histomicrographs of ovaries of northern pikeminnow representing early development stages. (Stage 1) consisting mainly of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E and F; Arrows).

Multivariate Analysis of Ovary Se Data

Analysis of historical ovary Se data for NPM suggests there are significant relationships with sampling location, fish size, and GSI (Figures 1-3). However, the historical data set lacks information on GSI for fish from the Montana side of the reservoir, may be confounded by correlations between fish size and GSI, and is limited for fish with a relatively high GSI (>5%). The sampling program for this study was designed to address these limitations and provide a robust dataset for evaluating the influence of multiple factors on ovary Se concentrations in NPM.

Historical sampling data (collected 2013-2018) were collated with data collected from 2019 (Appendix B). An initial exploratory analysis of natural log (ln)-transformed data was conducted by Principal Component Analysis (PCA) (prcomp, R) using z-scores of independent variables (total length, GSI, body weight, and gonad weight) to identify correlations among these variables and select the most appropriate variables for linear

modeling. The first two axes of the PCA with four input variables explained 99% of the variance in the four variables. Bivariate relationships among independent variables, and bivariate relationships between ovary Se and independent variables were plotted by area and year to help visualize effects of area and year on relationships. Natural log (ln) transformations of total length and body weight were highly correlated (R = 0.98), and a biplot from the first PCA with all four variables showed very similar relationship between body weight and total length and final PC scores. Because GSI includes body weight in its denominator, and body weight and total length were highly correlated, body weight was removed from the independent variables used in the MLR and total length was used as a measure of fish size in the model to reduce collinearity (Figure 8).

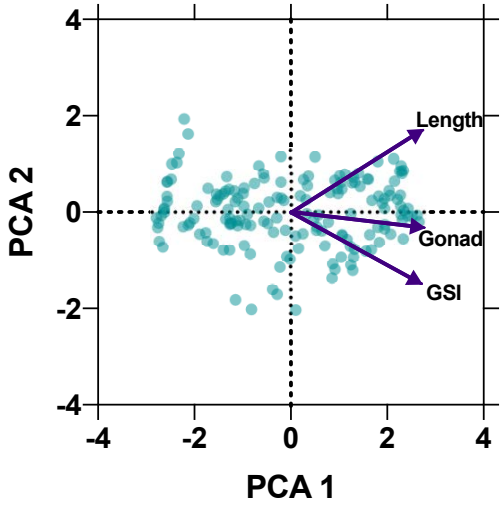

Figure 8. Biplot from PCA using z-scores of ln-transformed total length, gonad weight, and GSI.

After selecting initial independent variables (length, gonad weight, and GSI), exploratory linear and multiple linear regressions (MLR) were conducted to predict ln ovary Se for various subsets of the data. For example, models using one or more independent variables were developed for data sets for individual years, different combinations of years, individual locations, and different combinations of locations. These exploratory analyses were intended to gain a better understanding of how the data were distributed as a function of the independent variables, location, and sampling year. Based on these exploratory analyses, we concluded that the initial models should be developed using only the 2019 data because these data had been collected with a more balanced design of GSI and fish size classes than earlier data. Developing an initial model based on data from multiple years could introduce biases due to the incomplete sampling design with respect to the independent variables being evaluated.

The first model was developed to test for differences between area-specific slopes and intercepts with stepwise analysis using Bayesian Information Criterion (BIC) and to identify final models (Eq. 1). The contrasts used to test for area-specific intercepts tested for differences between Elk influenced sites and other sites.

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) +

Ln(GW) + area* Ln(GW) (Eq. 1)

where, OvSe = ovary Se, TL = total length (cm), GSI = gonadosomatic index, and GW = gonad weight. Variance inflation factors (VIFs) were relatively high for this model (>7) (Zuur et al., 2010) and gonad weight was not retained in the BIC version of the model, so gonad weight was removed and a second model was developed (Eq. 2).

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) (Eq. 2)

Area-specific slopes were not retained in the BIC model, resulting in a final model with area-specific intercepts and pooled slopes. Exclusion of area-specific slopes means that relationships between independent variables (total length and GSI) and ovary Se are statistically similar between sites. Retention of area-specific intercepts indicates that while differences in fish size and GSI between sites explains some of the observed differences in ovary Se concentrations, there are also statistically significant differences in ovary Se concentrations between some sites independent of the influence of fish size and GSI. This model performed reasonably well in terms of predicting ovary Se concentrations for the data on which it was based (Adj. R^2 = 0.72; Figure 9). Further, the predicted R^2 of 0.69 is just slightly lower than the adjusted R^2 of 0.72, indicating the model is not over-parameterized or unduly influenced by individual data points.

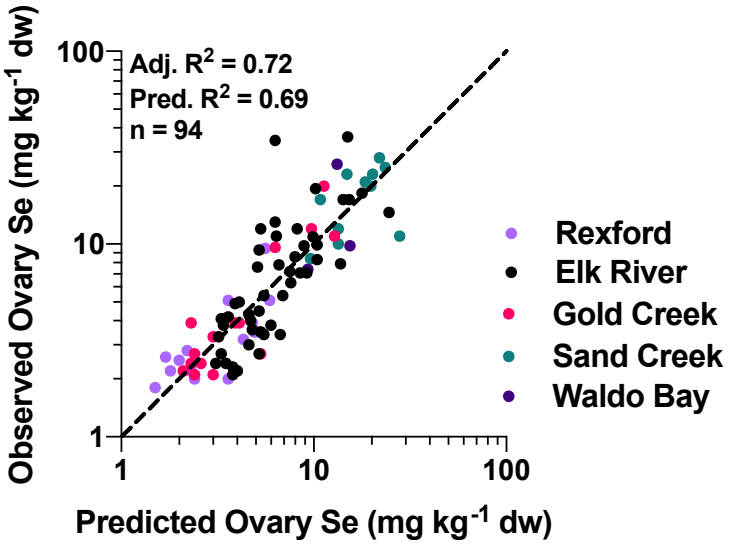

Figure 9. Ovary Se MLR model based on 2019 data only (see Eq. 2).

Once the MLR model based on data from 2019 only was developed, data from years prior to 2019 were then evaluated using the 2019 MLR model. Exploratory analyses indicated that data from 2014 and 2015 did not fit the model well. The majority of samples for 2014 were collected in February with the remaining 2014 samples and all 2015 samples collected in April. As would be expected given the sampling times, GSI was low in both data sets. The 2015 data set in particular consisted of fish with GSI <0.5%, which appears to introduce non-linearities into the overall relationship between GSI and ovary Se (Figure 10). Consequently, we opted to exclude the 2015 data from further analysis. It may have been possible to include the 2014 data set in the model, but given the limited amount of data (n=5) and limited ranged of GSI, we opted to exclude it from the analysis as well. Consequently, a model was fit using data from 2016-2019. The full model included "year" as a term to test for differences in ovary Se concentrations between years and as before was evaluated using BIC to select the most parsimonious variables for inclusion (Eq. 3).

$$\text{Ln(OvSe)} = \text{area} + \text{year} + \text{Ln(TL)} + \text{area*}\ \text{Ln(TL)} + \text{Ln(GSI)} + \text{area*}\ \text{Ln(GSI)} \quad \text{(Eq. 3)}$$

The final model selected by BIC using all data from 2016-2019 (n=141) retained the same variables as the model using only 2019 data with only slight differences in the model coefficients (Table 4). Adjusted and predicted R^2 for the BIC model were 0.67 and 0.65, respectively (Figure 11). The model tested for effects of year and area as well as area-specific slopes. Again, area-specific slopes were not retained in the BIC model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (ovary Se) across sites. Similarly, year was not retained as a factor in the model indicating there were no significant differences across the three sampling years (2016, 2018, and 2019) included in the analysis. Significant differences in area-specific intercepts were observed and retained in the model. The intercepts for both Gold Creek and Rexford were significantly lower than the Elk intercept ($p = 0.01$ and $p < 0.01$, respectively) indicating that after accounting for the influence of fish length and GSI, ovary Se concentrations in fish collected from Gold Creek and Rexford were significantly lower than those observed for fish collected near the Elk River (Table 4).

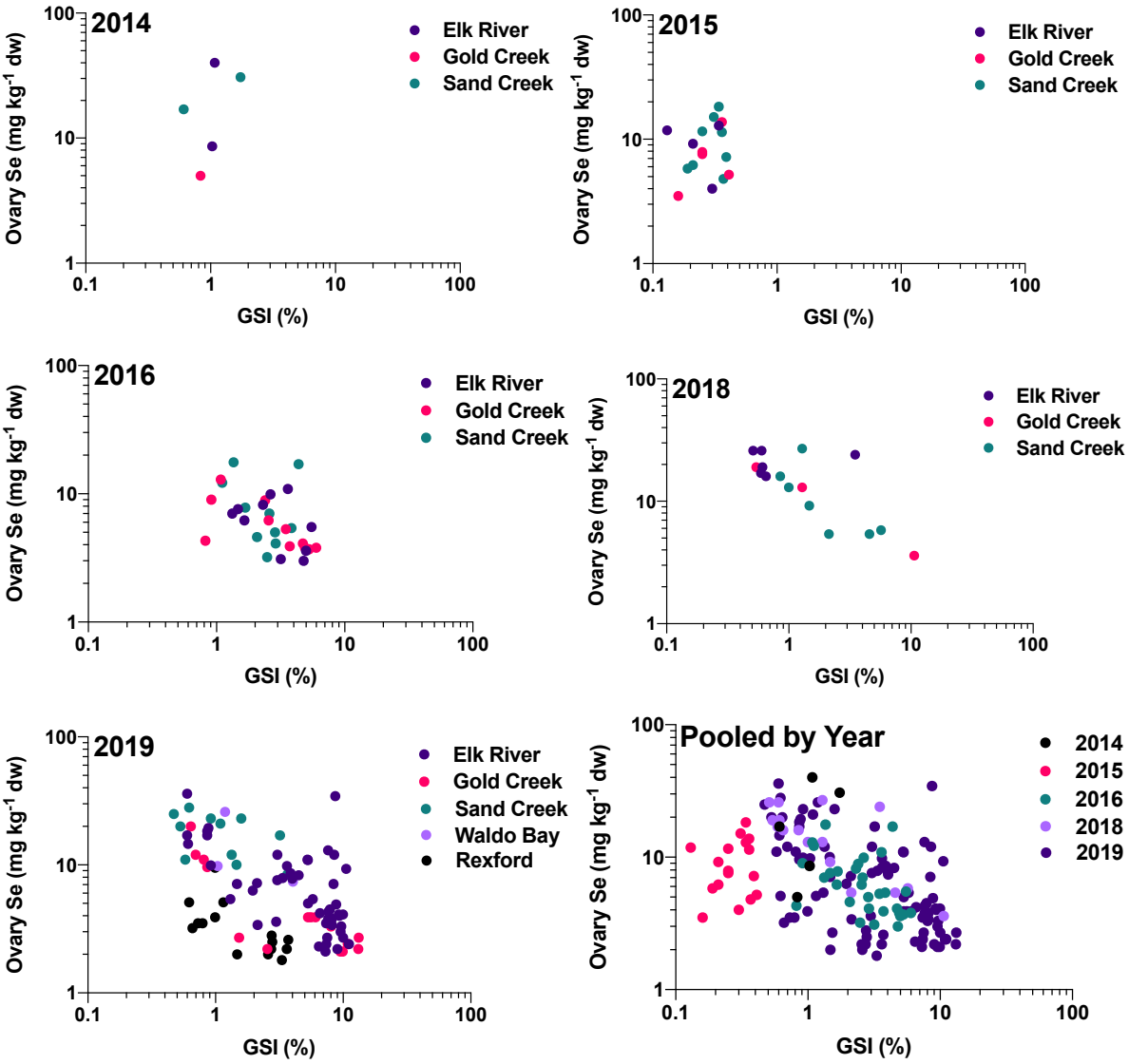

Figure 10. Relationship between GSI and Ovary Se by Year

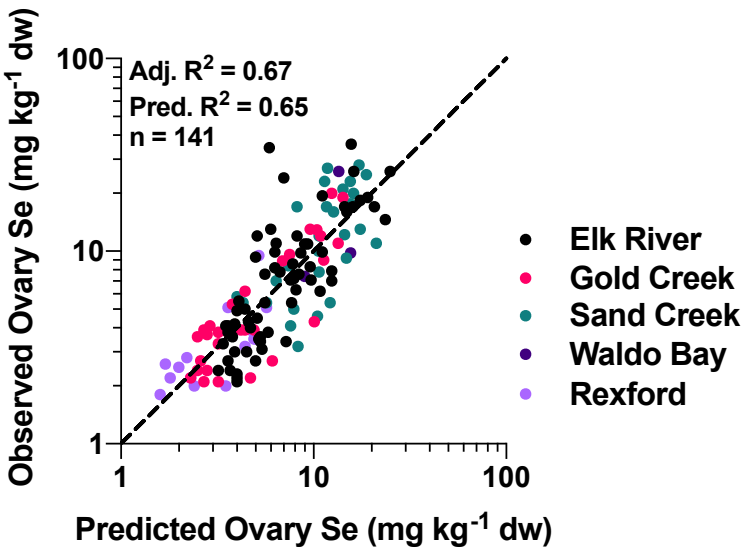

Figure 11. Final Ovary Se MLR model based on 2016-2019 data.

Table 4. Final ovary Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	7.94	0.96	8.31	-	
	Gold	7.66	0.10	-2.81	0.01	
	Sand	7.98	0.10	0.45	0.65	
	Waldo	8.02	0.26	0.33	0.75	
	Rexford	6.91	0.13	-7.62	<0.01	
Slopes	Ln Total Length	-1.45	0.26	-5.62	<0.01	-0.289
	Ln GSI	-0.39	0.05	-8.08	<0.01	-0.493

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that GSI (standardized slope = -0.49) has a stronger effect on ovary Se concentrations in the model than total length (standardized slope = -0.33) over the range sampled for these variables (Table 4). Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model appear to have equal variance (p = 0.145) but may not be normal (p = 0.031).

One potential caveat to this model is that the PCA analysis indicates a level of correlation between total length and GSI, as both variables have positive associations for PC1, though opposite associations for PC2 (Figure 8). A simple correlation analysis indicates these two variables are somewhat correlated (r=0.41; Figure 12). This correlation is primarily caused by the lack of data for fish with a total length >54 cm and GSI <3%. This observation is supported by the lack of a significant correlation (p >0.05) between total length and GSI for fish with a GSI >3%. It is unclear whether this data gap is due to sampling bias or some

mechanistic reason why fish in this category are not observed, though the former seems more likely. Regardless, this correlation introduces some uncertainty into the ovary Se model. While VIFs for the model were low (1.4 for both total length and GSI) suggesting the correlation is not unduly influencing the model, the full influence of this correlation can be difficult to characterize.

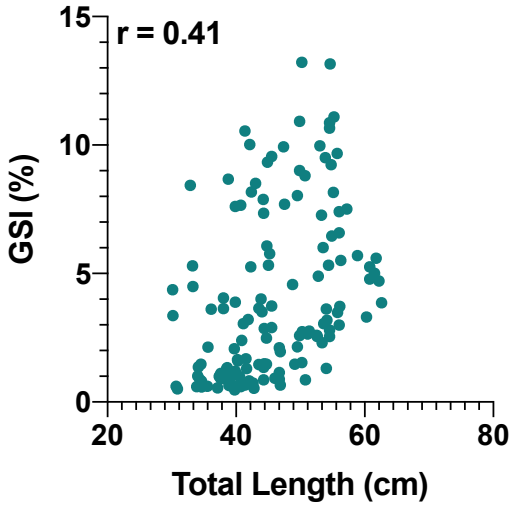

Figure 12. Correlation between total length and GSI in northern pikeminnow (2016-2019).

We further evaluated this issue by constraining the data set to only those data with GSI >3% (n = 69) where there is no correlation between fish length and GSI and re-parameterized the model. The resulting MLR model still retained both GSI and total length as variables (Adj. $R^2 = 0.55$), again supporting the premise that both variables are important predictors of ovary Se. However, the standardized model coefficients changed with total length (-0.49) now more important than GSI (-0.35). This reversal in relative importance of standardized model coefficients may simply be the result of constraining the original data set by ~50% or it could be an indication that the correlation between total length and GSI in the full data set is influencing the way variance is partitioned in the model.

Ultimately, the uncertainties associated with the correlation between total length and GSI appear to have relatively modest impacts on model predictions of ovary Se. Based on the final model using the full data set, differences in area-specific intercepts between sites would translate to predictions of ovary Se concentrations being, on average, 2.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold Creek and Elk River ovary Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.3 times higher than those from Gold Creek for a given fish length and GSI. Estimated mean ovary Se concentrations for small (30 cm) and large (60 cm) females with a

GSI of 6% (a conservatively low GSI for female fish ready to spawn) are below the BC ENV egg Se guideline (11 mg^{-1} kg dw) at all sampling locations using the MLR model based on the full data set. In comparison, the MLR model based on the data set constrained to a GSI >3% (i.e., the data set with no correlation between total length and GSI) provides higher estimates of mean ovary Se for small (30 cm) fish (Table 5), but estimates are generally within 30% of those using the MLR model based on the full data set. The somewhat larger increase in estimated mean ovary Se for Sand is the result of a higher intercept using the constrained data set.

Table 5. MLR model estimated mean ovary Se concentrations in female northern pikeminnow collected from different locations in Koocanusa Reservoir as a function of fish size and GSI. Estimated provided for the MLR model based on all data and the model based only on data where GSI was >3%.

Site	Fish Length (cm)	GSI (%)	Estimated Mean Ovary Se (mg kg^{-1} dw) All Data	Estimated Mean Ovary Se (mg kg^{-1} dw) Data with GSI >3%
Elk River	30	6	10.1	11.6
	60	6	3.7	3.5
Gold Creek	30	6	7.6	9.3
	60	6	2.8	2.8
Sand Creek	30	6	10.5	15.0
	60	6	3.8	4.6
Waldo Bay	30	6	10.9	9.0
	60	6	4.0	2.7
Rexford	30	6	3.6	5.0
	60	6	1.3	1.5

6.4 Multivariate Analysis of Muscle Se Data

Concurrent with ovary sampling, muscle samples have also been collected and analyzed for Se concentrations. The muscle Se data is a potential second line of evidence to support the observations and conclusions from the ovary Se analysis. As has been demonstrated in other species (USEPA, 2016), ovary Se and muscle Se concentrations in NPM are correlated (Figure 13). Consequently, observations based on ovary Se concentrations regarding the effects of fish size and sampling location are expected to also be observed for muscle Se data. While there is no identified mechanistic link between GSI and muscle Se concentrations, it is possible a correlation between GSI and muscle Se might be observed given the correlations between ovary Se and muscle Se, as well as total length and GSI in the data set.

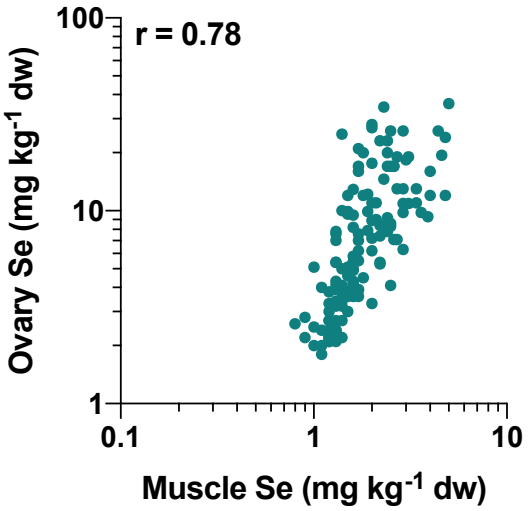

Figure 13. Correlation between muscle Se and ovary Se concentrations in NPM collected from Koocanusa Reservoir (2016-2019).

The multivariate analysis of muscle Se data used the same general multivariate approach described above for ovary Se data. The same data used in the ovary Se analysis was used in the muscle Se analysis for comparability except for a single fish collected from the mouth of the Elk River in 2016 for which no muscle data were collected (n = 140). Given the results of the ovary Se analysis, an initial model using only the 2019 data was not developed for muscle. Instead, the full data set (2016-2019) was used to evaluate the same general full model:

Ln(muscle Se) = area + year + Ln(TL) + area* Ln(TL) + Ln(GSI) +

area* Ln(GSI) (Eq. 4)

The model selected by BIC retained both total fish length and GSI as variables. Adjusted and predicted R^2 for the BIC muscle Se model were lower than for the ovary Se model at 0.47 and 0.45, respectively (Figure 14). The lower performance of the muscle Se model appears to be driven by underprediction of the relatively high muscle Se data for fish collected from the mouth of Elk River, although area-specific slopes were not retained in the model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (muscle Se) across sites (Table 6). Significant differences in area-specific intercepts were identified in the model. The intercepts for Gold Creek, Sand Creek and Rexford were all significantly lower than the Elk intercept ($p < 0.01$) indicating that after accounting for the influence of fish length and GSI, muscle Se concentrations in fish collected from all three locations were significantly lower than for fish collected near the Elk River (Table 6). Based on the final model, differences in area-specific intercepts between sites would translate to predictions of muscle Se concentrations being, on average, 1.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold and

Sand Creeks versus Elk River muscle Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.2-1.3 times higher than those from Gold and Sand Creeks for a given fish length and GSI.

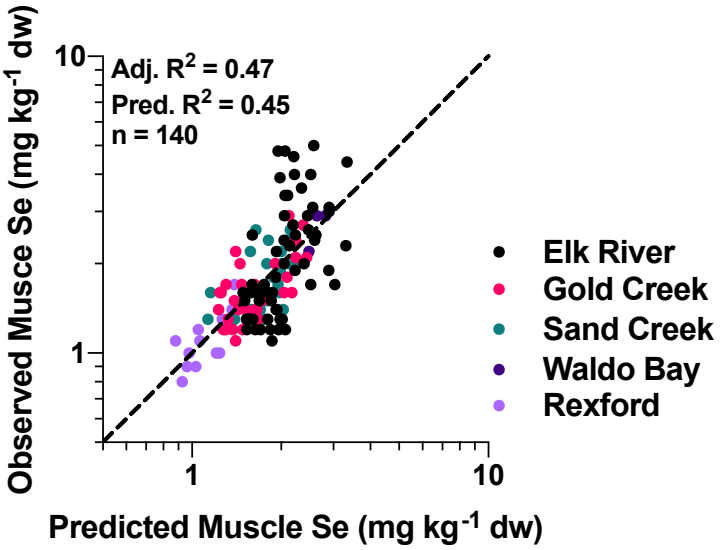

Figure 14. Final Muscle Se MLR model based on 2016-2019 data.

Table 6. Final muscle Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	4.197	0.607	6.92	-	
	Gold	4.022	0.063	-2.77	<0.01	
	Sand	3.919	0.066	-4.21	<0.01	
	Waldo	4.263	0.167	0.40	0.69	
	Rexford	3.619	0.085	-6.76	<0.01	
Slopes	Ln Total Length	-0.889	0.164	-5.41	<0.01	-0.406
	Ln GSI	-0.086	0.030	-2.81	<0.01	-0.215

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that total fish length (standardized slope = -0.41) has a stronger effect on muscle Se concentrations in the model than GSI (standardized slope = -0.21) over the range sampled for these variables (Table 5). This is the opposite of what was observed for ovary Se, but again, should be treated with caution given the correlation between total length and GSI. Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model have unequal variance (p = 0.001) and are not normally distributed (p = 0.001) again demonstrating the model has some systematic biases.

7. DISCUSSION AND CONCLUSIONS

The objectives of this study were to: 1.) determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations, and 2.) to evaluate the effects of GSI, fish size, and sampling location on ovary and muscle Se concentrations. The first objective was not achieved due to the inability to collect a sufficient number of ripe female NPM. In the remainder of this report, the success in achieving the second objective and implications of study findings are discussed.

7.1 Effects of GSI, Fish Size, and Sampling Location on Ovary Se Concentrations

Historical monitoring data suggest GSI, fish size, and sampling location may influence ovary Se concentrations, but the data are confounded by relatively small sample sizes, are unevenly distributed for some variables (e.g., GSI), and potentially auto-correlated. To address these limitations, a total of 94 additional ovary Se samples were collected in 2019 that were relatively evenly distributed across size classes and to a lesser extent GSIs.

Using 2019 data and incorporating most of the data from historical monitoring (total n = 141), an MLR model that characterizes ovary Se concentrations as a function of fish size (total length) and GSI was developed. While the model has some uncertainties related to the correlation between total fish length and GSI in the data set used for model development, the conclusion that total length and GSI are important predictors of ovary Se concentrations in NPM appears robust and predictions using a constrained data set with no correlation between independent variables are generally within 30% of the model based on the full data set.

There were several key findings from this model. First, the model indicates that fish with lower GSI have higher ovary Se concentrations independent of any other variables. This indicates that fish collected early in the year (e.g., February-May) have ovary Se concentrations that overestimate the egg Se concentrations that the fish will have at the time of spawning. The mechanism underlying this reduction in egg Se with development is currently unclear. Transfer of Se into the eggs is known to be associated with vitellogenesis (Janz et al., 2010) and the ovarian histology component of this study demonstrates vitellogenesis coincides with egg development and an increase in GSI, as is typical of most teleost fish. Consequently, an increase in egg Se rather than a decrease in egg Se would be expected with increasing GSI. However, there are many species-specific complexities to the process of vitellogenesis including variations in the use of multiple vitellogenin isoforms, variations in timing of primary and secondary vitellogenesis, and the level of processing of vitellogenin in the egg and associated level of water absorption (Hara et al., 2016). These processes could all influence how Se concentrations in eggs change during the course of egg development and to the best our knowledge, have not been studied in any detail in NPM or closely related species. However, regardless of the mechanism, the reduction in ovary Se concentrations with increasing GSI is important for assessing potential Se risks to NPM in the

reservoir as it is the ovary/egg Se concentration at the time of spawning that should be compared to an egg Se effect concentration.

Given this finding, ovary Se data from fish with low GSI (i.e., <5%) should be excluded from the data set when assessing potential risk to NPM. The oocyte maturation study demonstrated a strong positive relationship ($R^2 = 0.81$) between GSI and oocyte development. Fish where the majority of oocytes in an ovary were stage 3 (late vitellogenic) oocytes were associated with a GSI >5% (Figure 6). Consequently, only ovaries collected from fish with a GSI ≥5% should be used in assessing Se risks to NPM as these ovaries are likely to provide a relatively unbiased estimate of egg Se concentrations for comparison to egg Se toxicity thresholds. Using this data usability qualifier (GSI ≥5%) restricts the ovary Se data set. All data prior to 2016 are eliminated from assessment due to either low GSI or GSI not being reported and the distribution of ovary Se concentrations are significantly lower (Figure 15). Of all the samples collected from fish with a GSI >5% (n=45), only a single fish has exceeded the USEPA egg Se criteria of 15.1 mg kg^{-1} dw and only 4 fish have exceeded the BC ENV guideline of 11 mg kg^{-1} dw.

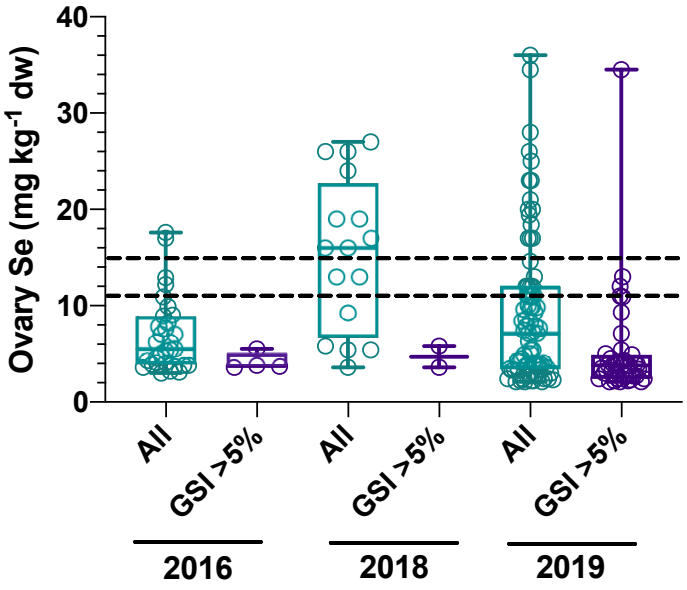

Figure 15. Comparison of ovary Se concentrations for all fish versus only fish with GSI ≥5%. Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines.

The second significant finding from development of the ovary MLR model was that fish size has a significant effect on ovary Se concentrations in NPM, with smaller fish having higher ovary Se concentrations. This is likely the result of differences in dietary preferences between small and large adult NPM. Small adult NPM (<30 cm) typically have a primarily insectivorous diet, but become increasingly piscivorous with increasing size, feeding

primarily on juvenile salmonids (Clarke et al., 2005; Petersen, 2001; Zimmerman, 1999). Whole body trophic transfer factors (TTFs) for fish (i.e., invertebrate to fish or fish to fish) are typically <1 except at very low (<1 mg kg^{-1} dw) dietary Se concentrations (DeForest et al., 2007). A consequence of TTFs <1 is that consumers at progressively higher trophic levels will have progressively lower whole body Se concentrations (i.e., biodilution). This may explain the size effect observed in the current analysis as small NPM feeding on insects would be expected to have higher Se exposure than large NPM which have a higher trophic level and are feeding on juvenile salmonids.

The third, and final, significant finding resulting from the ovary Se MLR model was identification of effects of sampling location on ovary Se concentrations. By accounting for the influence of fish size and GSI on ovary Se, the model was able to test for differences in ovary Se concentrations between sampling locations. Results from this analysis indicate that fish collected from Gold Creek and Rexford have significantly lower ovary Se concentrations than locations sampled higher in the reservoir. Locations higher in the reservoir are generally closer to the Se input from the Elk River although the Sand Creek sampling location is further from the Elk River than the Gold Creek sampling location (Figure 4).

7.2 Effects of GSI, Fish Size, and Sampling Location on Muscle Se Concentrations

The muscle Se MLR model was not as robust as the ovary Se MLR model (Figures 11 and 13). There are likely several reasons for this outcome. First, the range in muscle Se concentrations (0.8-5.0 mg kg^{-1} dw) is much less than observed for ovary Se concentrations (1.8-36 mg kg^{-1} dw). Consequently, small errors in analytical precision will introduce significantly more variance in the muscle Se MLR model. Second, although apparently not significant enough to be detected by the BIC analysis, the muscle Se data collected from the mouth of the Elk River qualitatively appear to have a systematic bias (i.e., different slope) with respect to the MLR model, over-predicting low muscle Se concentrations and under-predicting high muscle Se concentrations.

Despite the muscle Se model being less robust, it did generally support the observations of the ovary Se model. Specifically, the muscle model supports that fish size is an important variable in determining NPM Se concentrations in Koocanusa Reservoir (Table 5). It also supports observations that fish collected from the mouth of the Elk River have higher Se tissue concentrations than fish from most other locations sampled in the reservoir (Table 5).

The finding that GSI is a significant variable in the muscle Se model was somewhat unexpected. Mechanistically, there is no obvious reason why GSI would be an important determinant of muscle Se concentrations. It is possible that retention of GSI in the model is simply an artifact of GSI being an important variable in predicting ovary Se and ovary Se being generally strongly correlated to muscle Se, or that total length and GSI are somewhat correlated. Supporting this hypothesis is the observation that the standardized slope for GSI is

only half of the slope for fish size (Table 6), indicating it has proportionally less influence on muscle Se concentrations while whereas the opposite is true for ovary Se where the standardized slope for GSI is twice as steep as for fish size (Table 4).

7.3 Conclusions and Recommendations

A primary objective of this study was to determine the sensitivity of embryo-larval NPM to maternally transferred egg Se, which was not achieved due to the limited number of ripe female fish collected. However, the second objective of this study to evaluate the effects of fish size, GSI and sampling location on NPM ovary Se was successfully accomplished. Results from this effort indicates that all three variables influence ovary Se concentrations. Importantly, the study concludes that ovary Se data collected from fish with a GSI <5% should not be used to assess Se risks to NPM as these data over-estimate egg Se concentrations. However, the study also demonstrates that small adult NPM have higher egg Se concentrations than large NPM likely due to a predominantly insectivorous diet and that NPM near the Elk River and further upstream (i.e., Sand Creek) have higher egg Se concentrations than those collected further down the reservoir.

Based on these results, this sub-population (small adult fish that reside in the upper reservoir) of NPM likely exhibit higher egg Se concentrations than the overall NPM population in the reservoir, although the mean ovary Se concentration is still predicted to be below the BC ENV egg Se guideline. The relative size of this sub-population and distribution of egg Se concentrations within it is not well characterized, but current results suggests understanding the sensitivity of NPM to maternally transferred egg Se concentrations may still be important. Consequently, additional sampling to characterize the distribution of ovary Se concentrations in NPM in the upper reservoir and to conduct a toxicity study to determine their sensitivity to egg Se concentrations is recommended.

The main limitation of the 2019 Se toxicity study was an inability to capture a sufficient number of ripe female NPM. It is currently unclear why there was so much difficulty collecting a greater number of ripe females. Relatively large numbers of females were collected in the second half of June with GSIs in the range expected for ripe females (Table 2). This continued into early July, but despite relatively high GSIs, only a few fish manually expressed eggs. By mid-July, the CPUE began to drop rapidly and fish that had already spawned began to be captured. As the field season progressed, it was apparent that NPM were not continually congregating in the same areas during the presumed spawning period. Where abundant ripe males were found one day, no ripe males were present only two days later. It was expected that ripe females would be present in these congregations of males or join them days after they were located. This was not the case. Considerable effort (2,644 fishing hours) was invested using four different capture methods across a large spatial scale (~30 km reach of the reservoir). Although an abundance of mature females with high GSIs were captured in

the first four weeks of sampling, CPUE dropped drastically through the last two weeks without locating the desired numbers of ripe females.

A clearer understanding of where Koocanusa NPM populations are spawning is needed, including whether it occurs in congregations and whether tributaries are possible spawning habitat areas. Some changes in gear use, particularly setting gill nets during dusk and dawn, may increase capture rates, but this introduces new safety issues for crews, which will need to be addressed in planning. Greater capture success may result from investing efforts in tracking NPM movements within Koocanusa Reservoir. Understanding NPM movements provides possibilities for improving understanding of habitat use during spawning and allows more focused fishing efforts in suspected spawning sites.

8. CLOSURE

We trust this report provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact Kevin Brix at (305) 773-8347.

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APPENDIX A

UNIVERSITY OF SASKATCHEWAN SELENIUM TOXICITY REPORT

APPENDIX B

NORTHERN PIKEMINNOW OVARY SELENIUM, MUSCLE SELENIUM, AND GSI DATA FOR KOOCANUSA RESERVOIR: 2008-2019

APPENDIX C

NORTHERN PIKEMINNOW METAL TISSUE DATA: 2019

Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

Report

Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

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March 2020

Table of Contents

1 Study Rationale

Ongoing monitoring in the transboundary Koocanusa Reservoir located in British Columbia (BC) indicated a range of selenium (Se) concentrations in wild northern pikeminnow (NPM; *Ptychocheilus oregonensis*) that in some cases exceeded the U.S. Environmental Protection Agency (USEPA) criterion for fish egg/ovary of 15.1 mg/Kg dry weight [dw] and the British Columbia Ministry of the Environment and Climate Change Strategy (ENV) guideline of 11 mg/Kg dw (Brix et al. 2019). Embryonic life-stages of fishes are particularly susceptible to Se exposure via maternal transfer (Janz et al. 2010). However, to the best of our knowledge no studies have investigated the sensitivity of NPM to Se. Also, recent data suggest that there is a negative correlation between relative gonad size of females (as represented by gonadosomatic index [GSI]) and ovary Se concentrations. While this trend could indicate lower exposures of embryos under the assumption that GSI is directly related to maturation stage (later maturation stages are assumed to have greater GSIs, which were reported to have lower Se concentrations), little is known about gonadal phenotypes and their correlation with GSI in this species.

Therefore, this study aimed to investigate 1) the potential effects of maternal transfer of Se to embryos of NPM collected from several sites on the BC side of Koocanusa Reservoir, representing a gradient of Se concentrations, and 2) to characterize gonadal maturation phenotypes prior to and during the reproductive season of NPM in Koocanusa Reservoir. Unfortunately, despite extensive efforts, an insufficient number of female NPM in spawning condition were collected to properly characterize the relationship between egg Se concentrations and NPM embryo-larval development. Consequently, this report only presents the methods and results of the gonadal maturation characterization.

2 Objective

The main objective of this study was to determine whether egg Se concentrations found in NPM from different locations in Koocanusa Reservoir as well as the Elk River, BC may have effects on developing embryos and larvae of NPM. The secondary objective of this study was to characterize ovarian phenotypes of NPMs prior to and during their reproductive season using histology. To accomplish this,

Specific objectives to be addressed during the 2019 NPM early life stage (ELS) studies were:

- Characterize concentrations of Se in parent fish and embryos collected from the BC portion of Koocanusa Reservoir.
- Collect gonadal tissues (representing a range of GSIs) from NPM of different sizes prior to and during the reproductive season to characterize ovarian maturation and oocyte developmental stages.
- Establish a field-fertilization, and an on-site and laboratory culture protocol for NPM embryos and fry.

- Characterize survival, growth, and development of ELS of NPM related to tissue Se concentrations in ovaries of parent fish and eggs/embryos.
- Describe (if detectable) the toxicity threshold concentration (LC_{10} [mortality] and/or EC_{10} [time to hatch, time to swim-up, teratogenicity, growth]) of maternally transferred Se in NPM embryos.

Unfortunately, an insufficient number of female NPM in spawning condition were collected during the study to allow for full development of a protocol and characterization of the effect of maternally transferred Se on developing NPM embryos and larvae. Consequently, only the methods and results for the ovarian histology assessment are provided in this report.

3 Methods

3.1 Ovarian Histology to Assess Gonadal Maturation Stages

All methods for histology preparation followed the UofS Toxicology Centre's draft standard operating procedure (Appendix A). Field-collected NPM were dissected on site and gonads were excised, weighed and then immediately preserved in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed with an automated unit by the UofS Health Sciences Histology Core Facility, to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings, and cooled for 20 minutes to allow sufficient hardening. Because the ovary samples were fragile, blocks were pre-sectioned to expose the tissues and soaked in a glycerin-ethanol solution for 24 hours before section collection. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section, and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

Oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads (2009) - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish. Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

Gonadosomatic indices (GSIs) were calculated for all fish from which gonads were collected for histological assessment as follows (Eq. 1):

GSI = gonad weight [g] / body weight [g] *100 (1)

4 Results & Discussion

4.1 Ovarian Histology to Assess Gonadal Maturation Stages

Between July 8, 2019 and July 19, 2019, ovaries from a total of 15 NPM were collected for histological analyses of maturation stages of oocytes across fish of different sizes (weight range: 250 – 1800 g; fork length range: 33.2 – 61.8 cm), and GSIs (range 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (*Figure 4-1*; ***Table 4-1***). While there was no obvious relationship between the size of fish and GSIs, there was a clear positive correlation between GSI and ovarian maturation stage (*Figure 4-1*) with fish having GSIs greater than or equal to 5% all grouping in the final maturation stage (3) with one exception. Similarly, there was a significant and linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($R^2 = 0.81$), revealing that ovaries of mature fish with a GSI greater than 5% consisted of over 50% LVOs (*Figure 4-2B*). Finally, there was a negative relationship between ovarian Se concentrations and GSI as well as oocyte development stages (*Figure 4-3*).

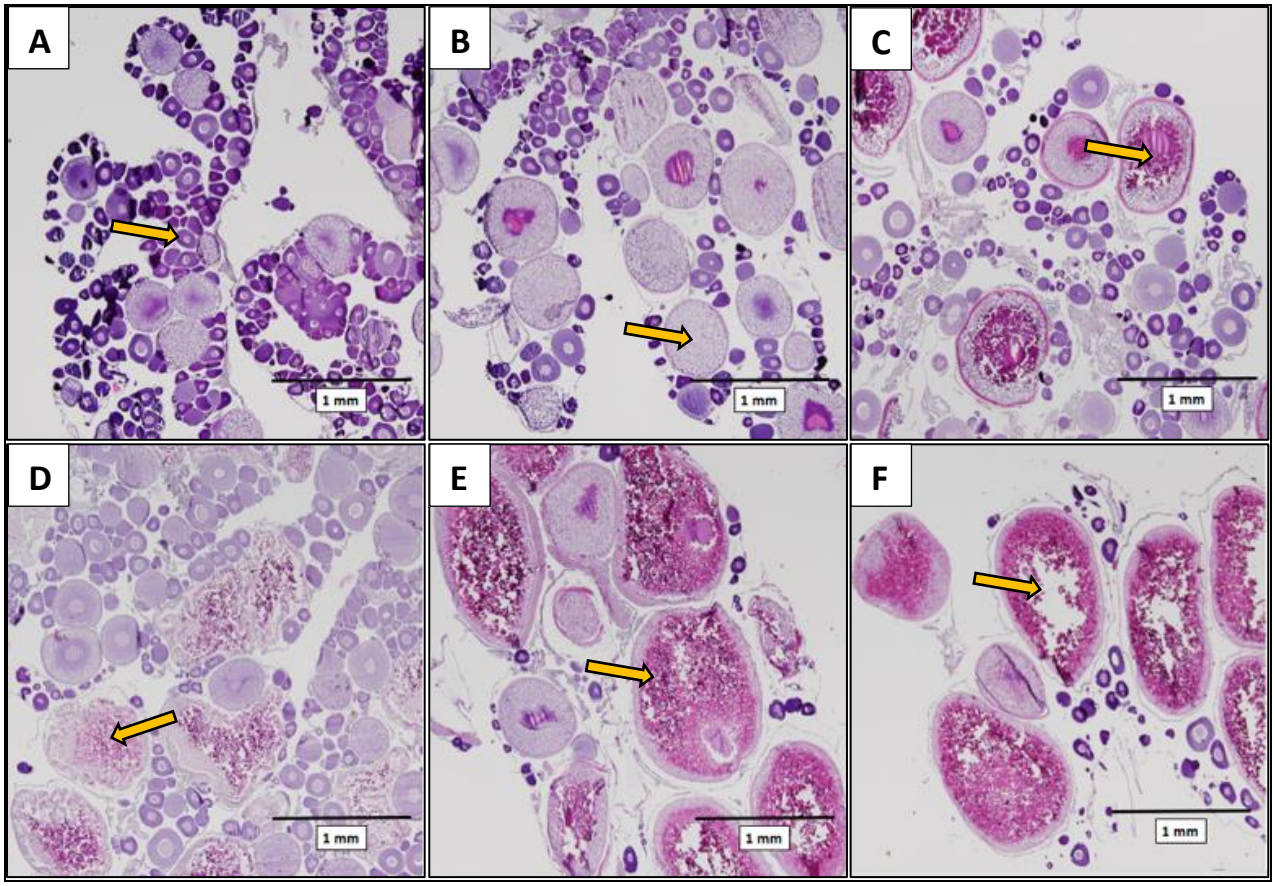

Figure 4-1. Histomicrographs of ovaries of northern pikeminnow representing early development stages (Stage 1) predominantly consisting of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E&F; Arrows).

Table 4-1. Summary of northern pikeminnow oocyte histology analysis detailing maternal morphometric characteristics, oocyte development counts, percent area covered by cell development categories, assessed gonadal developmental stage, and notes. Abbreviations are defined as follows: GSI – gonadosomatic index; PO – perinucleolar oocytes; CAO – cortical alveolar oocytes; EVO – early vitellogenic oocytes; LVO – late vitellogenic oocytes.

Sample ID	Maternal Assessment				Histological Assesment									
	Total Length (cm)	Total Weight (g)	GSI (%)	Ovary Se (µg/g)	Cell Type Count				Percent Area of Cell Type				Developmental Stage	Notes
					PO	CAO	EVO	LVO	PO	CAO	EVO	LVO		
SC-06	43.5	495	1.46	10	341	40	25	10	61.8	16.9	12.7	8.5	2	Atresia Present
GC-14	50.8	1030	0.86	9.6	364	38	4	0	67.7	28.9	3.4	0.0	1	
GC-15	61.8	1800	5.60	3.9	34	12	4	10	6.3	8.6	4.8	80.3	3	
ER-31	33.2	300	5.30	10.9	62	9	8	20	9.0	4.6	10.7	75.7	3	
ER-34	54.0	1470	1.31	5.4	156	46	10	8	37.4	27.6	13.1	21.9	2	
ER-35	40.7	530	7.65	3.8	47	9	4	18	11.2	3.7	5.3	79.7	3	
ER-36	41.4	650	10.54	9.3	19	8	5	14	3.9	6.9	9.8	79.4	3	
ER-37	45.2	750	5.77	5.4	67	8	3	3	32.5	14.2	17.6	35.7	2	
ER-38	41.1	560	3.05	12	54	11	12	9	11.5	11.6	38.5	38.4	2	
ER-39	42.1	580	10.02	2.7	42	10	4	12	10.6	7.4	7.5	74.4	3	Atresia Present
ER-40	34.4	250	0.86	18.4	367	37	0	0	73.5	26.5	0.0	0.0	1	
ER-41	42.4	530	8.17	3.4	48	6	3	10	18.3	5.2	6.8	69.7	3	
ER-42	42.3	620	5.25	11	35	7	4	5	12.0	10.9	22.8	54.3	3	
ER-44	40.7	610	0.60	36	255	64	0	0	41.7	58.3	0.0	0.0	1	
ER-45	49.9	1200	9.01	2.2	11	3	1	6	7.1	8.8	5.2	79.0	3	

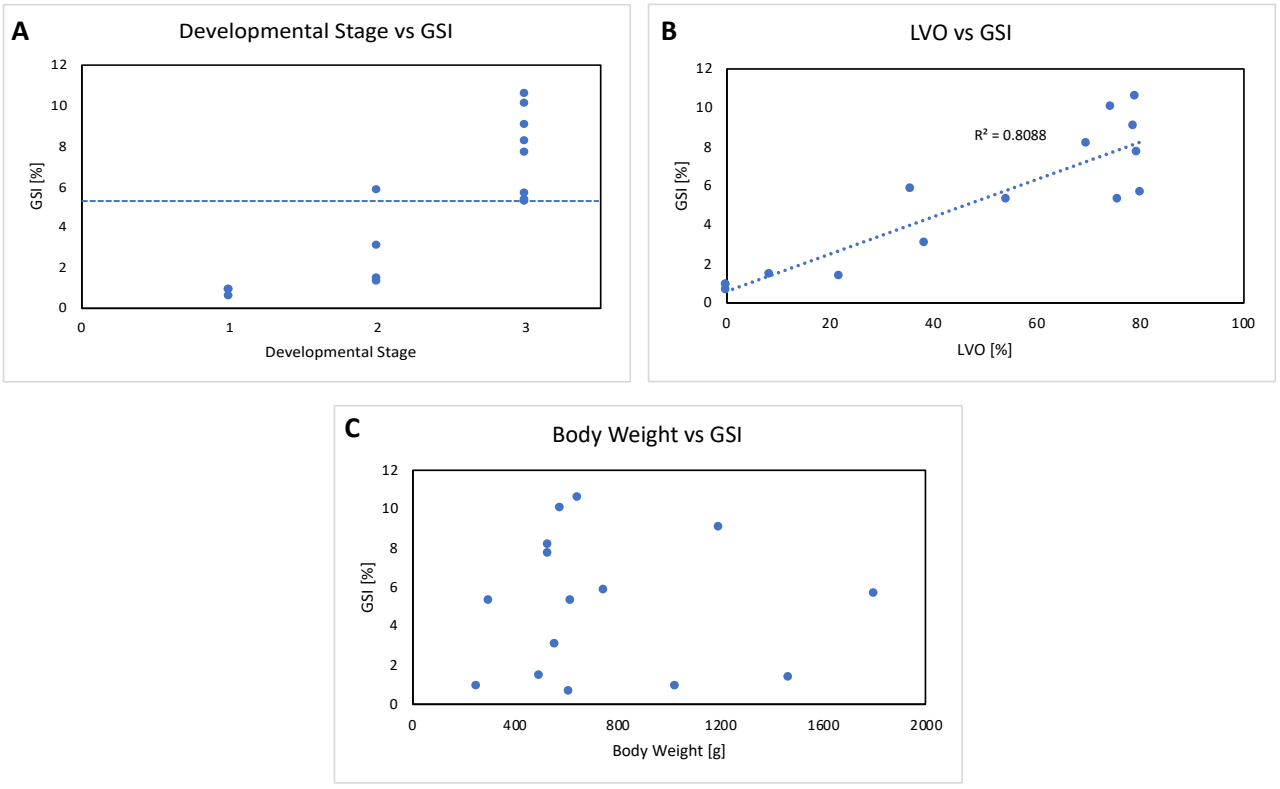

Figure 4-2. Relationships between gonadosomatic indices (GSIs; %) and A) body weight, B) LVO, and C) gonadal development stage in northern pikeminnow. Dotted line represents the 5% GSI level.

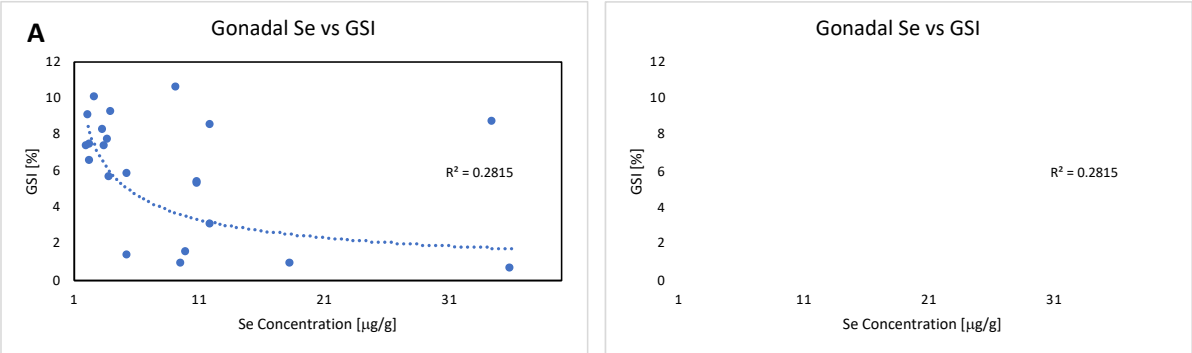

Figure 4-3. Relationship between ovarian Se concentration (µg/g d.w.) and A) gonadosomatic index (GSI), or B) Developmental Stage of oocytes in northern pikeminnow collected from the Koocanusa Reservoir. **Note:** Panel A includes data from 6 additional fish for which no histological evaluation was conducted.

5 Conclusions

This study successfully characterized, for the first time, the phenotypes of different ovarian maturation stage prior to spawning. Clear correlations between histologically determined proportion of follicular stages and GSI were described, demonstrating that fish in the final maturation stage (3) all had GSIs greater than or equal to 5%. Finally, there was significant, albeit weak, negative correlation between ovarian Se concentrations and maturation stage and GSI, indicating greater exposure of immature females. However, sample size and variability were such that future studies are required to confirm this relationship.

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Appendix A – Histological Procedures

University of Saskatchewan

Toxicology Centre

STANDARD OPERATING PROCEDURE

Histological Procedures

Draft

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DEFINITIONS AND ACRONYMS

PLEASE NOTE:

This protocol was produced for and by highly-qualified personnel of the Toxicology Centre of the University of Saskatchewan. It is therefore an in-house document and it should not be distributed without previous consent.

1.0 TISSUE SAMPLING & FIXATION

Morphometric measurements must be recorded as quickly as possible after the experimental animal is euthanized, because rapid degradation of tissues interferes with subsequent histological analyses. Record the following as applicable: individual ID#, sex, length (total length, fork length, standard length, snout-to-vent length, etc.), total weight, gonad weight, liver weight, appearance of secondary sex characteristics, and deformities or other external abnormalities.

There are several options for collecting histology samples, depending on the size of the organism:

1.1 Wole body/intact:

Fix the animal whole, and leave it intact for subsequent processing. In this case, the whole organism must be small enough to fit in a histocasette/standard paraffin block/microscope slide (e.g. fathead minnow at 30 days post-hatch);

1.2 Whole body/dissect

Fix the animal whole, and excise the tissues of interest at a later date prior to processing. For example, an adult fathead minnow or *Xenopus* metamorph can be fixed whole, transferred to 70% ethanol for storage, and then dissected to remove tissues of interest such as liver, gonad, thyroid etc. In this case, during sampling it is necessary to make an incision to expose the internal organs and allow rapid penetration of the fixative.

- Make a shallow mid-ventral incision through the body wall the entire length of the body cavity, being careful to not damage any of the internal organs;
- For adult fathead minnows, make a lateral incision up one side of the body wall to allow the fixative to penetrate the viscera. If possible, using fine forceps, gently move the viscera aside, grasp the swim bladder and discard;
- Attach an individual paper tag with ID# to the body using a needle, fishing line and a waterproof ‘rite-in-the-rain’ paper tag. Label with pencil only, because ethanol removes ink. Attach the tag by passing the needle and line through the body and tying it off.

1.3 Tissue necropsy:

Excise tissue samples from the freshly euthanized animal prior to fixation. This is done for large-bodied specimens that can’t be fixed whole;

- In some cases an entire organ can be excised completely intact and fixed whole for histological analysis. Alternately, it may be necessary to remove only a portion of the tissue of interest, which should be done in a standardised manner, e.g.:

- middle portion of the left or right gonad;
- a particular lobe of the liver;
- right 2nd gill arch;
- middle portion of the posterior kidney;

- When possible, tissue samples should not exceed 1 cm in any direction, although there are exceptions.

When collecting samples for histology, it is preferable to use chemical overdose, because physical methods of euthanasia can sometimes damage histological samples. It is possible to collect different types of samples from a given individual, e.g. remove the fresh liver for biochemical analysis, and then fix the remaining tissues for histology.

1.4 Fixation:

Proper fixation of tissues is one of the most crucial steps in routine histology, and should be kept consistent across samples. The histology samples (i.e. tissue samples in histocassettes, or whole body samples) should be placed in fixative within 2-3 minutes of euthanasia. Ensure that samples are fully submerged, using a minimum of 10 volumes of fixative to 1 volume of tissue. Use Nalgene wide-mouth leak-proof polyethylene containers.

Samples should remain submerged in fixative for 48 hours, and are then transferred to 70% ethanol for storage. Fixative cannot be re-used, and should be disposed appropriately. The 70% ethanol should be poured off and replenished two more times (minimum 1-2 days each) to remove excess fixative prior to tissue processing.

Commonly used histological fixatives include: 10% Neutral Buffered Formalin, Cal-Ex™ II (Fisher), Davidson's Fixative, and Bouin's fluid. Those containing acids have superior tissue penetration with the added advantage of de-calcifying bone, which can improve tissue sectioning. Cal-Ex is therefore preferred for whole body fixation. Davidson's Fixative is also popular; it can be prepared in advance using stock chemicals, and has a reasonable shelf life for longer-term storage.

Davidson's Fixative

Formalin	200 ml
100% Ethanol	300 ml
Glycerin	100 ml
Glacial Acetic Acid	100 ml
Distilled Water	300 ml

2.0 SPECIMEN GROSSING

Once fixed and stored in 70% ethanol, the specimens can be further trimmed if necessary prior to tissue processing. Whole body samples can be dissected to remove tissues of interest. In some cases, an entire organ can be excised (e.g. the gonad, liver), or alternately, a representative portion of the tissue of interest can be removed. Note: fixed weights and lengths can be used to generate condition factor, gonado- and hepatosomatic indices.

3.0 TISSUE PROCESSING and EMBEDDING

Fixed tissue specimens (stored in 70% ethanol) are loaded into a Vacuum Infiltration Processor (aka "**Tissue Processor**"). This programmable, automated unit contains reservoirs of various solvents as well as molten paraffin wax. The tissue processor can be programmed to control temperature, stir the solutions, and create pressure/vacuum cycles during sample processing, all of which can enhance the penetration of solutions through the tissues. The purpose of the process is: (1) **dehydration** - the tissues are bathed in a series of progressively stronger alcohols (70% up to 100%) to remove excess water from the cells, (2) **clearing** - the alcohol is flushed from the tissues and replaced with xylene or toluene (which are capable of dissolving paraffin) (3) **infiltration** - the tissues are infiltrated with molten paraffin. The final result is an intact tissue sample perfused with paraffin, which is immediately placed in a paraffin-filled mould and allowed to cool.

4.1 Tissue Processing:

It takes 14 hours to run a batch of samples through the tissues processor; this is typically done overnight, with sample embedding happening the following morning. Ensure that the samples are stored in a third rinse of 70% ethanol prior to loading them in the processor.

4.2 Embedding

1) Arrive at the Lab 20-30 minutes before the end of the processing run to prepare for embedding.

- Ensure that the Cryo station is turned on;
- Label an embedding ring for each sample to be embedded;
- Coat the embedding moulds with a thin layer of Mould Release, and place them on the warming console;

2) When the processing run is complete, remove the samples from the tissue processor and place them in the 'holding basin' full of molten paraffin wax in the embedding

console. Ensure that the samples do not cool down and solidify at this point, i.e. get the cassettes into the melted wax as quickly as possible, and keep the lid closed;

3) Using the heated paraffin dispenser, place a small amount of paraffin in the bottom of the mould. (Note that the paraffin dispenser flow rate can be adjusted);

4) Open a histocassette and spill the tissue sample out into the holding wax. Set the cassette aside.

5) Using heated forceps, gently grab the tissue and place in the bottom of the mould to attain the appropriate ***orientation for sectioning***. Place it on the cooling pad for 5-10 seconds, to ensure that the wax gels, and the tissue is held in place.

6) Place the labelled embedding ring on the mould with and fill with paraffin. The wax level should be above the rim of the embedding ring to account for shrinkage during cooling. Set it on the Cryo console to cool.

7) Repeat until all samples are embedded, working as quickly as possible.

8) Leave the blocks on the Cryo console for ~20 minutes. Gently pull the mould off and set the block on the benchtop to cool. Transport solidified blocks back to the Toxicology Centre, and let sit overnight prior to attempting any trimming or sectioning.

Table 1. Tissue processing program used at the University of Saskatchewan Histology Core Facility. Tissues are dehydrated in graded alcohols (Station 1 to 7), cleared in xylene (Station 8 to 10), and infiltrated with molten paraffin (Station 11 to 14).

Station	Reagent	Time	Temp (°C)	Pres/Vac Cycle	Mix
1	Ethanol 70%	1 hr	ambient	V	On
2	Ethanol 80%	1 hr	ambient	V	On
3	Ethanol 95%	1 hr	ambient	V	On
4	Ethanol 95%	1 hr	ambient	V	On
5	100% ethanol	1 hr	ambient	V	On
6	100% ethanol	1 hr	ambient	V	On
7	100% ethanol	1 hr	ambient	V	On
8	Ethanol/Xylene	1 hr	ambient	V	On
9	Xylene	1 hr	ambient	V	On
10	Xylene	1 hr	ambient	V	On
11	Paraffin	1 hr	60	V	On
12	Paraffin	1 hr	60	V	On
13	Paraffin	1 hr	60	V	On
14	Paraffin	1 hr	60	V	On

5.0 MICROTOMY (aka SECTIONING)

The embedding ring of the paraffin block is mounted on a rotary microtome. Ribbons of thin sections are created, and these are placed on glass microscope slides. The user can control the thickness of the sections (usually 5-7 μm, thinner is generally better), as well as the number and spacing of the sections retained on the microscope slide. There are several options for sectioning:

- **Single representative section** – one section is retained from each block, this is considered to be representative of the entire tissue;
- **Serial sectioning** - the user cuts through the entire tissue, and all sections are retained (labour-intensive);
- **Step sectioning** - the user cuts through the tissue and retains representative sections at pre-defined intervals;

5.1 General Methods for Sectioning:

- Turn on slide warming table, let it warm up to 40°C (temperature is generally pre-set, and shouldn't require adjustments);
- In a small beaker, prepare ~40mL of distilled water containing ~4 drops of Mayer's Albumin mounting medium, stored in fridge. This should be sufficient for 1 day of sectioning; fresh solution should be made up daily (1 drop Mayer's per 10 mL dH20);
- Use a razor blade to trim excess wax from the tissue blocks to within 2mm of the tissue edge. Maintain square sides on trimmed portion;
- Wipe down a fresh microtome blade with xylene to remove the oil coating, and mount it in the knife holder;
- Ensure microtome is clean and lubricated (see user manual);
- Pre-label a slide for the first paraffin block using solvent resistant marker (slides will be dipped in solvents during staining).
- Place the block firmly in the microtome chuck. Section the block according to the specific protocol (i.e. a single 'representative' section per block, step sections, or serial secions). The sections should come off the blade in continuous ribbons. Note that if the blocks are trimmed small, numerous sections and multiple rows of sections can be placed on a single slide;
- Place the labelled slide on the warming table and flood with the mounting medium. Float the sections of interest on the slide until they appear smooth and free of wrinkles;

- Once the sections are smooth, wipe away excess mounting medium from the slide (Kimwipe), and place it in a slide holder. Full racks of slides are stored in the 40C oven (minimum overnight) prior to staining;
- If scratches or nicks appear in the ribbons during sectioning, move the blade to an unused area, or replace entirely;

6.0 SLIDE STAINING - HEMATOXYLIN and EOSIN

Once the tissue sections have been allowed to dry overnight in a 40°C oven, they can be stained for light microscopy. Myriad staining techniques exist; Hematoxylin and Eosin ("**H&E**") is a common 2-part staining technique routinely used for basic paraffin sections. A rack full of slides is immersed in a series of solvents and stains, resulting in de-waxing of the sections and differential uptake of the 2 stains in various cellular components.

6.1 Staining:

- The stains and solvents can be used to stain app. 10 – 12 racks of slides, and then must be replaced. Check with other lab users regarding the status of the stain series, or if necessary check the quality of the most recently stained slides for fading or loss of contrast. Solvents can be topped up if they have evaporated down;
- Do not stain paraffin sections unless the slides have dried in 40°C oven for minimum 24 hours;
- Multiple racks can be stained at once. When the first boat is in the hematoxylin, a second rack can be started;
- It takes ~45 minutes to stain and coverglass one rack of slides;
- Staining and coverglassing are done in the fume hood;
- Before starting, check supplies of cytoseal and coverglass (use #1 thickness).

Table 2: Step-by-step staining process.

Station	Solution	Time	Notes
1	Xylene 1	2 min	
2	Xylene 2	2 min	
3	Xylene / 100% Ethanol	2 min	1:1 Ratio
4	100% Ethanol	2 min	
5	95% Ethanol	2 min	
6	70% Ethanol	2 min	
7	Tap Water	2 min	Replace often
8	Distilled Water	2 min	Replace often
9	Hematoxylin	5 min	
10	Tap water	rinse 4x	Water should run clear
11	Acid Alcohol (0.1%)	15 sec	0.1ml HCl/100ml 70% EtOH
12	Water	rinse 2x	
13	Phosphotungstic Acid (0.33%)	30 sec	(0.33 g/100ml Water)
14	Citric Acid (0.33%)	30 sec	(0.33 g/100ml Water)
15	Running Tap Water	5 min	
16	Eosin Y	2.5 min	
17	Tap Water	rinse 4x	Water should run clear
	70% Ethanol		
18	95% Ethanol	1 min	
19	100% Ethanol	2 min	
20	100% Ethanol	2 min	
21	Xylene / 100% Ethanol	2 min	1:1 Ratio
22	Xylene	2 min	
23	Xylene	Holding	

6.2 Coverglassing:

- Slides should be coverglassed as soon as possible after staining is completed. The slide rack is held in the last Xylene station until coverglassing is completed;
- Place a coverglass on a cork, add thin line of cytoseal full length;
- Remove slide from Xylene, blot slide edge on paper towel, do not allow Xylene to evaporate completely;
- Turn slide upside down, slowly lower it onto the coverglass at a slight angle, avoid trapping air bubbles in the cytoseal;
- Wipe off the back of the slide, and place flat on trays to dry, ensuring that the slide edges are not touching each other. Slides should air dry minimum 1 week before placing in slide boxes.

Appendix B. Northern Pikeminnow Ovary Selenium, Muscle Selenium, and GSI data for Koocanusa Reservoir: 2008-2019

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
MT	2008	May	14	-	Rexford	2.8	1.0	54.8	-	1973.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.5	1.0	48.3	-	1340.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.1	51.3	-	1347.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.1	51.8	-	1740.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.7	1.2	53.7	-	1708.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.2	1.2	56.2	-	1705.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.8	1.2	50.2	-	1592.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.2	50.0	-	1226.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.9	1.2	50.7	-	1306.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.9	1.3	53.1	-	1720.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.9	1.3	55.8	-	1789.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.3	49.5	-	1303.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.3	47.6	-	1183.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.4	50.9	-	1557.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.6	52.3	-	1586.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.7	51.8	-	1728.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.0	1.9	60.3	-	2259.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.5	1.9	47.0	-	1140.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.7	1.5	56.3	-	1851.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.4	1.5	48.6	-	1134.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.7	1.7	51.7	-	1297.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	1.7	47.0	-	1039.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.3	1.8	52.2	-	1465.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.4	1.8	53.8	-	1506.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.3	1.8	50.2	-	1190.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.4	1.9	61.2	-	2696.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	6.0	2.0	46.8	-	953.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	8.1	2.3	47.6	-	1043.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.1	2.3	51.0	-	1361.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.0	2.3	45.1	-	925.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	2.4	48.8	-	1052.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.5	56.2	-	1860.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.7	1.5	51.0	-	1343.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.3	1.6	50.4	-	1148.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.5	1.7	46.3	-	898.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.7	42.2	-	662.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.9	51.9	-	1134.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.2	1.9	44.4	-	776.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.9	1.9	46.4	-	1116.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.7	2.3	46.6	-	762.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	40.1	5.0	41.0	37.2	505.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	25.7	1.6	38.2	34.9	465.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	3.3	2.4	29.5	27.2	316.0	-	-	-	-	-
BC	2014	April	-	-	Elk River	21.9	4.6	39.3	35.2	440.0	-	-	-	-	-
BC	2014	April	-	ER-PM-14G-Apr-14	Elk River	40.1	6.2	34.1	30.8	312.0	-	3.4	-	-	1.08
BC	2014	April	-	ER-PM-11G-Apr-14	Elk River	8.6	2.5	37.3	33.5	438.0	-	4.5	-	-	1.03
BC	2014	February	-	-	Gold Creek	7.6	2.8	39.3	35.7	495.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	15.4	2.2	35.5	32.3	360.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	10.3	2.5	38.6	34.9	500.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	4.1	2.4	38.0	-	580.0	-	-	-	-	-
BC	2014	April	-	GC-PM-10G-Apr-14	Gold Creek	5.0	2.3	40.4	36.5	522.0	-	4.3	-	-	0.83

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2014	February	-	-	Sand Creek	13.7	1.6	35.5	32.1	320.0	-	-	-	-	-
BC	2014	February	-	-	Sand Creek	3.8	1.2	49.9	45.4	1200.0	-	-	-	-	-
BC	2014	April	-	SC-PM-10G-Apr-14	Sand Creek	17.0	1.6	34.4	31.2	300.0	-	1.8	-	-	0.61
BC	2014	April	-	SC-PM-01G-Apr-14	Sand Creek	30.7	2.9	36.1	32.8	355.0	-	6.2	-	-	1.74
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	4.0	-	35.8	32.3	380.0	-	1.1	-	-	0.30
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	12.9	4.1	34.2	30.8	320.0	13.0	1.1	4.01	315	0.34
BC	2015	April	-	ER-NSC-25-Apr-15	Elk River	9.2	3.1	40.4	36.5	560.0	13.0	1.2	7.81	551	0.21
BC	2015	April	-	ER-NSC-13-Apr-15	Elk River	11.8	6.0	46.7	42.7	885.0	13.0	1.1	12.97	871	0.13
BC	2015	April	-	GC-NSC-13-Apr-15	Gold Creek	5.2	1.9	32.1	28.9	252.0	9.0	1.0	5.50	245	0.41
BC	2015	April	-	GC-NSC-31-Apr-15	Gold Creek	13.8	2.0	33.0	30.2	261.0	12.0	0.9	2.30	258	0.36
BC	2015	April	-	GC-NSC-33-Apr-15	Gold Creek	7.9	2.3	37.4	34.0	390.0	10.0	1.0	2.41	387	0.25
BC	2015	April	-	GC-NSC-34-Apr-15	Gold Creek	7.6	1.6	37.5	34.0	390.0	9.0	1.0	2.10	387	0.25
BC	2015	April	-	GC-NSC-49-Apr-15	Gold Creek	3.5	1.3	44.8	40.1	880.0	12.0	1.4	17.22	861	0.16
BC	2015	April	-	SC-NSC-37-Apr-15	Sand Creek	7.2	1.7	32.6	29.4	261.0	9.0	1.0	3.22	257	0.39
BC	2015	April	-	SC-NSC-36-Apr-15	Sand Creek	4.8	1.9	33.5	30.0	275.0	10.0	1.0	2.51	271	0.37
BC	2015	April	-	SC-NSC-33-Apr-15	Sand Creek	11.4	1.4	34.0	30.2	300.0	8.0	1.1	3.30	296	0.36
BC	2015	April	-	SC-NSC-47-Apr-15	Sand Creek	18.3	2.4	34.4	30.8	325.0	13.0	1.1	3.66	320	0.34
BC	2015	April	-	SC-NSC-44-Apr-15	Sand Creek	15.1	2.4	35.2	31.7	370.0	13.0	1.2	5.49	363	0.31
BC	2015	April	-	SC-NSC-39-Apr-15	Sand Creek	11.6	2.0	38.0	34.0	445.0	12.0	1.1	3.34	441	0.25
BC	2015	April	-	SC-NSC-13-Apr-15	Sand Creek	6.2	1.5	40.0	36.3	492.0	14.0	1.0	8.33	483	0.21
BC	2015	April	-	SC-NSC-46-Apr-15	Sand Creek	5.8	1.7	41.6	37.5	630.0	13.0	1.2	14.58	614	0.19
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	3.1	-	54.1	49.5	1500.0		47.7			3.18
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	10.9	2.9	36.1	32.9	455.0	16.0	16.4	5.00	434	3.60
BC	2016	April	-	ER-NSC-19 O-Apr-16	Elk River	7.0	1.7	38.6	35.2	555.0	14.0	7.4	25.12	522	1.33
BC	2016	April	-	ER-NSC-17 O-Apr-16	Elk River	6.2	2.0	40.2	36.2	615.0	14.0	10.2	9.28	596	1.65
BC	2016	April	-	ER-NSC-27 O -Apr-16	Elk River	7.6	1.3	49.1	45.0	1200.0	15.0	17.6	24.42	1,158	1.47
BC	2016	April	-	ER-NSC-38 O-Apr-16	Elk River	9.9	1.5	51.1	47.0	1400.0	15.0	36.9	24.67	1,338	2.64
BC	2016	April	-	ER-NSC-16 O-Apr-16	Elk River	8.2	1.6	53.4	48.0	1540.0	14.0	35.5	17.35	1,487	2.31
BC	2016	April	-	ER-NSC-28 O-Apr-16	Elk River	5.5	1.7	56.3	50.8	1900.0	22.0	104.8	31.83	1,763	5.51
BC	2016	April	-	ER-NSC-15 O-Apr-16	Elk River	3.0	1.5	60.8	55.9	2600.0	20.0	124.3	46.89	2,429	4.78
BC	2016	April	-	ER-NSC-29 O-Apr-16	Elk River	3.6	1.6	61.5	56.7	2640.0	21.0	132.0	55.87	2,452	5.00
BC	2016	April	-	GC-NSC-17 O-Apr-16	Gold Creek	9.0	2.1	37.6	33.9	435.0	12.0	4.0	7.86	423	0.91
BC	2016	April	-	GC-NSC-01 O-Apr-16	Gold Creek	12.9	1.6	38.2	34.9	450.0	13.0	4.9	8.31	437	1.08
BC	2016	April	-	GC-NSC-14 O-Apr-16	Gold Creek	8.9	2.0	40.9	36.9	585.0	13.0	14.1	5.36	566	2.40
BC	2016	April	-	GC-NSC-16 O-Apr-16	Gold Creek	4.3	1.6	42.0	38.3	610.0	12.0	5.0	6.28	599	0.82
BC	2016	April	-	GC-NSC-12 O-Apr-16	Gold Creek	3.9	1.3	45.5	41.6	940.0	14.0	35.1	15.21	890	3.73
BC	2016	April	-	GC-NSC-26 O-Apr-16	Gold Creek	3.8	1.5	53.5	48.0	1600.0	15.0	96.2	42.73	1,461	6.01
BC	2016	April	-	GC-NSC-24 O-Apr-16	Gold Creek	3.8	1.4	52.8	48.2	1640.0	14.0	80.3	25.92	1,534	4.89
BC	2016	April	-	GC-NSC-06 O-Apr-16	Gold Creek	6.2	1.7	54.5	48.8	1400.0	15.0	35.7	22.15	1,342	2.55
BC	2016	April	-	GC-NSC-08 O-Apr-16	Gold Creek	5.3	2.2	55.8	50.6	1870.0	17.0	65.1	30.61	1,774	3.48
BC	2016	April	-	GC-NSC-13 O -Apr-16	Gold Creek	3.7	1.6	60.8	55.6	2360.0	17.0	124.2	62.56	2,173	5.26
BC	2016	April	-	GC-NSC-05 O-Apr-16	Gold Creek	4.1	1.6	62.2	57.0	2500.0	15.0	117.8	58.55	2,324	4.71
BC	2016	April	-	SC-NSC-25 O-Apr-16	Sand Creek	17.0	2.6	30.1	27.2	257.0	12.0	11.2	3.32	242	4.37
BC	2016	April	-	SC-NSC-09 O-Apr-16	Sand Creek	17.6	2.0	34.1	30.5	315.0	13.0	4.3	3.97	307	1.36
BC	2016	April	-	SC-NSC-21 O-Apr-16	Sand Creek	12.2	1.9	37.6	33.8	520.0	13.0	5.8	9.69	505	1.11
BC	2016	April	-	SC-NSC-29 O-Apr-16	Sand Creek	4.6	1.5	39.7	35.9	550.0	10.0	11.4	13.65	525	2.07
BC	2016	April	-	SC-NSC-03 O-Apr-16	Sand Creek	7.8	1.3	41.5	37.5	760.0	14.0	12.7	9.50	738	1.68
BC	2016	April	-	SC-NSC-35 O-Apr-16	Sand Creek	5.0	1.4	44.4	39.8	935.0	14.0	26.8	20.35	888	2.86
BC	2016	April	-	SC-NSC-36 O-Apr-16	Sand Creek	3.2	1.4	44.7	40.4	875.0	14.0	21.7	21.84	831	2.48
BC	2016	April	-	SC-NSC-32 O-Apr-16	Sand Creek	4.1	1.4	45.5	41.2	996.0	15.0	28.8	20.28	947	2.89
BC	2016	April	-	SC-NSC-34 O-Apr-16	Sand Creek	7.0	1.3	52.5	47.8	1420.0	15.0	36.8	19.82	1,363	2.59

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2016	April	-	SC-NSC-33 O-Apr-16	Sand Creek	5.4	1.3	62.6	57.4	2530.0	21.0	97.4	48.72	2,384	3.85
BC	2018	June	7	RG_ER_NSC06O_20180607	Elk River	26.0	4.4	30.9	27.7	205.0	6.0	1.1	2.93	201	0.51
BC	2018	June	7	RG_ER_NSC03O_20180607	Elk River	17.0	1.7	33.9	30.2	275.0	10.0	1.6	2.06	271	0.59
BC	2018	June	6	RG_ER_NSC02O_20180606	Elk River	19.0	3.1	35.5	31.8	315.0	9.0	1.9	2.70	310	0.61
BC	2018	June	7	RG_ER_NSC05O_20180607	Elk River	26.0	2.5	39.9	35.7	445.0	11.0	2.7	5.16	437	0.60
BC	2018	June	6	RG_ER_NSC01O_20180606	Elk River	16.0	4.0	41.4	37.0	545.0	10.0	3.6	6.42	535	0.65
BC	2018	June	7	RG_ER_NSC04O_20180607	Elk River	24.0	4.8	44.0	39.8	755.0	12.0	26.4	4.40	724	3.50
BC	2018	June	7	RG_GC_NSC02O_20180607	Gold Creek	19.0	2.7	37.1	33.5	350.0	9.0	1.9	4.32	344	0.54
BC	2018	June	7	RG_GC_NSC01O_20180607	Gold Creek	13.0	2.9	38.5	34.7	475.0	9.0	6.1	8.25	461	1.29
BC	2018	June	7	RG_GC_NSC03O_20180607	Gold Creek	3.6	1.7	54.5	50.1	1800.0	15.0	191.6	47.82	1,561	10.65
BC	2018	June	5	RG_SC_NSC05O_20180605	Sand Creek	13.0	2.7	34.0	31.0	280.0	8.0	2.8	2.76	274	1.00
BC	2018	June	5	RG_SC_NSC03O_20180605	Sand Creek	9.2	2.4	34.5	31.2	330.0	10.0	4.9	2.93	322	1.47
BC	2018	June	5	RG_SC_NSC04O_20180605	Sand Creek	5.4	1.6	35.6	32.4	340.0	9.0	7.2	3.65	329	2.13
BC	2018	June	10	RG_SC_NSC06O_20180610	Sand Creek	27.0	2.0	41.6	37.7	530.0	11.0	6.8	4.65	519	1.29
BC	2018	June	5	RG_SC_NSC01O_20180605	Sand Creek	16.0	1.7	44.3	40.4	685.0	12.0	5.8	10.55	669	0.85
BC	2018	June	5	RG_SC_NSC02O_20180605	Sand Creek	5.4	1.3	48.8	44.8	1140.0	13.0	52.1	24.00	1,064	4.57
BC	2018	June	10	RG_SC_NSC07O_20180610	Sand Creek	5.8	1.6	58.9	53.9	1690.0	17.0	96.2	29.16	1,565	5.69
MT	2018	May	8	-	Rexford	3.5	1.1	49.0	-	1215.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.2	1.1	47.4	-	1090.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	5.5	1.3	52.5	-	1575.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	4.6	1.4	48.3	-	1155.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.5	1.4	45.3	-	1110.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.7	1.4	51.6	-	1290.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.4	1.6	53.6	-	1360.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	6.7	1.6	44.2	-	760.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.9	1.9	51.3	-	1395.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.3	1.4	48.8	-	1220.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.5	1.1	49.3	-	1190.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.9	1.1	46.8	-	915.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.4	1.1	44.1	-	960.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.1	1.2	48.2	-	1070.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	1.8	1.2	56.4	-	1575.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.3	49.8	-	1145.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.6	48.2	-	1150.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.8	1.6	47.1	-	955.0	-	-	-	-	-
BC	2019	June	14	6/14/2019 RG_ER-NPM-01_20190614	Elk River	3.8	1.6	55.1	50.2	1680.0	-	137.0	-	-	8.15
BC	2019	June	14	6/14/2019 RG_ER-NPM-02_20190614	Elk River	2.7	1.3	57.2	53.0	1520.0	-	114.0	-	-	7.50
BC	2019	June	17	6/17/2019 RG_ER-NPM-03_20190617	Elk River	3.3	1.2	55.7	50.5	1550.0	-	149.9	-	-	9.67
BC	2019	June	17	6/17/2019 RG_ER-NPM-04_20190617	Elk River	3.0	1.2	45.5	41.6	1050.0	-	100.3	-	-	9.55
BC	2019	June	18	6/18/2019 RG_ER-NPM-05_20190618	Elk River	4.9	1.6	50.7	46.6	1140.0	-	100.3	-	-	8.80
BC	2019	June	18	6/18/2019 RG_ER-NPM-06_20190618	Elk River	5.0	1.6	54.4	49.6	1540.0	-	81.9	-	-	5.32
BC	2019	June	18	6/18/2019 RG_ER-NPM-07_20190618	Elk River	4.3	1.3	47.5	43.0	1140.0	-	87.7	-	-	7.69
BC	2019	June	18	6/18/2019 RG_ER-NPM-08_20190618	Elk River	4.2	1.6	56.0	51.2	1400.0	-	92.1	-	-	6.58
BC	2019	June	19	6/19/2019 RG_ER-NPM-09_20190619	Elk River	7.2	2.0	46.7	42.4	880.0	-	18.6	-	-	2.11
BC	2019	June	19	6/19/2019 RG_ER-NPM-10_20190619	Elk River	9.9	1.9	46.0	42.2	720.0	-	6.6	-	-	0.92
BC	2019	June	19	6/19/2019 RG_ER-NPM-11_20190619	Elk River	17.0	2.5	40.2	36.5	620.0	-	3.7	-	-	0.60
BC	2019	June	20	6/20/2019 RG_ER-NPM-12_20190620	Elk River	2.4	1.3	55.2	50.3	1950.0	-	216.3	-	-	11.09
BC	2019	June	20	6/20/2019 RG_ER-NPM-13_20190620	Elk River	3.6	1.5	56.0	51.5	1590.0	-	47.6	-	-	2.99
BC	2019	June	20	6/20/2019 RG_ER-NPM-14_20190620	Elk River	7.6	1.7	53.6	48.8	1520.0	-	46.2	-	-	3.04
BC	2019	June	20	6/20/2019 RG_ER-NPM-15_20190620	Elk River	8.6	2.5	39.9	36.2	480.0	-	18.6	-	-	3.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-16_20190620	Elk River	17.0	2.4	39.0	34.9	430.0	-	3.7	-	-	0.86

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_ER-NPM-17_20190620	Elk River	4.5	1.8	44.2	40.0	840.0	-	66.2	-	-	7.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-18_20190620	Elk River	4.1	2.5	53.0	48.0	1540.0	-	153.5	-	-	9.97
BC	2019	June	25	6/25/2019 RG_ER-NPM-19_20190625	Elk River	7.9	1.9	30.2	28.3	200.0	-	6.7	-	-	3.35
BC	2019	June	25	6/25/2019 RG_ER-NPM-20_20190625	Elk River	6.3	2.9	46.9	42.4	740.0	-	14.5	-	-	1.96
BC	2019	June	27	6/27/2019 RG_ER-NPM-21_20190627	Elk River	13.0	3.4	39.9	36.9	710.0	-	54.0	-	-	7.61
BC	2019	June	28	6/28/2019 RG_ER-NPM-22_20190628	Elk River	7.1	2.6	32.9	29.5	295.0	-	24.9	-	-	8.43
BC	2019	June	28	6/28/2019 RG_ER-NPM-23_20190628	Elk River	9.8	3.6	38.0	34.2	440.0	-	16.0	-	-	3.63
BC	2019	June	28	6/28/2019 RG_ER-NPM-24_20190628	Elk River	7.8	2.4	43.9	39.6	740.0	-	29.6	-	-	4.00
BC	2019	June	28	6/28/2019 RG_ER-NPM-25_20190628	Elk River	8.3	2.5	33.3	30.4	340.0	-	15.3	-	-	4.49
BC	2019	June	28	6/28/2019 RG_ER-NPM-26_20190628	Elk River	4.0	1.1	44.9	41.5	915.0	-	85.3	-	-	9.33
BC	2019	June	28	6/28/2019 RG_ER-NPM-27_20190628	Elk River	14.6	2.3	30.7	27.6	180.0	-	1.1	-	-	0.61
BC	2019	July	3	7/3/2019 RG_ER-NPM-28_20190703	Elk River	34.5	2.3	38.8	35.7	550.0	-	47.7	-	-	8.67
BC	2019	July	4	7/4/2019 RG_ER-NPM-29_20190704	Elk River	19.4	4.6	46.7	42.4	700.0	-	6.1	-	-	0.88
BC	2019	July	4	7/4/2019 RG_ER-NPM-30_20190704	Elk River	7.1	2.7	44.7	40.5	760.0	-	11.1	-	-	1.47
BC	2019	July	8	7/8/2019 RG_ER-NPM-31_20190708	Elk River	10.9	3.1	33.2	29.9	300.0	-	15.9	-	-	5.30
BC	2019	July	9	7/9/2019 RG_ER-NPM-32_20190709	Elk River	4.1	1.3	54.8	50.0	1550.0	-	143.1	-	20.09	9.23
BC	2019	July	9	7/9/2019 RG_ER-NPM-33_20190709	Elk River	3.5	1.4	44.3	41.6	780.0	-	57.3	-	-	7.35
BC	2019	July	9	7/9/2019 RG_ER-NPM-34_20190709	Elk River	5.4	1.6	54.0	49.0	1470.0	-	19.3	-	-	1.31
BC	2019	July	9	7/9/2019 RG_ER-NPM-35_20190709	Elk River	3.8	1.2	40.7	36.0	530.0	-	40.6	-	-	7.65
BC	2019	July	9	7/9/2019 RG_ER-NPM-36_20190709	Elk River	9.3	3.9	41.4	37.9	650.0	-	68.5	-	-	10.54
BC	2019	July	9	7/9/2019 RG_ER-NPM-37_20190709	Elk River	5.4	2.2	45.2	40.5	750.0	-	43.3	-	-	5.77
BC	2019	July	10	7/10/2019 RG_ER-NPM-38_20190710	Elk River	12.0	4.0	41.1	37.1	560.0	-	17.1	-	-	3.05
BC	2019	July	10	7/10/2019 RG_ER-NPM-39_20190710	Elk River	2.7	1.2	42.1	38.3	580.0	-	58.1	-	-	10.02
BC	2019	July	10	7/10/2019 RG_ER-NPM-40_20190710	Elk River	18.4	3.0	34.4	30.4	250.0	-	2.1	-	-	0.86
BC	2019	July	10	7/10/2019 RG_ER-NPM-41_20190710	Elk River	3.4	1.3	42.4	38.3	530.0	-	43.3	-	-	8.17
BC	2019	July	10	7/10/2019 RG_ER-NPM-42_20190710	Elk River	11.0	3.4	42.3	38.8	620.0	-	32.6	-	-	5.25
BC	2019	July	11	7/11/2019 RG_ER-NPM-43_20190711	Elk River	12.0	4.8	43.0	39.3	825.0	-	70.1	-	-	8.50
BC	2019	July	12	7/12/2019 RG_ER-NPM-44_20190712	Elk River	36.0	5.0	40.7	36.6	610.0	-	3.7	-	-	0.60
BC	2019	July	12	7/12/2019 RG_ER-NPM-45_20190712	Elk River	2.2	1.2	49.9	45.3	1200.0	-	108.1	-	-	9.01
BC	2019	July	13	7/13/2019 RG_ER-NPM-46_20190713	Elk River	2.4	1.3	56.0	50.6	1575.0	-	116.5	-	-	7.40
BC	2019	July	15	7/15/2019 RG_ER-NPM-47_20190715	Elk River	2.1	1.2	53.3	48.2	1200.0	-	87.2	-	-	7.27
BC	2019	July	16	7/16/2019 RG_ER-NPM-48_20190716	Elk River	2.3	1.3	54.9	50.5	1240.0	-	80.1	-	-	6.46
BC	2019	July	26	7/26/2019 RG_ER-NPM-49_20190726	Elk River	3.4	1.4	49.5	45.0	590.0	-	12.6	-	-	2.14
BC	2019	June	26	6/26/2019 RG_GC-NPM-01_20190626	Gold Creek	2.4	1.2	54.5	49.0	1375.0	-	149.5	-	-	10.87
BC	2019	June	26	6/26/2019 RG_GC-NPM-02_20190626	Gold Creek	2.1	1.3	53.9	49.3	1425.0	-	135.5	-	-	9.51
BC	2019	June	26	6/26/2019 RG_GC-NPM-03_20190626	Gold Creek	2.1	1.2	47.4	43.1	1075.0	-	106.7	-	-	9.93
BC	2019	June	26	6/26/2019 RG_GC-NPM-04_20190626	Gold Creek	20.0	2.4	38.9	34.9	460.0	-	2.9	-	-	0.64
BC	2019	June	26	6/26/2019 RG_GC-NPM-05_20190626	Gold Creek	3.9	1.7	44.8	40.4	915.0	-	55.5	-	-	6.07
BC	2019	June	26	6/26/2019 RG_GC-NPM-06_20190626	Gold Creek	2.4	1.1	49.9	44.9	1060.0	-	115.8	-	-	10.92
BC	2019	June	26	6/26/2019 RG_GC-NPM-07_20190626	Gold Creek	11.0	2.1	34.6	31.3	375.0	-	3.0	-	-	0.81
BC	2019	June	27	6/27/2019 RG_GC-NPM-08_20190627	Gold Creek	2.2	1.2	54.6	49.8	1600.0	-	210.4	-	-	13.15
BC	2019	June	27	6/27/2019 RG_GC-NPM-09_20190627	Gold Creek	2.2	1.4	52.6	47.8	1060.0	-	27.0	-	-	2.55
BC	2019	June	27	6/27/2019 RG_GC-NPM-10_20190627	Gold Creek	2.7	1.2	50.2	45.4	1280.0	-	169.2	-	-	13.22
BC	2019	June	27	6/27/2019 RG_GC-NPM-11_20190627	Gold Creek	12.0	1.8	41.6	37.4	600.0	-	4.2	-	-	0.70
BC	2019	June	27	6/27/2019 RG_GC-NPM-12_20190627	Gold Creek	3.9	1.4	45.0	40.5	920.0	-	48.9	-	-	5.32
BC	2019	June	27	6/27/2019 RG_GC-NPM-13_20190627	Gold Creek	3.3	2.0	49.5	44.8	1150.0	-	92.4	-	-	8.03
BC	2019	July	18	7/18/2019 RG_GC-NPM-14_20190718	Gold Creek	9.6	1.5	50.8	46.2	1030.0	-	8.8	-	-	0.86
BC	2019	July	19	7/19/2019 RG_GC-NPM-15_20190719	Gold Creek	3.9	1.4	61.8	57.0	1800.0	-	100.9	-	-	5.60
BC	2019	July	25	7/25/2019 RG_GC-NPM-16_20190725	Gold Creek	2.7	1.4	50.2	46.0	1200.0	-	18.3	-	-	1.53
BC	2019	June	20	6/20/2019 RG_SC-NPM-01_20190620	Sand Creek	8.4	2.2	43.5	39.3	740.0	-	26.9	-	-	3.64
BC	2019	June	20	6/20/2019 RG_SC-NPM-02_20190620	Sand Creek	20.0	1.8	42.8	38.0	600.0	-	3.2	-	-	0.53

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_SC-NPM-03_20190620	Sand Creek	11.0	2.0	34.6	31.6	340.0	-	2.0	-	-	0.58
BC	2019	June	20	6/20/2019 RG_SC-NPM-04_20190620	Sand Creek	17.0	2.6	41.9	38.9	640.0	-	20.5	-	-	3.20
BC	2019	June	20	6/20/2019 RG_SC-NPM-05_20190620	Sand Creek	28.0	2.0	39.2	35.5	490.0	-	3.1	-	-	0.62
BC	2019	July	24	7/24/2019 RG_SC-NPM-06_20190724	Sand Creek	10.0	1.4	43.5	34.2	495.0	-	7.2	-	-	1.46
BC	2019	July	25	7/25/2019 RG_SC-NPM-07_20190725	Sand Creek	21.0	1.7	38.4	35.0	525.0	-	5.7	-	-	1.09
BC	2019	July	25	7/25/2019 RG_SC-NPM-08_20190725	Sand Creek	23.0	2.4	40.3	37.3	540.0	-	8.6	-	-	1.59
BC	2019	July	25	7/25/2019 RG_SC-NPM-09_20190725	Sand Creek	12.0	1.5	44.3	39.9	790.0	-	10.6	-	-	1.34
BC	2019	July	26	7/26/2019 RG_SC-NPM-10_20190726	Sand Creek	25.0	1.4	39.8	35.7	510.0	-	2.4	-	-	0.47
BC	2019	July	26	7/26/2019 RG_SC-NPM-11_20190726	Sand Creek	23.0	2.2	37.9	34.2	495.0	-	4.6	-	-	0.92
BC	2019	June	21	6/21/2019 RG_WB-NPM-01_20190621	Waldo Bay	7.4	2.2	38.0	34.5	406.0	-	16.4	-	-	4.04
BC	2019	June	26	6/26/2019 RG-WB-NPM-02_20190626	Waldo Bay	26.0	2.9	39.9	35.9	480.0	-	5.7	-	-	1.19
BC	2019	June	26	6/26/2019 RG-WB-NPM-03_20190626	Waldo Bay	9.8	2.9	37.5	33.7	440.0	-	4.6	-	-	1.04
MT	2019	May	15	Rexford_NSC_01	Rexford	3.9	1.7	40.5	37.0	540.0	-	5.3	7.4	527.28	0.99
MT	2019	May	15	Rexford_NSC_02	Rexford	2.5	1.0	54.5	50.3	1785.0	-	49.7	32.04	1703.24	2.79
MT	2019	May	15	Rexford_NSC_03	Rexford	5.1	1.5	39.9	36.2	495.0	-	3.1	7.74	484.17	0.62
MT	2019	May	15	Rexford_NSC_04	Rexford	2.2	1.2	50.3	46.0	1500.0	-	41.0	29.007	1430.033	2.73
MT	2019	May	15	Rexford_NSC_05	Rexford	1.8	1.1	60.3	54.8	2060.0	-	68.2	29.69	1962.14	3.31
MT	2019	May	15	Rexford_NSC_06	Rexford	3.5	1.4	42.6	38.8	760.0	-	5.5	27.71	726.76	0.73
MT	2019	May	15	Rexford_NSC_07	Rexford	3.2	1.3	46.9	42.5	1070.0	-	7.1	20.16	1042.76	0.66
MT	2019	May	15	Rexford_NSC_08	Rexford	3.5	1.4	40.9	36.4	610.0	-	4.8	13.94	591.22	0.79
MT	2019	May	15	Rexford_NSC_09	Rexford	2.6	0.8	56.1	51.3	1620.0	-	60.3	28.73	1530.992	3.72
MT	2019	May	15	Rexford_NSC_10	Rexford	2.0	1.1	49.9	44.9	1200.0	-	30.9	14.03	1155.03	2.58
MT	2019	May	15	Rexford_NSC_11	Rexford	9.5	1.6	37.4	33.5	475.0	-	4.7	7.27	463.01	0.99
MT	2019	May	15	Rexford_NSC_12	Rexford	5.1	1.0	46.7	42.0	940.0	-	10.8	16.07	913.11	1.15
MT	2019	May	15	Rexford_NSC_13	Rexford	2.0	1.0	44.4	40.1	890.0	-	13.1	22.17	854.689	1.48
MT	2019	May	15	Rexford_NSC_14	Rexford	2.8	0.9	51.4	47.2	1540.0	-	42.4	20.822	1476.813	2.75
MT	2019	May	15	Rexford_NSC_15	Rexford	2.2	0.9	54.0	49.3	1490.0	-	53.9	21.269	1414.834	3.62

Appendix C. Northen Pikeminnow Tissue Data: 2019

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG-ER-NPM-32-BF-20190709	Eggs	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	0.01	2.2	21	<0.01	0.6	0.037	0.03	<0.05	2.8	<0.01	0.10	<0.005	<0.05	<0.2	<0.005	<0.1	66	64.02
7/9/2019 RG-ER-NPM-33-BF-20190709	Eggs	<5	<0.02	<0.05	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.6	56	<0.05	0.9	0.04	<0.05	<0.5	3.0	<0.02	0.2	<0.01	<0.2	<0.5	<0.02	<0.2	80	65.11
7/12/2019 RG-ER-NPM-43-BF-20190712	Eggs	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.02	2.9	45	<0.01	2.4	0.046	0.04	0.07	11.7	<0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	69	64.87
7/13/2019 RG-ER-NPM-46-BF-20190713	Eggs	<2	<0.01	0.11	0.13	<0.01	<1	<0.01	<0.05	0.02	2.1	30	<0.01	0.3	0.042	0.03	<0.05	2.7	<0.01	0.10	0.010	<0.05	<0.2	<0.005	<0.1	98	63.44
7/16/2019 RG-ER-NPM-48-BF-20190716	Eggs	<2	<0.01	0.10	0.36	<0.01	<1	<0.01	0.06	0.02	2.6	22	<0.01	0.8	0.051	0.04	<0.05	2.3	<0.01	0.15	0.009	<0.05	<0.2	<0.005	<0.1	77	69.18
7/3/2019 RG-ER-NPM-28-AF-20190703	Eggs	120	<0.01	0.04	1.2	<0.01	<1	<0.01	0.06	0.04	2.4	74	0.07	2.9	0.019	0.04	0.10	8.4	<0.01	2.9	<0.005	<0.05	1.0	0.031	<0.1	77	95.04
7/9/2019 RG-ER-NPM-32-AF-20190709	Eggs	260	<0.02	0.17	2.6	<0.02	<2	<0.02	<0.1	0.04	2.2	140	0.51	7.4	0.03	<0.05	0.2	2.9	<0.02	3.0	<0.01	<0.1	4.3	0.05	<0.2	68	96.25
7/13/2019 RG-ER-NPM-46-AF-20190713	Eggs	100	<0.01	0.08	3.3	<0.01	<1	<0.01	0.07	0.04	2.2	130	0.20	12	0.041	0.05	0.10	2.3	<0.01	2.6	0.011	<0.05	3.8	0.028	<0.1	88	94.15
7/15/2019 RG-ER-NPM-47-AF-20190715	Eggs	60	<0.1	<0.5	<5	<0.02	<50	<0.02	<5	<5	<5	<50	<0.5	<5	0.07	<0.5	<5	1.8	<0.02	1	<0.1	<2	<5	<0.1	<1	70	94.79
7/16/2019 RG-ER-NPM-48-AF-20190716	Eggs	560	0.01	0.31	15	0.02	<1	0.01	0.09	0.11	2.6	760	1.1	85	0.048	0.12	0.21	2.7	0.01	6.7	0.015	<0.05	24	0.14	0.4	86	91.84
7/3/2019 RG-ER-NPM-28-PF_20190703	Eggs	<5	<0.02	0.25	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.1	34	<0.05	2.3	0.02	<0.05	<0.5	9.6	<0.02	0.1	<0.01	<0.2	<0.5	<0.02	<0.2	62	66.93
6/14/2019 RG_ER-NPM-01-M_20190614	Muscle	2	<0.01	0.15	0.25	<0.01	<1	<0.01	0.24	0.01	2.0	25	<0.01	0.3	1.3	<0.02	0.05	1.6	<0.01	3.8	0.016	<0.05	<0.2	<0.005	<0.1	19	72.64
6/14/2019 RG_ER-NPM-02-M_20190614	Muscle	<2	<0.01	0.21	0.95	<0.01	<1	<0.01	<0.05	0.02	2.6	24	0.02	0.8	2.2	<0.02	<0.05	1.3	<0.01	6.2	0.014	<0.05	<0.2	<0.005	<0.1	33	72.76
6/17/2019 RG_ER-NPM-03-M_20190617	Muscle	<2	<0.01	0.22	0.92	<0.01	<1	<0.01	0.15	0.02	3.5	33	0.06	0.6	1.2	<0.02	<0.05	1.2	<0.01	3.6	0.013	<0.05	<0.2	<0.005	<0.1	28	71.78
6/17/2019 RG_ER-NPM-04-M_20190617	Muscle	<2	<0.01	0.16	0.90	<0.01	<1	<0.01	<0.05	0.02	2.6	26	<0.01	0.6	0.66	<0.02	<0.05	1.2	<0.01	3.0	0.021	<0.05	0.2	<0.005	<0.1	22	71.16
6/18/2019 RG_ER-NPM-05-M_20190618	Muscle	<2	<0.01	0.04	0.72	<0.01	<1	<0.01	0.20	0.01	0.85	9	<0.01	0.6	1.7	<0.02	<0.05	1.6	<0.01	3.6	0.010	<0.05	<0.2	<0.005	<0.1	19	77.76
6/18/2019 RG_ER-NPM-06-M_20190618	Muscle	<2	<0.01	0.07	0.58	<0.01	<1	<0.01	<0.05	0.03	0.79	8	<0.01	0.8	2.1	<0.02	<0.05	1.6	<0.01	3.0	0.008	<0.05	<0.2	<0.005	<0.1	17	78.52
6/18/2019 RG_ER-NPM-07-M_20190618	Muscle	<2	<0.01	0.17	1.2	<0.01	<1	<0.01	<0.05	0.02	2.4	23	<0.01	1.0	0.90	<0.02	0.08	1.3	<0.01	5.7	0.013	<0.05	<0.2	<0.005	<0.1	24	72.59
6/18/2019 RG_ER-NPM-08-M_20190618	Muscle	<2	<0.01	0.03	0.75	<0.01	<1	<0.01	<0.05	0.01	0.95	13	0.01	0.4	1.9	<0.02	<0.05	1.6	<0.01	3.1	0.009	<0.05	<0.2	<0.005	<0.1	22	78.06
6/19/2019 RG_ER-NPM-09-M_20190619	Muscle	3	<0.01	0.06	1.7	<0.01	<1	<0.01	<0.05	0.03	2.5	27	0.09	1.2	0.94	<0.02	0.07	2.0	<0.01	8.5	0.017	<0.05	<0.2	<0.005	<0.1	38	76.79
6/19/2019 RG_ER-NPM-10-M_20190619	Muscle	<2	<0.01	0.03	0.95	<0.01	<1	<0.01	<0.05	0.02	1.7	16	0.02	0.7	0.98	<0.02	<0.05	1.9	<0.01	3.0	0.009	<0.05	<0.2	<0.005	<0.1	29	76.48
6/19/2019 RG_ER-NPM-11-M_20190619	Muscle	<2	<0.01	0.07	0.36	<0.01	<1	<0.01	<0.05	0.01	1.5	16	0.03	0.6	0.87	<0.02	<0.05	2.5	<0.01	1.4	0.012	<0.05	<0.2	<0.005	<0.1	18	78.43
6/20/2019 RG_ER-NPM-12-M_20190620	Muscle	<2	<0.01	0.17	0.20	<0.01	<1	<0.01	<0.05	<0.01	0.96	9	0.01	0.2	1.5	<0.02	<0.05	1.3	<0.01	0.61	0.010	<0.05	<0.2	<0.005	<0.1	15	72.41
6/20/2019 RG_ER-NPM-13-M_20190620	Muscle	<2	<0.01	0.08	0.78	<0.01	<1	<0.01	<0.05	0.02	1.1	11	0.01	0.7	1.5	<0.02	<0.05	1.5	<0.01	4.5	0.006	<0.05	<0.2	<0.005	<0.1	19	75.51
6/20/2019 RG_ER-NPM-14-M_20190620	Muscle	<2	<0.01	0.14	0.65	<0.01	<1	<0.01	<0.05	0.01	1.1	11	<0.01	0.6	1.3	<0.02	<0.05	1.7	<0.01	2.6	0.010	<0.05	<0.2	<0.005	<0.1	16	74.20
6/20/2019 RG_ER-NPM-15-M_20190620	Muscle	<2	<0.01	0.02	0.85	<0.01	<1	<0.01	<0.05	0.01	0.92	14	0.02	0.9	1.1	<0.02	<0.05	2.5	<0.01	2.0	0.008	<0.05	<0.2	<0.005	<0.1	24	79.46
6/20/2019 RG_ER-NPM-16-M_20190620	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	0.09	0.03	1.9	29	0.01	1.3	1.0	<0.02	<0.05	2.4	<0.01	4.8	0.011	<0.05	<0.2	<0.005	<0.1	34	80.14
6/20/2019 RG_ER-NPM-17-M_20190620	Muscle	<2	<0.01	0.08	1.0	<0.01	<1	<0.01	<0.05	0.02	2.2	22	<0.01	1.0	1.1	<0.02	<0.05	1.8	<0.01	3.7	0.013	<0.05	<0.2	<0.005	<0.1	26	78.24
6/20/2019 RG_ER-NPM-18-M_20190620	Muscle	<2	<0.01	0.07	0.46	<0.01	<1	<0.01	<0.05	<0.01	0.83	7	<0.01	0.4	1.7	<0.02	<0.05	2.5	<0.01	1.7	<0.005	<0.05	<0.2	<0.005	<0.1	20	74.74
6/25/2019 RG_ER-NPM-19-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.02	1.8	17	<0.01	1.0	0.41	<0.02	<0.05	1.9	<0.01	2.2	0.021	<0.05	<0.2	<0.005	<0.1	38	78.49
6/25/2019 RG_ER-NPM-20-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.03	2.7	27	0.01	1.4	0.92	<0.02	<0.05	2.9	<0.01	7.1	0.018	<0.05	<0.2	<0.005	<0.1	29	76.00
6/27/2019 RG_ER-NPM-21-M_20190627	Muscle	<2	<0.01	0.04	1.3	<0.01	<1	<0.01	<0.05	0.02	2.6	35	<0.01	1.9	0.78	<0.02	<0.05	3.4	<0.01	7.1	0.011	<0.05	<0.2	<0.005	<0.1	28	76.67
6/28/2019 RG_ER-NPM-22-M_20190628	Muscle	<2	<0.01	0.02	0.84	<0.01	<1	<0.01	<0.05	0.02	0.92	9	<0.01	1.2	0.61	<0.02	0.05	2.6	<0.01	3.3	0.016	<0.05	<0.2	<0.005	<0.1	18	76.61
6/28/2019 RG_ER-NPM-23-M_20190628	Muscle	<2	<0.01	0.01	2.4	<0.01	<1	<0.01	0.08	0.02	2.3	21	<0.01	1.3	1.9	<0.02	<0.05	3.6	<0.01	3.4	0.012	<0.05	<0.2	<0.005	<0.1	25	74.79
6/28/2019 RG_ER-NPM-24-M_20190628	Muscle	<2	<0.01	0.06	2.0	<0.01	<1	<0.01	0.22	0.02	1.5	20	<0.01	1.4	0.88	<0.02	<0.05	2.4	<0.01	8.4	0.008	<0.05	<0.2	<0.005	<0.1	28	78.90
6/28/2019 RG_ER-NPM-25-M_20190628	Muscle	<2	<0.01	0.02	0.50	<0.01	<1	<0.01	0.06	<0.01	1.1	10	<0.01	0.7	1.0	<0.02	<0.05	2.5	<0.01	1.5	0.012	<0.05	<0.2	<0.005	<0.1	17	75.15
6/28/2019 RG_ER-NPM-26-M_20190628	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	0.07	0.02	1.7	21	<0.01	1.5	1.1	<0.02	<0.05	1.1	<0.01	7.1	0.010	<0.05	<0.2	<0.005	<0.1	24	77.37
6/28/2019 RG_ER-NPM-27-M_20190628	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	0.02	0.83	11	<0.01	1.2	0.35	<0.02	<0.05	2.3	<0.01	4.7	0.013	<0.05	<0.2	<0.005	<0.1	22	77.97
7/3/2019 RG_ER-NPM-28-M_20190703	Muscle	<2	<0.01	0.03	1.1	<0.01	<1	<0.01	<0.05	0.01	1.2	12	0.02	1.4	0.86	<0.02	<0.05	2.3	<0.01	4.7	0.010	<0.05	<0.2	<0.005	<0.1	19	76.85
7/4/2019 RG_ER-NPM-29-M_20190704	Muscle	<2	<0.01	0.03	0.37	<0.01	<1	<0.01	<0.05	0.01	1.7	21	<0.01	0.6	0.82	<0.02	<0.05	4.6	<0.01	0.75	0.011	<0.05	<0.2	<0.005	<0.1	21	78.32

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/4/2019 RG_ER-NPM-30-M_20190704	Muscle	<2	<0.01	0.03	0.58	<0.01	<1	<0.01	<0.05	<0.01	0.73	9	0.02	0.8	1.6	<0.02	<0.05	2.7	<0.01	2.3	0.006	<0.05	<0.2	<0.005	<0.1	15	77.17
7/8/2019 RG_ER-NPM-31-M_20190708	Muscle	3	<0.01	0.04	1.4	<0.01	<1	<0.01	13	0.05	2.3	170	0.02	1.6	0.72	<0.02	0.40	3.1	<0.01	2.7	0.019	<0.05	<0.2	<0.005	<0.1	33	78.99
7/9/2019 RG_ER-NPM-32-M_20190709	Muscle	<2	<0.01	0.12	0.77	<0.01	<1	<0.01	0.77	0.02	1.8	26	<0.01	0.5	1.2	<0.02	<0.05	1.3	<0.01	3.2	0.010	<0.05	<0.2	<0.005	<0.1	25	72.74
7/9/2019 RG_ER-NPM-33-M_20190709	Muscle	<2	<0.01	0.11	0.92	<0.01	<1	<0.01	0.11	0.02	2.2	21	<0.01	0.8	0.76	<0.02	<0.05	1.4	<0.01	3.9	0.021	<0.05	<0.2	<0.005	<0.1	26	75.45
7/9/2019 RG_ER-NPM-34-M_20190709	Muscle	<2	<0.01	0.12	1.5	<0.01	<1	<0.01	0.17	0.02	2.0	20	<0.01	1.1	1.4	<0.02	<0.05	1.6	<0.01	5.4	0.011	<0.05	<0.2	<0.005	<0.1	28	74.77
7/9/2019 RG_ER-NPM-35-M_20190709	Muscle	<2	<0.01	0.32	1.8	<0.01	<1	<0.01	0.14	0.02	2.2	24	<0.01	1.3	1.0	<0.02	<0.05	1.2	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	31	77.08
7/9/2019 RG_ER-NPM-36-M_20190709	Muscle	<2	<0.01	0.06	1.4	<0.01	<1	<0.01	0.17	0.03	2.7	25	<0.01	1.4	2.0	<0.02	<0.05	3.9	<0.01	4.3	0.020	<0.05	<0.2	<0.005	<0.1	34	78.53
7/9/2019 RG_ER-NPM-37-M_20190709	Muscle	4	<0.01	0.11	1.8	<0.01	<1	<0.01	0.07	0.03	2.2	26	0.02	2.0	0.87	<0.02	<0.05	2.2	<0.01	6.6	0.009	<0.05	<0.2	<0.005	<0.1	33	79.86
7/10/2019 RG_ER-NPM-38-M_20190710	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	0.10	0.02	2.4	25	0.02	1.4	0.80	<0.02	0.05	4.0	<0.01	4.0	0.011	<0.05	<0.2	<0.005	<0.1	29	78.21
7/10/2019 RG_ER-NPM-39-M_20190710	Muscle	<2	<0.01	0.11	0.46	<0.01	<1	<0.01	0.06	0.01	1.6	15	<0.01	0.5	0.92	<0.02	<0.05	1.2	<0.01	1.7	0.027	<0.05	<0.2	<0.005	<0.1	20	76.75
7/10/2019 RG_ER-NPM-40-M_20190710	Muscle	5	<0.01	0.09	0.74	<0.01	<1	<0.01	0.11	0.02	1.6	27	0.04	0.9	0.66	<0.02	0.09	3.0	<0.01	1.2	0.010	<0.05	<0.2	<0.005	<0.1	32	79.51
7/10/2019 RG_ER-NPM-41-M_20190710	Muscle	<2	<0.01	0.12	0.91	<0.01	<1	<0.01	0.18	0.01	1.0	11	<0.01	0.8	0.87	<0.02	<0.05	1.3	<0.01	3.1	0.024	<0.05	<0.2	<0.005	<0.1	21	76.75
7/10/2019 RG_ER-NPM-42-M_20190710	Muscle	<2	<0.01	0.03	1.5	<0.01	<1	<0.01	0.08	0.02	1.7	15	<0.01	1.4	0.92	<0.02	<0.05	3.4	<0.01	5.2	0.009	<0.05	<0.2	<0.005	<0.1	29	78.08
7/11/2019 RG_ER-NPM-43-M_20190711	Muscle	<2	<0.01	0.05	1.1	<0.01	<1	<0.01	0.13	0.02	2.6	17	0.02	1.3	0.69	<0.02	<0.05	4.8	<0.01	4.0	0.022	<0.05	<0.2	<0.005	<0.1	26	79.26
7/12/2019 RG_ER-NPM-44-M_20190712	Muscle	<2	<0.01	0.05	0.14	<0.01	<1	<0.01	<0.05	<0.01	0.73	10	<0.01	0.5	1.1	<0.02	<0.05	5.0	<0.01	0.10	0.008	<0.05	<0.2	<0.005	<0.1	17	78.91
7/12/2019 RG_ER-NPM-45-M_20190712	Muscle	<2	<0.01	0.10	0.19	<0.01	<1	<0.01	0.05	<0.01	0.77	6	<0.01	0.2	1.0	<0.02	<0.05	1.2	<0.01	0.80	0.015	<0.05	<0.2	<0.005	<0.1	14	76.31
7/13/2019 RG_ER-NPM-46-M_20190713	Muscle	2	<0.01	0.19	0.60	<0.01	<1	<0.01	0.06	0.02	2.2	23	0.01	0.4	1.3	<0.02	<0.05	1.3	<0.01	2.8	0.022	<0.05	<0.2	<0.005	<0.1	28	75.43
7/15/2019 RG_ER-NPM-47-M_20190715	Muscle	<2	<0.01	0.17	1.0	<0.01	<1	<0.01	0.07	0.02	1.6	16	<0.01	0.7	1.1	<0.02	0.12	1.2	<0.01	5.2	0.014	<0.05	<0.2	<0.005	<0.1	26	76.36
7/16/2019 RG_ER-NPM-48-M_20190716	Muscle	<2	<0.01	0.14	1.2	<0.01	<1	<0.01	<0.05	0.02	1.4	12	<0.01	0.8	1.7	<0.02	<0.05	1.3	<0.01	4.9	0.021	<0.05	<0.2	<0.005	<0.1	27	78.26
7/26/2019 RG_ER-NPM-49-M_20190726	Muscle	<2	<0.01	0.09	0.71	<0.01	<1	<0.01	0.06	0.02	1.5	15	<0.01	0.6	1.6	<0.02	<0.05	1.4	<0.01	3.6	0.014	<0.05	<0.2	<0.005	<0.1	24	77.86
6/26/2019 RG_GC-NPM-01-M_20190626	Muscle	<2	<0.01	0.18	0.17	<0.01	<1	<0.01	0.05	<0.01	0.84	8	<0.01	0.1	2.6	<0.02	<0.05	1.2	<0.01	0.51	0.007	<0.05	<0.2	<0.005	<0.1	12	75.39
6/26/2019 RG_GC-NPM-02-M_20190626	Muscle	<2	<0.01	0.07	0.07	<0.01	<1	<0.01	<0.05	<0.01	0.75	7	<0.01	0.1	1.5	<0.02	<0.05	1.3	<0.01	0.12	0.015	<0.05	<0.2	<0.005	<0.1	15	77.80
6/26/2019 RG_GC-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	0.54	<0.01	<1	<0.01	<0.05	<0.01	0.68	7	<0.01	0.5	1.1	<0.02	<0.05	1.2	<0.01	2.3	0.010	<0.05	<0.2	<0.005	<0.1	18	77.49
6/26/2019 RG_GC-NPM-04-M_20190626	Muscle	<2	<0.01	0.06	0.60	<0.01	<1	<0.01	<0.05	<0.01	0.82	12	<0.01	0.8	1.1	<0.02	<0.05	2.4	<0.01	2.3	0.011	<0.05	<0.2	<0.005	<0.1	22	78.87
6/26/2019 RG_GC-NPM-05-M_20190626	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.8	20	0.01	1.4	1.1	<0.02	<0.05	1.7	<0.01	3.4	0.009	<0.05	<0.2	<0.005	<0.1	25	77.49
6/26/2019 RG_GC-NPM-06-M_20190626	Muscle	<2	<0.01	0.12	1.0	<0.01	<1	<0.01	<0.05	<0.01	1.7	17	<0.01	0.7	0.92	<0.02	<0.05	1.1	<0.01	3.8	0.021	<0.05	<0.2	<0.005	<0.1	22	75.90
6/26/2019 RG_GC-NPM-07-M_20190626	Muscle	<2	<0.01	0.02	1.1	<0.01	<1	<0.01	0.06	<0.01	1.3	20	0.01	1.2	1.1	<0.02	<0.05	2.1	<0.01	4.6	0.015	<0.05	<0.2	<0.005	<0.1	26	79.07
6/27/2019 RG_GC-NPM-08-M_20190627	Muscle	2	<0.01	0.10	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.4	12	0.23	0.7	1.2	<0.02	<0.05	1.2	<0.01	6.6	0.022	<0.05	0.4	<0.005	<0.1	31	75.59
6/27/2019 RG_GC-NPM-09-M_20190627	Muscle	2	<0.01	0.04	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.91	12	<0.01	0.2	1.5	<0.02	<0.05	1.4	<0.01	0.78	0.017	<0.05	<0.2	<0.005	<0.1	14	78.43
6/27/2019 RG_GC-NPM-10-M_20190627	Muscle	<2	<0.01	0.09	0.92	<0.01	<1	<0.01	<0.05	<0.01	1.6	13	<0.01	0.7	1.2	<0.02	<0.05	1.2	<0.01	4.8	0.022	<0.05	<0.2	<0.005	<0.1	20	75.35
6/27/2019 RG_GC-NPM-11-M_20190627	Muscle	<2	<0.01	0.09	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.63	10	<0.01	0.3	1.1	<0.02	<0.05	1.8	<0.01	0.60	0.005	<0.05	<0.2	<0.005	<0.1	14	77.35
6/27/2019 RG_GC-NPM-12-M_20190627	Muscle	<2	<0.01	0.10	1.3	<0.01	<1	<0.01	<0.05	<0.01	2.2	21	<0.01	1.4	1.0	<0.02	<0.05	1.4	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	26	78.38
6/27/2019 RG_GC-NPM-13-M_20190627	Muscle	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	<0.01	0.82	11	<0.01	0.3	1.6	<0.02	<0.05	2.0	<0.01	0.57	<0.005	<0.05	<0.2	<0.005	<0.1	14	78.91
7/18/2019 RG_GC-NPM-14-M_20190718	Muscle	<2	<0.01	0.11	0.78	<0.01	<1	<0.01	<0.05	<0.01	2.9	34	<0.01	0.4	1.7	<0.02	<0.05	1.5	<0.01	1.3	0.014	<0.05	<0.2	<0.005	<0.1	27	76.81
7/19/2019 RG_GC-NPM-15-M_20190719	Muscle	2	<0.01	0.04	0.44	<0.01	<1	<0.01	<0.05	<0.01	0.84	6	<0.01	0.3	2.6	<0.02	<0.05	1.4	<0.01	1.8	0.011	<0.05	<0.2	<0.005	<0.1	20	80.92
7/25/2019 RG_GC-NPM-16-M_20190725	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	<0.01	1.2	9	<0.01	0.8	1.3	<0.02	<0.05	1.4	<0.01	5.6	0.016	<0.05	<0.2	<0.005	<0.1	27	77.46
6/20/2019 RG_SC-NPM-01-M_20190620	Muscle	<2	<0.01	0.05	0.70	<0.01	<1	<0.01	<0.05	<0.01	2.5	31	<0.01	0.9	1.0	<0.02	<0.05	2.2	<0.01	2.7	0.013	<0.05	<0.2	<0.005	<0.1	27	78.73
6/20/2019 RG_SC-NPM-02-M_20190620	Muscle	<2	<0.01	0.03	0.69	<0.01	<1	<0.01	1.2	<0.01	1.2	24	<0.01	0.9	0.99	0.04	0.12	1.8	<0.01	3.8	0.010	<0.05	<0.2	<0.005	<0.1	23	80.68
6/20/2019 RG_SC-NPM-03-M_20190620	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	<0.05	<0.01	2.1	21	0.02	1.6	0.55	<0.02	<0.05	2.0	<0.01	5.1	0.012	<0.05	<0.2	<0.005	<0.1	34	79.58
6/20/2019 RG_SC-NPM-04-M_20190620	Muscle	<2	<0.01	0.07	0.77	<0.01	<1	<0.01	<0.05	<0.01	2.1	25	<0.01	0.8	1.1	<0.02	<0.05	2.6	<0.01	2.9	0.016	<0.05	<0.2	<0.005	<0.1	34	79.13
6/20/2019 RG_SC-NPM-05-M_20190620	Muscle	<2	<0.01	0.04	0.93	<0.01	<1	<0.01	<0.05	0.01	1.1	13	<0.01	0.9	0.81	<0.02	<0.05	2.0	<0.01	3.4	0.011	<0.05	<0.2	<0.005	<0.1	24	79.40

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/24/2019 RG_SC-NPM-06-M_20190724	Muscle	<2	<0.01	0.01	0.79	<0.01	<1	<0.01	0.32	0.01	0.85	17	<0.01	1.3	1.4	<0.02	<0.05	1.4	<0.01	4.8	<0.005	<0.05	<0.2	<0.005	<0.1	18	79.65
7/25/2019 RG_SC-NPM-07-M_20190725	Muscle	3	<0.01	0.06	1.1	<0.01	<1	<0.01	0.06	0.01	0.95	15	0.02	1.6	1.3	<0.02	<0.05	1.7	<0.01	4.7	0.007	<0.05	<0.2	<0.005	<0.1	44	80.43
7/25/2019 RG_SC-NPM-08-M_20190725	Muscle	<2	<0.01	0.10	1.0	<0.01	<1	<0.01	0.05	0.02	1.0	15	<0.01	1.8	1.3	<0.02	0.05	2.4	<0.01	6.6	0.014	<0.05	<0.2	<0.005	<0.1	24	78.64
7/25/2019 RG_SC-NPM-09-M_20190725	Muscle	<2	<0.01	0.02	0.24	<0.01	<1	<0.01	<0.05	0.01	1.8	19	<0.01	0.5	1.2	<0.02	<0.05	1.5	<0.01	1.1	0.006	<0.05	<0.2	<0.005	<0.1	19	76.51
7/26/2019 RG_SC-NPM-10-M_20190726	Muscle	<2	<0.01	0.07	0.39	<0.01	<1	<0.01	<0.05	0.01	0.76	10	<0.01	0.6	1.0	<0.02	0.06	1.4	<0.01	1.4	0.006	<0.05	<0.2	<0.005	<0.1	17	79.18
7/26/2019 RG_SC-NPM-11-M_20190726	Muscle	<2	<0.01	0.06	0.28	<0.01	<1	<0.01	<0.05	<0.01	1.1	14	<0.01	0.4	0.90	<0.02	<0.05	2.2	<0.01	1.1	0.013	<0.05	<0.2	<0.005	<0.1	21	79.53
6/21/2019 RG_WB-NPM-01-M_20190621	Muscle	<2	<0.01	0.02	0.97	<0.01	<1	<0.01	<0.05	0.02	1.7	23	0.04	0.9	1.1	<0.02	<0.05	2.2	<0.01	2.8	0.010	<0.05	<0.2	<0.005	<0.1	28	77.69
6/26/2019 RG-WB-NPM-02-M_20190626	Muscle	<2	<0.01	0.09	2.4	<0.01	<1	<0.01	<0.05	0.02	2.6	28	0.01	1.7	0.99	<0.02	<0.05	2.9	<0.01	9.4	0.015	<0.05	<0.2	<0.005	<0.1	44	79.29
6/26/2019 RG-WB-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	1.5	<0.01	<1	<0.01	<0.05	0.02	2.5	26	<0.01	1.5	1.0	<0.02	<0.05	2.9	<0.01	3.9	0.006	<0.05	<0.2	<0.005	<0.1	32	79.01
6/14/2019 RG_ER-NPM-01-O_20190614	Ovary	<2	<0.01	0.07	0.12	<0.01	<1	<0.01	<0.05	0.02	3.2	43	<0.01	0.6	0.10	0.05	<0.05	3.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	97	64.38
6/14/2019 RG_ER-NPM-02-O_20190614	Ovary	<2	<0.01	0.07	0.14	<0.01	<1	<0.01	<0.05	0.03	3.1	38	<0.01	3.0	0.16	0.06	<0.05	2.7	0.01	0.19	0.007	<0.05	<0.2	<0.005	<0.1	110	65.57
6/17/2019 RG_ER-NPM-03-O_20190617	Ovary	<2	<0.01	0.06	0.22	<0.01	<1	<0.01	<0.05	0.03	2.8	45	0.01	1.3	0.10	0.05	<0.05	3.3	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	94	65.63
6/17/2019 RG_ER-NPM-04-O_20190617	Ovary	<2	<0.01	0.09	0.13	<0.01	<1	<0.01	<0.05	0.02	2.8	41	<0.01	0.7	0.051	0.04	<0.05	3.0	<0.01	0.11	0.009	<0.05	<0.2	<0.005	<0.1	87	61.36
6/18/2019 RG_ER-NPM-05-O_20190618	Ovary	<2	<0.01	0.04	0.21	<0.01	<1	<0.01	<0.05	0.03	3.0	37	0.01	2.2	0.12	0.04	<0.05	4.9	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	78	62.62
6/18/2019 RG_ER-NPM-06-O_20190618	Ovary	<2	<0.01	0.26	0.14	<0.01	<1	<0.01	<0.05	0.03	2.6	52	<0.01	2.2	0.14	0.07	<0.05	5.0	<0.01	0.20	0.006	<0.05	<0.2	<0.005	<0.1	120	67.92
6/18/2019 RG_ER-NPM-07-O_20190618	Ovary	<2	<0.01	0.06	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	41	<0.01	1.3	0.11	0.04	<0.05	4.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	87	63.03
6/18/2019 RG_ER-NPM-08-O_20190618	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.03	2.3	91	0.01	2.6	0.13	0.08	<0.05	4.2	<0.01	0.27	0.009	<0.05	<0.2	<0.005	<0.1	120	67.65
6/19/2019 RG_ER-NPM-09-O_20190619	Ovary	3	<0.01	0.06	0.16	<0.01	<1	<0.01	<0.05	0.03	2.8	60	0.18	2.2	0.054	0.07	<0.05	7.2	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	68.24
6/19/2019 RG_ER-NPM-10-O_20190619	Ovary	2	<0.01	0.04	0.26	<0.01	<1	<0.01	0.21	0.05	2.8	96	0.13	3.4	0.17	0.14	<0.05	9.9	<0.01	0.32	0.023	<0.05	<0.2	<0.005	<0.1	260	76.39
6/19/2019 RG_ER-NPM-11-O_20190619	Ovary	4	<0.01	0.06	0.32	<0.01	<1	0.01	0.11	0.06	4.1	130	0.18	2.7	0.096	0.12	0.06	17	<0.01	0.41	0.049	<0.05	<0.2	<0.005	<0.1	500	79.40
6/20/2019 RG_ER-NPM-12-O_20190620	Ovary	<2	<0.01	0.07	0.15	<0.01	<1	<0.01	<0.05	0.02	3.0	33	<0.01	0.5	0.081	0.04	<0.05	2.4	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	84	63.59
6/20/2019 RG_ER-NPM-13-O_20190620	Ovary	<2	<0.01	0.06	0.14	<0.01	<1	<0.01	<0.05	0.03	2.4	54	<0.01	1.9	0.23	0.06	<0.05	3.6	<0.01	0.22	0.008	<0.05	<0.2	<0.005	<0.1	120	71.25
6/20/2019 RG_ER-NPM-14-O_20190620	Ovary	<2	<0.01	0.06	0.12	<0.01	<1	<0.01	<0.05	0.04	2.6	60	<0.01	1.8	0.21	0.08	<0.05	7.6	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	72.31
6/20/2019 RG_ER-NPM-15-O_20190620	Ovary	<2	<0.01	0.04	0.36	<0.01	<1	<0.01	<0.05	0.05	2.9	95	<0.01	5.5	0.078	0.10	<0.05	8.6	<0.01	0.24	0.009	<0.05	<0.2	<0.005	<0.1	150	70.05
6/20/2019 RG_ER-NPM-16-O_20190620	Ovary	2	<0.01	0.08	0.35	<0.01	<1	0.01	0.09	0.07	3.6	150	0.02	2.5	0.13	0.12	0.06	17	<0.01	0.26	0.033	<0.05	<0.2	<0.005	<0.1	520	83.66
6/20/2019 RG_ER-NPM-17-O_20190620	Ovary	<2	<0.01	0.07	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	2.3	0.085	0.04	<0.05	4.5	<0.01	0.16	0.005	<0.05	<0.2	<0.005	<0.1	74	65.19
6/20/2019 RG_ER-NPM-18-O_20190620	Ovary	<2	<0.01	0.05	0.22	<0.01	<1	<0.01	<0.05	0.02	1.8	32	<0.01	2.5	0.14	0.04	<0.05	4.1	<0.01	0.17	<0.005	<0.05	<0.2	<0.005	<0.1	80	65.88
6/25/2019 RG_ER-NPM-19-O_20190625	Ovary	<2	<0.01	0.08	0.38	<0.01	<1	<0.01	<0.05	0.04	2.6	66	<0.01	8.3	0.024	0.07	<0.05	7.9	<0.01	0.17	0.018	<0.05	<0.2	<0.005	<0.1	120	66.61
6/25/2019 RG_ER-NPM-20-O_20190625	Ovary	<2	<0.01	0.04	0.08	<0.01	<1	<0.01	<0.05	0.06	3.2	110	<0.01	2.6	0.12	0.08	<0.05	6.3	<0.01	0.21	0.030	<0.05	<0.2	<0.005	<0.1	180	75.96
6/27/2019 RG_ER-NPM-21-O_20190627	Ovary	<2	<0.01	0.03	0.11	<0.01	<1	<0.01	<0.05	0.02	2.7	49	<0.01	2.3	0.039	0.03	<0.05	13	<0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	80	60.36
6/28/2019 RG_ER-NPM-22-O_20190628	Ovary	<2	<0.01	0.05	0.17	<0.01	<1	<0.01	<0.05	0.02	2.2	37	<0.01	2.5	0.021	0.03	<0.05	7.1	<0.01	0.09	0.008	<0.05	<0.2	<0.005	<0.1	79	61.41
6/28/2019 RG_ER-NPM-23-O_20190628	Ovary	<2	<0.01	0.01	0.32	<0.01	<1	<0.01	<0.05	0.03	2.7	44	<0.01	4.1	0.12	0.04	<0.05	9.8	0.02	0.14	0.006	<0.05	<0.2	<0.005	<0.1	100	65.42
6/28/2019 RG_ER-NPM-24-O_20190628	Ovary	<2	<0.01	0.05	0.19	<0.01	<1	<0.01	<0.05	0.04	3.1	55	<0.01	1.5	0.12	0.05	<0.05	7.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	120	67.86
6/28/2019 RG_ER-NPM-25-O_20190628	Ovary	<2	<0.01	0.02	0.22	<0.01	<1	<0.01	<0.05	0.03	3.3	51	<0.01	4.7	0.050	0.06	<0.05	8.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	97	63.20
6/28/2019 RG_ER-NPM-26-O_20190628	Ovary	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	0.02	3.0	54	<0.01	2.1	0.037	0.04	<0.05	4.0	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	77	62.47
6/28/2019 RG_ER-NPM-27-O_20190628	Ovary	<2	<0.01	0.86	1.5	<0.01	<1	0.10	0.06	0.06	1.5	47	0.07	0.4	0.023	<0.02	0.06	14.6	<0.01	0.10	0.021	<0.05	<0.2	<0.005	<0.1	300	56.47
7/3/2019 RG_ER-NPM-28-O_20190703	Ovary	4	<0.01	0.03	0.20	<0.01	<1	0.01	<0.05	0.04	2.8	170	0.06	2.8	0.088	0.08	<0.05	34.5	<0.01	0.27	0.018	<0.05	<0.2	<0.005	<0.1	180	77.60
7/4/2019 RG_ER-NPM-29-O_20190704	Ovary	6	<0.01	0.05	0.39	<0.01	<1	0.04	0.22	0.07	3.9	150	0.06	2.8	0.097	0.15	0.10	19.4	<0.01	0.57	0.034	<0.05	0.3	<0.005	<0.1	330	77.92
7/4/2019 RG_ER-NPM-30-O_20190704	Ovary	14	<0.01	0.04	0.44	<0.01	<1	0.02	0.05	0.05	4.2	110	0.15	3.7	0.11	0.07	<0.05	7.1	<0.01	0.36	0.014	<0.05	0.3	<0.005	0.1	200	76.76
7/8/2019 RG_ER-NPM-31-O_20190708	Ovary	<2	<0.01	0.04	0.20	<0.01	<1	<0.01	0.09	0.03	3.3	64	<0.01	3.5	0.068	0.05	<0.05	10.9	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	100	65.74
7/9/2019 RG_ER-NPM-32-O_20190709	Ovary	<2	<0.01	0.08	0.13	<0.01	<1	<0.01	0.10	0.04	3.3	81	0.01	1.2	0.13	0.08	<0.05	4.1	<0.01	0.36	0.016	<0.05	<0.2	<0.005	<0.1	160	74.41

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG_ER-NPM-33-O_20190709	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.6	47	<0.01	0.9	0.030	<0.02	<0.05	3.5	<0.01	0.18	0.010	<0.05	<0.2	<0.005	<0.1	97	63.66
7/9/2019 RG_ER-NPM-34-O_20190709	Ovary	3	<0.01	0.07	0.13	<0.01	<1	0.01	<0.05	0.05	3.2	77	<0.01	3.1	0.21	0.08	<0.05	5.4	<0.01	0.24	0.029	<0.05	<0.2	<0.005	0.1	260	76.24
7/9/2019 RG_ER-NPM-35-O_20190709	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.02	2.4	57	<0.01	1.1	0.056	0.04	<0.05	3.8	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	82	65.42
7/9/2019 RG_ER-NPM-36-O_20190709	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	0.02	3.0	43	<0.01	2.4	0.12	0.04	<0.05	9.3	0.01	0.10	0.006	<0.05	<0.2	<0.005	<0.1	78	62.62
7/9/2019 RG_ER-NPM-37-O_20190709	Ovary	<2	<0.01	0.06	0.23	<0.01	<1	<0.01	0.21	0.03	3.1	48	<0.01	1.5	0.050	0.05	<0.05	5.4	<0.01	0.24	<0.005	<0.05	<0.2	<0.005	<0.1	92	63.31
7/10/2019 RG_ER-NPM-38-O_20190710	Ovary	3	<0.01	0.04	0.19	<0.01	<1	<0.01	<0.05	0.04	3.6	57	<0.01	5.9	0.071	0.08	<0.05	12	0.01	0.28	0.007	<0.05	<0.2	<0.005	<0.1	130	69.92
7/10/2019 RG_ER-NPM-39-O_20190710	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.02	3.3	48	<0.01	2.0	0.038	0.04	<0.05	2.7	0.01	0.14	0.010	<0.05	<0.2	<0.005	<0.1	110	62.30
7/10/2019 RG_ER-NPM-40-O_20190710	Ovary	5	<0.01	0.06	0.35	<0.01	<1	0.02	0.12	0.04	4.0	110	0.07	1.4	0.060	0.07	0.06	18.4	<0.01	0.24	0.036	<0.05	0.2	<0.005	<0.1	540	79.88
7/10/2019 RG_ER-NPM-41-O_20190710	Ovary	<2	<0.01	0.07	0.17	<0.01	<1	<0.01	<0.05	0.02	2.6	52	<0.01	1.3	0.030	0.05	<0.05	3.4	<0.01	0.13	0.015	<0.05	<0.2	<0.005	<0.1	110	64.12
7/10/2019 RG_ER-NPM-42-O_20190710	Ovary	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.03	3.2	55	<0.01	5.1	0.079	0.07	<0.05	11	0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	100	65.47
7/11/2019 RG_ER-NPM-43-O_20190711	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.03	3.0	42	<0.01	2.5	0.049	0.04	<0.05	12	0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	78	64.30
7/12/2019 RG_ER-NPM-44-O_20190712	Ovary	3	<0.01	0.05	0.15	<0.01	<1	0.01	0.11	0.06	3.6	110	0.02	2.9	0.068	0.14	<0.05	36	<0.01	0.34	0.032	<0.05	<0.2	<0.005	<0.1	410	78.06
7/12/2019 RG_ER-NPM-45-O_20190712	Ovary	<2	<0.01	0.09	0.22	<0.01	<1	<0.01	<0.05	0.02	2.7	33	<0.01	0.5	0.049	0.04	<0.05	2.2	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	91	62.46
7/13/2019 RG_ER-NPM-46-O_20190713	Ovary	<2	<0.01	0.12	0.07	<0.01	<1	<0.01	<0.05	0.04	3.3	91	<0.01	0.6	0.17	0.09	<0.05	2.4	<0.01	0.22	0.046	<0.05	<0.2	<0.005	<0.1	210	75.90
7/15/2019 RG_ER-NPM-47-O_20190715	Ovary	<2	<0.01	0.12	0.14	<0.01	<1	<0.01	<0.05	0.02	2.5	31	<0.01	0.7	0.091	0.03	<0.05	2.1	<0.01	0.12	0.011	<0.05	<0.2	<0.005	<0.1	99	66.82
7/16/2019 RG_ER-NPM-48-O_20190716	Ovary	<2	<0.01	0.11	0.06	<0.01	<1	0.01	<0.05	0.05	3.7	69	<0.01	1.4	0.37	0.11	<0.05	2.3	<0.01	0.33	0.059	<0.05	<0.2	<0.005	<0.1	230	81.71
7/26/2019 RG_ER-NPM-49-O_20190726	Ovary	2	<0.01	0.15	0.32	<0.01	<1	0.02	0.06	0.05	3.7	240	<0.01	1.2	0.23	0.07	<0.05	3.4	<0.01	0.43	0.051	<0.05	<0.2	0.008	0.1	380	82.16
6/26/2019 RG_GC-NPM-01-O_20190626	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	41	<0.01	0.6	0.14	0.05	<0.05	2.4	<0.01	0.17	0.005	<0.05	<0.2	<0.005	<0.1	100	64.51
6/26/2019 RG_GC-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	0.6	0.096	0.06	<0.05	2.1	<0.01	0.14	0.012	<0.05	<0.2	<0.005	<0.1	110	65.08
6/26/2019 RG_GC-NPM-03-O_20190626	Ovary	2	<0.01	0.05	0.18	<0.01	<1	<0.01	0.40	0.04	3.1	47	<0.01	0.6	0.049	0.04	<0.05	2.1	<0.01	0.12	0.006	<0.05	<0.2	<0.005	<0.1	77	63.05
6/26/2019 RG_GC-NPM-04-O_20190626	Ovary	<2	<0.01	0.17	0.19	<0.01	<1	<0.01	0.07	<0.01	2.5	75	0.01	1.1	0.096	0.04	<0.05	20	<0.01	0.21	0.032	<0.05	<0.2	<0.005	<0.1	360	72.82
6/26/2019 RG_GC-NPM-05-O_20190626	Ovary	<2	<0.01	0.03	0.26	<0.01	<1	<0.01	<0.05	<0.01	2.2	46	<0.01	1.9	0.076	0.03	<0.05	3.9	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	83	63.49
6/26/2019 RG_GC-NPM-06-O_20190626	Ovary	<2	<0.01	0.06	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.7	40	<0.01	1.9	0.048	0.04	<0.05	2.4	<0.01	0.12	0.012	<0.05	<0.2	<0.005	<0.1	100	63.02
6/26/2019 RG_GC-NPM-07-O_20190626	Ovary	<2	<0.01	0.11	0.13	<0.01	<1	0.01	0.12	<0.01	3.2	120	0.01	1.4	0.082	0.08	<0.05	11	<0.01	0.19	0.047	<0.05	<0.2	<0.005	<0.1	360	77.70
6/27/2019 RG_GC-NPM-08-O_20190627	Ovary	<2	<0.01	0.15	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	30	<0.01	0.4	0.078	0.03	<0.05	2.2	<0.01	0.11	0.012	<0.05	<0.2	<0.005	<0.1	100	62.90
6/27/2019 RG_GC-NPM-09-O_20190627	Ovary	<2	<0.01	0.10	0.06	<0.01	<1	<0.01	<0.05	<0.01	3.8	68	<0.01	1.5	0.25	0.10	<0.05	2.2	<0.01	0.30	0.036	<0.05	<0.2	<0.005	<0.1	180	77.62
6/27/2019 RG_GC-NPM-10-O_20190627	Ovary	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	40	<0.01	1.1	0.093	0.04	<0.05	2.7	<0.01	0.11	0.010	<0.05	<0.2	<0.005	<0.1	90	61.14
6/27/2019 RG_GC-NPM-11-O_20190627	Ovary	<2	<0.01	0.04	0.12	<0.01	<1	<0.01	0.11	0.01	2.8	92	<0.01	2.4	0.13	0.10	<0.05	12	<0.01	0.29	0.018	<0.05	<0.2	<0.005	<0.1	340	78.10
6/27/2019 RG_GC-NPM-12-O_20190627	Ovary	<2	<0.01	0.04	0.25	<0.01	<1	<0.01	<0.05	<0.01	2.3	47	<0.01	3.3	0.070	0.03	<0.05	3.9	<0.01	0.12	<0.005	<0.05	<0.2	<0.005	<0.1	95	64.74
6/27/2019 RG_GC-NPM-13-O_20190627	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.1	33	<0.01	1.3	0.078	0.03	<0.05	3.3	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	65	61.68
7/18/2019 RG_GC-NPM-14-O_20190718	Ovary	7	<0.01	0.09	0.18	<0.01	<1	0.02	<0.05	<0.01	3.3	140	0.02	1.5	0.28	0.08	<0.05	9.6	<0.01	0.27	0.045	<0.05	0.2	<0.005	0.1	430	79.54
7/19/2019 RG_GC-NPM-15-O_20190719	Ovary	2	<0.01	0.10	0.21	<0.01	<1	<0.01	<0.05	<0.01	2.2	37	<0.01	0.5	0.27	0.04	<0.05	3.9	<0.01	0.11	0.008	<0.05	<0.2	<0.005	<0.1	120	67.63
7/25/2019 RG_GC-NPM-16-O_20190725	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.8	39	<0.01	1.1	0.083	0.03	<0.05	2.7	<0.01	0.11	0.011	<0.05	0.4	<0.005	<0.1	91	64.09
6/20/2019 RG_SC-NPM-01-O_20190620	Ovary	<2	<0.01	0.04	0.13	<0.01	<1	<0.01	<0.05	<0.01	3.1	56	<0.01	3.3	0.071	0.06	<0.05	8.4	<0.01	0.20	0.008	<0.05	<0.2	<0.005	<0.1	120	68.02
6/20/2019 RG_SC-NPM-02-O_20190620	Ovary	7	<0.01	0.06	0.26	<0.01	<1	<0.01	0.05	<0.01	2.6	130	0.03	1.2	0.12	0.06	<0.05	20	<0.01	0.84	0.041	<0.05	<0.2	<0.005	<0.1	490	80.46
6/20/2019 RG_SC-NPM-03-O_20190620	Ovary	<2	<0.01	0.09	0.07	<0.01	<1	<0.01	<0.05	<0.01	3.0	93	0.02	2.1	0.041	0.08	<0.05	11	<0.01	0.20	0.025	<0.05	<0.2	<0.005	<0.1	320	73.78
6/20/2019 RG_SC-NPM-04-O_20190620	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	<0.01	3.6	110	0.02	3.0	0.098	0.09	<0.05	17	<0.01	0.72	0.012	<0.05	<0.2	<0.005	<0.1	140	69.48
6/20/2019 RG_SC-NPM-05-O_20190620	Ovary	<2	<0.01	0.10	0.33	<0.01	<1	<0.01	<0.05	0.05	3.0	98	<0.01	1.8	0.088	0.10	0.05	28	<0.01	0.23	0.034	<0.05	<0.2	<0.005	<0.1	360	75.91
7/24/2019 RG_SC-NPM-06-O_20190724	Ovary	<2	<0.01	0.08	0.18	<0.01	<1	<0.01	<0.05	0.05	2.5	120	<0.01	1.9	0.11	0.04	0.09	10	<0.01	0.78	0.008	<0.05	<0.2	<0.005	<0.1	250	77.04
7/25/2019 RG_SC-NPM-07-O_20190725	Ovary	<2	<0.01	0.07	0.09	<0.01	<1	<0.01	<0.05	0.04	3.0	100	<0.01	8.9	0.14	0.16	<0.05	21	<0.01	0.38	0.018	<0.05	<0.2	<0.005	<0.1	280	77.16
7/25/2019 RG_SC-NPM-08-O_20190725	Ovary	<2	<0.01	0.15	0.15	<0.01	<1	<0.01	<0.05	0.05	2.8	86	<0.01	5.1	0.092	0.10	<0.05	23	<0.01	0.57	0.018	<0.05	<0.2	<0.005	<0.1	200	74.33

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/25/2019 RG_SC-NPM-09-O_20190725	Ovary	<2	<0.01	0.08	0.07	<0.01	<1	<0.01	<0.05	0.05	2.7	75	<0.01	3.5	0.18	0.12	0.08	12	<0.01	0.33	0.007	<0.05	<0.2	<0.005	<0.1	220	75.90
7/26/2019 RG_SC-NPM-10-O_20190726	Ovary	3	<0.01	0.13	0.62	<0.01	<1	<0.01	0.09	0.05	3.2	100	<0.01	1.6	0.072	0.07	0.07	25	<0.01	0.28	0.023	<0.05	<0.2	<0.005	<0.1	440	75.75
7/26/2019 RG_SC-NPM-11-O_20190726	Ovary	<2	<0.01	0.12	0.15	<0.01	<1	<0.01	0.08	0.07	4.2	160	<0.01	2.8	0.12	0.05	<0.05	23	<0.01	0.62	0.028	<0.05	<0.2	<0.005	<0.1	320	80.08
6/21/2019 RG_WB-NPM-01-O_20190621	Ovary	5	<0.01	0.02	0.23	<0.01	<1	<0.01	<0.05	0.04	3.6	77	0.06	4.5	0.069	0.09	0.06	7.4	<0.01	0.40	0.006	<0.05	<0.2	<0.005	<0.1	120	67.47
7/13/2019 RG_ER-NPM-47-O_20190713	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	45	<0.01	0.9	0.045	0.03	<0.05	3.5	<0.01	0.10	0.009	<0.05	<0.2	<0.005	<0.1	94	64.10
6/26/2019 RG-WB-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.05	3.0	83	<0.01	4.4	0.12	0.13	<0.05	26	<0.01	0.28	0.030	<0.05	<0.2	<0.005	<0.1	260	76.76
6/26/2019 RG-WB-NPM-03-O_20190626	Ovary	<2	<0.01	0.14	0.12	<0.01	<1	<0.01	<0.05	0.03	2.0	65	<0.01	1.6	0.18	0.06	<0.05	9.8	<0.01	0.14	0.007	<0.05	<0.2	<0.005	<0.1	240	70.13

ATTACHMENT 2

Analysis of Historical Mountain Whitefish Data Memorandum

EcoTox

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MEMORANDUM

Date: June 10, 2020

To: Mariah Arnold

From: Kevin Brix (EcoTox), David DeForest (Windward), and Lucinda Tear (Windward)

Subject: Analysis of Historical Mountain Whitefish Data

This memorandum describes the methods and results of a multivariate analysis of historical monitoring data for mountain whitefish (MW) collected in the Elk Valley. The first objective of this analysis was to determine whether factors such as gonado-somatic index (GSI), fish size (as measured by fork length), and fish sampling location influence observed ovary selenium (Se) concentrations in MW in the Elk Valley. The second objective of the analysis was to identify sampling locations and estimate the sampling intensity required (i.e., number of fish) to have a high probability of collecting MW in spawning condition with egg Se concentrations >33 mg kg^{-1} dw, the maximum egg Se concentration observed in previous efforts to conduct a selenium toxicity study with this species (Nautilus Environmental 2017).

Introduction

Historical and ongoing monitoring of MW in the Elk Valley indicate individual, and in some cases mean, ovary Se concentrations exceed the BC ENV guideline of 11 mg kg^{-1} dw, as well as the Level 1 Benchmark (18 mg kg^{-1} dw) and in some cases the Interim Screening Benchmark (29 mg kg^{-1} dw). This is true for ovaries collected from both reference and mine-exposed locations, although ovaries collected from fish in mine-exposed areas are on average higher than those collected from reference areas.

Given the ovary Se concentrations observed historically, Teck conducted three rounds (2010, 2011, and 2013) of toxicity testing in an attempt to estimate the sensitivity of the embryo-larval stage of MW to maternally transferred Se (Nautilus Environmental 2017). Combined, these studies demonstrated no effects on MW embryo-larval survival, growth, or development up to the highest egg Se concentration obtained (33 mg kg dw^{-1}). However, through 2018, ovary Se concentrations as high as 81 mg kg^{-1} dw have been observed through sampling under the Regional Aquatics Effect Monitoring Program (RAEMP) (Figure 1). Consequently, it is

important to understand whether effects are occurring in MW with egg Se concentrations >33 mg kg^{-1} dw and Teck anticipates conducting a fourth toxicity study on MW in the Fall of 2020.

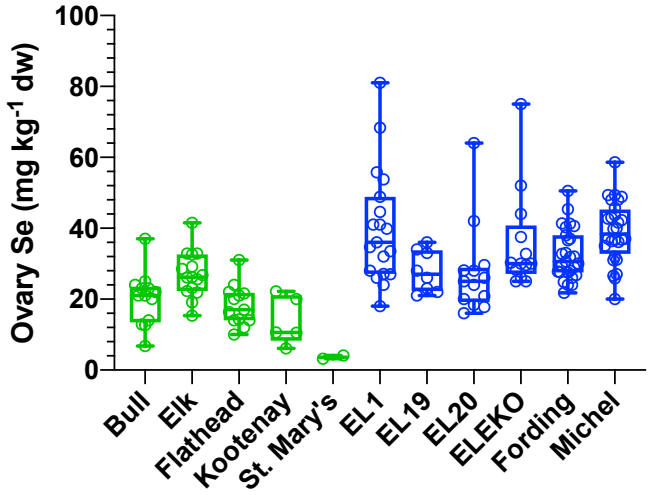


Figure 1. Ovary Se concentrations in mountain whitefish by location (2006-2018).

A preliminary review of the MW ovary Se data collected in 2018 demonstrates a negative correlation between GSI and ovary Se concentrations (Figure 2). The relationship is similar to that observed for northern pikeminnow (*Ptychocheilus oregonensis*; NPM). In NPM, females in spawning condition have a GSI >5%, while much of the historical ovary Se data were from fish with a GSI <5%, leading to an overestimation of egg Se concentrations likely to occur in NPM from Koocanusa Reservoir (EcoTox et al. 2020).

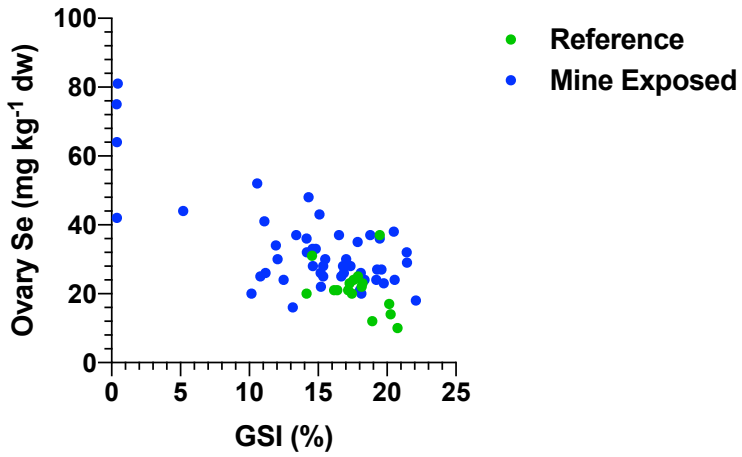


Figure 2. Relationship between GSI and ovary Se in mountain whitefish collected from Elk Valley in 2018.

Based on the preliminary analysis of the 2018 MW data, it is possible a similar bias exists in the ovary Se monitoring data for this species. This is a critical issue to understand with respect to developing a study plan for the 2020 MW Se toxicity study. If this negative relationship between GSI and ovary Se occurs across historical monitoring data, it will be important to understand the distribution of ovary Se data associated only with fish in spawning condition. This will inform the study design with respect to the sampling intensity required (i.e., number of females needing to be sampled) to have a high likelihood of collecting fish with egg Se concentrations >33 mg kg^{-1} dw. Similar to NPM, it is possible other factors (e.g., fish size and sampling location) also influence ovary Se concentrations (EcoTox et al. 2020). Understanding the importance of these factors can improve the sampling design for the 2020 MW Se toxicity study.

Data for Analysis

Historical monitoring data from 2006-2018 collected in the Elk River drainage and reference locations were considered in evaluating potential relationships between ovary Se concentrations and GSI, fish size (fork length), or sampling location. Data from 2012 were excluded from the analysis because information on GSI was not collected that year. Similarly, data from Koocanusa Reservoir were excluded from the analysis as GSI data were not available for fish collected from this location. In total, data from 156 fish were available for the analysis (Table 1).

Table 1. Ovary Se Sample Size by Location

	Year				
Location	2006	2009	2015	2018	Total
Mine Exposed Sites					
EL1	5	5		9	19
EL19				8	8
EL20			5	8	13
ELEKO			5	8	13
Fording	5	10	4	8	27
Michel	10	5	5	8	28
References Sites					
Bull River			5		5
Elk River	5	10			15
Flathead River			5	8	13
Kootenay River			5		5
St. Mary's River			2		2

Exploratory Analysis

An initial exploratory analysis of data was conducted using scatter plots and Principal Component Analysis (PCA) to provide visual and quantitative understanding of correlations among the variables of interest and how the relationships among those variables varied geographically. Fork length, GSI, and ovary Se were natural log (ln)-transformed, centered, and scaled for the PCA.

Scatter plots suggest that, similar to the 2018 data set (Figure 2), a relationship exists between GSI and ovary Se, while there does not appear to be a relationship with fish length (Figure 3).

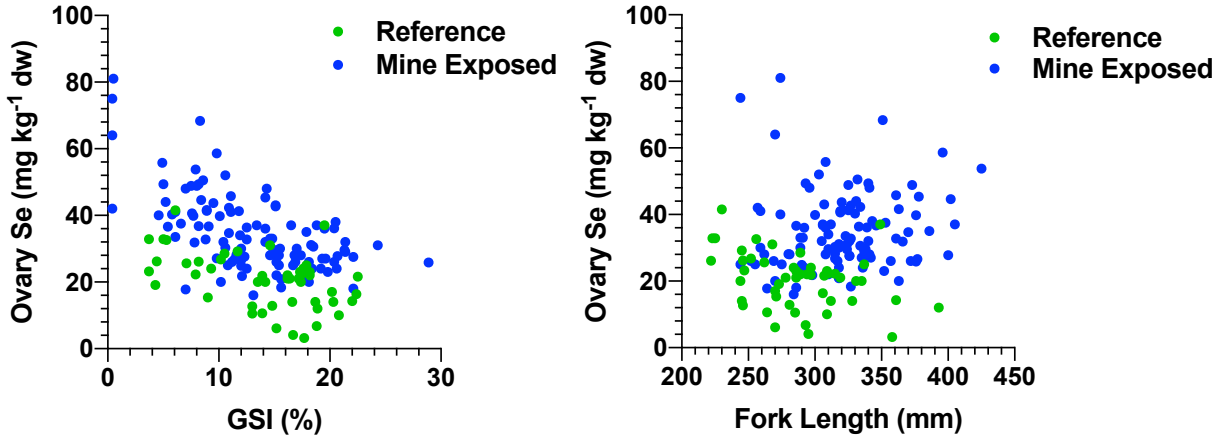


Figure 3. Relationships between GSI and fish length versus ovary Se using data summarized in Table 1.

The first two axes of the PCA explained approximately 88% of the variance. Axis 1 was most highly correlated with GSI (Figure 4) and explained 49% of the variance. Axis 2 was somewhat equally correlated with fork length and ovary Se and explained 38% of the variance. The biplot of PCA scores shows each of the variables having similar effects on the spread of the data (arrows indicating direction of increasing value of the associated variable are approximately equal length), and identifies four samples with very low GSI as quite separate from the rest of the data (far left of Axis 1).

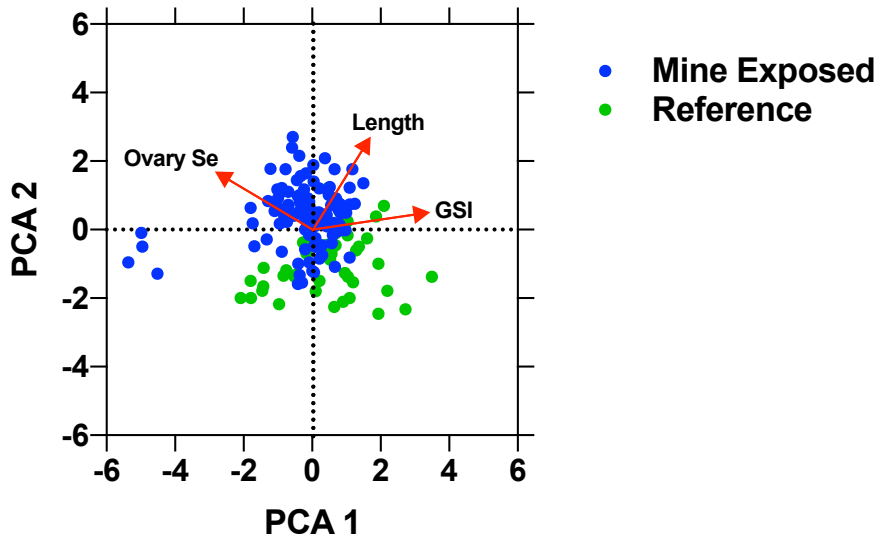


Figure 4. Biplot of Principal Component Analysis using ln-transformed fork length, GSI, and ovary Se. Arrows indicate direction of increasing values of the associated variable.

Multi-linear Regression (MLR) Analysis

Although the exploratory analyses indicated four data points differed from the bulk of the data, all data were retained in the initial MLR analyses. As in the exploratory analyses, all data were ln-transformed. A step-wise MLR model testing for linear effects of fork length and GSI with area-specific slopes and intercepts was run using the Akaike and Bayesian Information Criteria (AIC, BIC) to identify the most parsimonious models (R, stepAIC) (Equation 1).

$$\text{Ln(OvSe)} = \text{area} + \text{Ln(FL)} + \text{area*}\ \text{Ln(FL)} + \text{Ln(GSI)} + \text{area*}\ \text{Ln(GSI)} \qquad \text{(Eq. 1)}$$

where, OvSe = ovary Se, FL = fork length (mm), and GSI = gonadosomatic index.

Model residuals were tested for normality using Shapiro Wilks (shapiro.test, R) and Nonconstant Variance (ncv, R).

The models identified by both AIC and BIC were identical. Fish size, as measured by fork length, was not retained in the model, indicating it does not have a significant influence on ovary Se. In contrast, GSI was highly significant ($p < 0.001$), but no area-specific slopes were retained, indicating the relationship between GSI and ovary Se is similar across sites. Area-specific intercepts were retained in the model, indicating significant differences in ovary Se among sites after correcting for the effect of GSI.

Two samples with low ovary Se collected from St. Mary's River (reference location) did not fit the model well. These are not part of the four samples with very low GSI identified in the PCA. The two samples from St. Mary's River were removed and the model was rerun to evaluate how these two outliers might be unduly influencing model parameterization. Results were similar to the first run, with GSI highly significant ($p < 0.001$) and area-specific intercepts with the same differences as the previous model.

Adjusted and predicted R^2 for the final model were 0.59 and 0.58, respectively (Figure 5). Model residuals were not normally distributed and did not have constant variance across the range of predicted values (Shapiro Wilks $p < 0.001$, Nonconstant Variance Test $p < 0.001$). Overall, the model appears to predict the mine-exposed sites reasonably well, but performs more poorly for the reference locations.

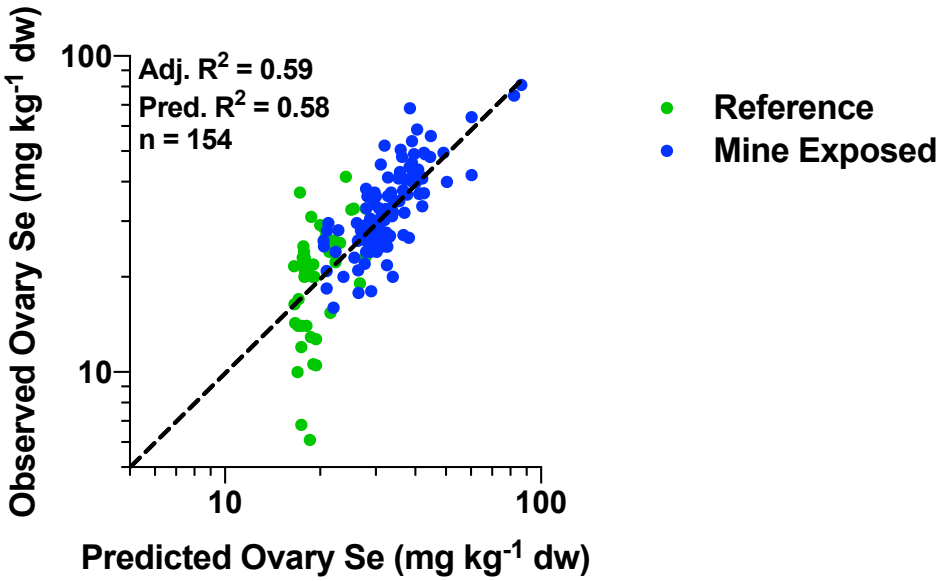


Figure 5. Final Linear Model Excluding Data from St. Mary's River.

The final model has a ln GSI slope of -0.288 and area-specific intercepts (Table 2). Treatment contrasts comparing Michel Creek to other locations indicate, with the exception of EL1, all areas have a significantly ($p < 0.05$) lower intercept than Michel Creek (Table 2). This means that after accounting for the effect of GSI on ovary Se concentrations, Michel Creek has significantly higher ovary Se concentrations than all locations except EL1.

Table 2. Final ovary Se model coefficients and significance. Note: The intercept t values and *p* values relate to testing for significant differences in intercepts relative to Michel Creek.

		Estimate	Std. Error	t Value	*p* Value
Intercepts	Reference	3.705	0.065	-10.10	<0.001
	EL1	4.261	0.081	-1.22	0.223
	EL19	4.106	0.109	-2.33	0.021
	EL20	3.836	0.092	-5.69	<0.001
	ELEKO	4.145	0.091	-2.34	0.020
	Fording	4.203	0.073	-2.15	0.033
	Michel	4.360	0.097	-	-
Slope	ln GSI	-0.288	0.033	-8.80	<0.001

Sample Size Analysis

The negative relationship between GSI and ovary Se provides evidence that this variable needs to be considered in evaluating sampling effort for future toxicity studies. Information on the GSI typically associated with MW in spawning conditioning is limited, but a recent study indicates a GSI of >15% is associated with spawning in this species (Irvine et al. 2017). The model also demonstrated that significant differences in ovary Se between sampling locations exist, with Michel Creek having the highest ovary Se on average. Given this information, we focused our sample size analysis on MW with a GSI >15% collected from Michel Creek.

The objective of the sample size analysis was to estimate the sampling intensity required to obtain egg Se concentrations with a specific level of confidence. Specifically, we wanted to estimate the number of female MW that will need to be sampled from Michel Creek to obtain eggs from at least 3 females with a given egg Se concentration with 90% confidence. Recalling that the highest egg Se concentration evaluated in previous toxicity testing efforts was 33 mg kg^{-1} dw, we estimated samples sizes needed to collect egg Se concentrations of 34, 36, 40, and 43 mg kg^{-1} dw. First, we calculated the proportion of fish captured with both a GSI >15% and specified ovary Se concentration as:

P(ovary Se >X and GSI >15%) = Count(ovary Se >X and GSI >15%)/Count(GSI >15%)

The proportions ranged from 0 to 0.50 with the highest proportions (as well as the highest number of fish) in Michel and Fording drainages. To estimate the sample size needed to collect at least 3 fish with a specific ovary Se, we assumed the proportion of fish with the specified P fit the binomial distribution.

P ~ Binomial(n,p)

where p = observed proportion of ovary Ovary Se fish = observed P, and n = sample size.

We estimated the number of samples required to be 90% confident of collecting at least 3 fish with the specified ovary Se fish for a range of p levels [P = 0.1, 0.2, 0.3, 0.4, 0.5] using the inverse of the binomial distribution [Excel function =BINOM.INV(*n, p, alpha*) or R function qbinom(1-*alpha*, *n*, *p*]. This was done by submitting a range of possible sample sizes (n = 5:100) to each function to find the minimum sample size at which 3 ovary Se samples would be collected with the desired confidence (*alpha* = 0.10). In parallel, we estimated the probability of collecting at least 3 ovary Se samples for the same range of sample sizes and p levels (Excel function =1-BINOM.DIST(2, P, *n*, cumulative = 1), pbinom(2, *n*, *alpha*, lower.tail = FALSE).

Results from this analysis indicate that even a relatively modest increase in the range of ovary Se concentrations of 5 mg kg^{-1} dw over the maximum concentration of the existing data can only be accomplished by sampling Michel Creek and would require sampling at least 28 female fish (Table 1). The sample size remains constant for ovary Se >38 mg kg^{-1} dw because the probability of capturing a fish remains constant. This is because the sample size for this analysis is small and unevenly distributed (n=11 for Michel Creek fish with GSI >15%). Consequently, there is some uncertainty in these estimates and caution should be exercised in using these values for sample design, i.e., sampling more fish than estimated by this analysis is recommended.

Table 1. Estimated Sample Size Required to Obtain 3 Female Mountain Whitefish with Specified Ovary Se Concentration with 90% Probability of Success Assuming GSI = 15%.

	Target Ovary Se Concentration				
Location	**34 mg kg^{-1} dw**	**36 mg kg^{-1} dw**	**38 mg kg^{-1} dw**	**40 mg kg^{-1} dw**	**43 mg kg^{-1} dw**
Michel Creek	9	13	28	28	28
Fording River	24	28	UC[1]	UC[1]	UC[1]

[1] Unable to calculate as no ovary samples with this Se concentration from fish with a GSI of at least 15% have been collected.

Conclusions

This analysis was conducted to provide information on the sampling design for the planned MW Se toxicity study. The objective of the toxicity study is to extend the range of concentration-response data above 33 mg kg dw^{-1}, the highest concentration observed over the course of three previous rounds of testing. This analysis demonstrated that ovary Se concentrations in MW are related to the GSI, but not the size, of female MW from which they are collected. After correcting for the effect of GSI using a linear model, differences in ovary Se between mine-

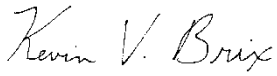
exposed and reference locations were detected, with fish collected from Michel Creek having the highest ovary Se.

A subsequent sample size analysis used historical monitoring data including only fish with a GSI >15%, the likely GSI associated with fish in spawning condition. The sample size analysis indicates that at least 28 female fish will need to be collected to have a high (90%) probability of collecting at least 3 fish that increase the range of the concentration-response data by 5-10 mg kg dw^{-1}. Based on this analysis, we recommend that at least 30 female fish from Michel Creek be sampled for the 2020 mountain whitefish toxicity study. We also recommend 5-10 fish from 2 reference locations be collected to provide controls for the study.

Closing

I trust that this analysis provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact me at (305) 773-8347.

Sincerely,



Kevin V. Brix, Ph.D.
Principal Scientist
EcoTox LLC

References

EcoTox, University of Saskatchewan and Minnow Environmental (2020). Draft Report - Evaluation of selenium concentrations in ovary of northern pikeminnow (*Ptychochelius oregonensis*). Miami, Florida, EcoTox LLC**:** 27 pp. + appendices.

Irvine, R. L., J. L. Thorley and L. Porto (2017). "When do mountain whitefish (*Prosopium williamsoni*) spawn? A comparison of estimates based on gonadosomatic indices and spawner and egg counts." Open Fish Sci. J. **10**: 12-22.

Nautilus Environmental (2017). Evaluation of the effects of selenium on early lifestage development of mountain whitefish from the Elk Valley, BC. Burnaby, British Columbia, Nautilus Environmental**:** 561 pp.

APPENDICES

2. Appendix B – Monitoring and Research Committee written recommendations

From: Gildea, Jason
To: Sullivan, Lauren; Reddekopp, Sheldon ENV:EX
Cc: Myla Kelly; Epps, Deb ENV:EX; Schmit, Ayn; McGrath, Patricia; McLaughlin, Julianne; Beaman, Joe
Subject: 2020 08 28 J.Gildea-USEPA_recmds
Date: Friday, August 28, 2020 4:10:24 PM
Attachments: image003.jpg

Disclaimer: *Please note the comments and recommendations contained in this document are strictly for Montana's and British Columbia's consideration. The views expressed in these comments and recommendations are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Regarding Montana's submission, the comments do not constitute approval or disapproval decisions under CWA Section 303(c). Neither are these comments a determination by the EPA Administrator under CWA Section 303(c)(4)(B) that revised or new standards are necessary to meet the requirements of the Act. These comments and recommendations do not impose any binding requirements, determine the obligations of the regulated community, change or substitute for any statutory provision or regulation requirement, represent, change or substitute for any Agency policy or guidance, or control in any case of conflict between this discussion and statute, regulation, policy or guidance.*

Lauren and Sheldon,
Thank you for the opportunity to provide comments on the technical analysis for setting a site-specific criterion for Lake Koocanusa. Please consider the following as my formal submission of comments as a member of the Lake Koocanusa Monitoring and Research Committee (MRC) to the chairs of the Selenium Technical Subcommittee (SeTSC).

First, I would like to acknowledge the extensive, well vetted process for getting to this point in the criterion development. The Lake Koocanusa Monitoring and Research Working Group (LKMRWG), which includes the MRC and SeTSC, has been meeting regularly for six years with the primary goal of developing and implementing a site-specific selenium criterion for Lake Koocanusa. Multiple in-person and phone-based meetings occurred during this time period allowing committee members and other stakeholders ample opportunity to discuss technical issues, provide input, collect data, analyze data, understand reservoir hydrodynamics and chemistry, assimilate information, and to develop and understand the selenium model. Specifically during this time period, I note that the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019) were produced and significantly added to the group's understanding of the process and available data. These reports, along with all data and information from the meetings, are well organized and archived on a website maintained by the Montana Department of Environmental Quality (DEQ) and B.C. Ministry of Environment and Climate Change Strategies (ENV) (available at http://lakekoocanusaconservation.pbworks.com/

w/page/100633354/FrontPage). The six-year process has provided all stakeholders with substantial background information and has prepared us to fully comment on the site-specific criterion model in a timely manner.

I note that it is seven years since B.C. ENV issued Ministerial Order #M113, which states that, "the Minister of Environment wishes to reduce calcite formation and to manage water quality to stabilize and reverse increasing trends in the water contaminant concentrations." Despite this order, pollutant concentrations and loads continue to increase into Lake Koocanusa, as demonstrated at the B.C. water quality monitoring site located at the Elk River Hwy 93 Crossing (Figure 1). This information is alarming and highlights the need to immediately stabilize and reverse pollutant loads into Lake Koocanusa.

Given the six-year process leading up to this point, I believe that it is now imperative for DEQ and B.C. to quickly adopt and implement a site-specific selenium criterion for Lake Koocanusa. I fully support Montana DEQ's proposed schedule to propose and adopt the criterion by December 2020 and I believe that the currently available science allows you to do that. The CWA and EPA's regulations at 40 CFR Part 131 require states and authorized tribes to establish water quality criteria that are protective of the designated use and scientifically sound, and then require those states and tribes to periodically (at least once every three years) review and revise the criteria as needed. Therefore, I encourage you to use the science available to you now to develop a site-specific selenium criterion. As you know, Lake Koocanusa currently has a site-specific selenium objective of 2 µg/L in B.C. and a water column chronic criterion of 5 µg/L in Montana. The modeling results provided by USGS in their 2020 report demonstrate that protective selenium concentrations for Lake Koocanusa are likely lower than either of these values, necessitating the need for immediate action to revise the criterion to a protective value. Presser and Naftz, 2020, note that, "78 percent of predictions are <1.5 µg/L and at least 46 percent of predictions are <1 µg/L for protection of this community of core benthic feeders." Delaying criterion development in any way will exacerbate on-going degradation of the Lake Koocanusa ecosystem, particularly given that pollutant loads are increasing.

Thank you again for the opportunity to comment on the site-specific selenium criterion and I look forward to working with you in the future on Lake Koocanusa issues.

Jason Gildea
Sent via email August 28, 2020
Hydrologist, USEPA Region 8, Montana Office
Gildea.Jason@epa.gov
406-457-5028

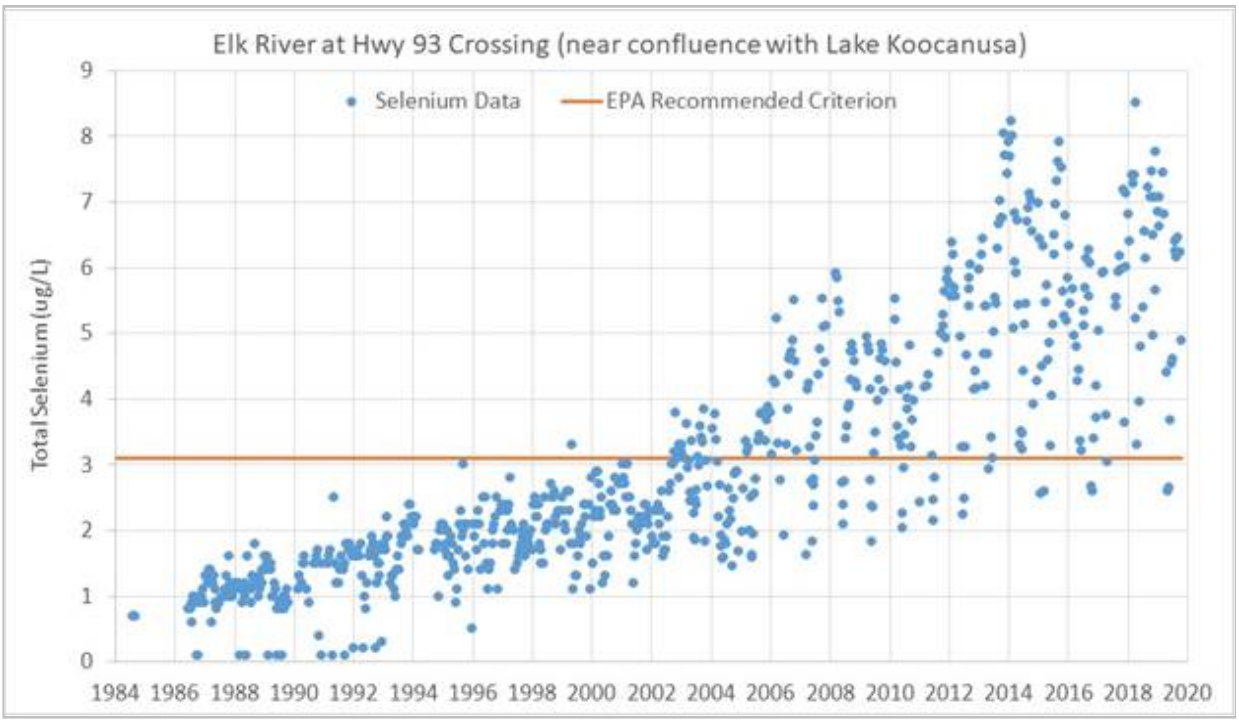


Figure 1. Selenium concentrations in the Elk River at HWY 93 crossing with EPA's recommended selenium criterion for lentic waters for comparison.

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Delivered via email to : TimDavis@mt.gov

August 28, 2020

Dear Mr. Davis and Mr. Moore:

Headwaters Montana works in northwest Montana to protect the water, wildlife, and traditional quiet outdoor recreation. We have over 2,000 subscribers. For the past several years we have been engaged in the Lake Koocanusa Monitoring and Research Committee (MRC) and the MRC's Selenium Technical Committee (SeTC) as an observer. We have appreciated being a part of the process and send this letter to provide you with our views and recommendation for the Montana selenium standard setting process that is now in its final phase.

First, we want to thank the DEQ and your dedicated staff for stepping up and taking on the significant task of working with BC to set this standard. It has proven to be an expensive and time-consuming process, but one which was necessary. Scientists are learning more and more about the toxic effects of selenium, and it has become apparent to us that the LKMRC process was necessary. Montana could have adopted the US EPA national standard. However, it was understood by many of the SeTC that selenium in Koocanusa had potentially more harmful and toxic effects. We think that the USGS model for selenium in Koocanusa proves this point and that the US EPA national standard would not have been protective of fish and aquatic life in the reservoir, and or would have had to be adjusted downward rather quickly after adoption.

Second, we would like to acknowledge that the standard-setting process is both a scientific and political one.

With respect to the science, it is clear that more and better data would have been helpful to the USGS modeling process. The USGS model was built with the scientific expertise of the most knowledgeable selenium experts in North America. We are confident that the essential components of the model reflect good scientific judgement. Headwaters Montana has played a role in securing congressional appropriations for the water quality monitoring on Lake Koocanusa, in particular the funding for the "super sipper" buoy deployed at the border. Our continuing efforts to secure addition federal funds for water quality monitoring should help ensure that the USGS has more robust data in the future.

The important point is that the USGS model represents the best available science at this point in time, and that future data will only make the model's predictions more reliable. Given that we can now expect BC and Teck to export even higher levels of selenium in the near future, we support the model and the goal of setting a selenium standard at the border in 2020 that is protective of aquatic life throughout the reservoir and downstream in the Kootenai River, including in Idaho and beyond.

During the August 25 MRC virtual meeting, some confusion was expressed by some on the MRC as to the "placement" of the selenium standard. Both BC and MT have used the motto, "one lake, one number" to describe the goal of the six-year effort. We want to be clear that we understand and expect the Montana standard will be set for the international border and does not represent a 'reservoir average' number.

With respect to the political decisions to be made by Montana on setting a selenium standard for the border, we understand that Montana will be in communication with both BC and Teck. We can anticipate some of their arguments and would like to address and correct several of them here. We think BC and Teck will argue for as high a number as possible, and one that will not protect US/Montana downstream interests. They will:

- Argue that the USGS is not scientifically valid. That no site-specific egg-ovary data or fish data were used. That 'generic' fish data was used. They will try to argue that the 'uncertainty' of the model should require additional data collection, study and analysis…. And delay. Our response to this, should BC and Teck make these arguments, is that 'uncertainty' argues for a more protective standard rather than a weak standard or delay, and that the standard should be set in 2020 and not delayed. The US and Montana will continue to collect data and the USGS model predictions will improve over time. After six years, further delay is not needed. It is better to approve now a protective standard that protects all aquatic life than study and delay.

In closing we would like to emphasize that:

- We support the MT-BC process
- We support the USGS model
- We support the timeframe for setting a selenium standard at the border before the end of 2020
- We support a standard that is conservative and protective of all aquatic life given the uncertainties of increased selenium loading, proposed new mines in the Elk Valley, and the future opportunity to refine the model – and the standard – as time goes by.
- We also support the Confederated Salish and Kootenai Tribes, the Kootenai Tribe of Idaho, and the Ktunaxa National Council's recommended number(s), that we anticipate will be at or below 1.0 ug/l.

Thank you for the opportunity to comment.

Sincerely,

Dave Hadden, Ex. Dir.
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Deborah Epps
British Columbia Ministry of Environment
Deb.Epps@gov.bc.ca

Myla Kelly
Montana Department of Environmental Quality
MKelly@mt.gov

August 28, 2020

RE: development of site-specific selenium criteria for Lake Koocanusa

Dear Deborah and Myla,

Montana Department of Fish, Wildlife & Parks (MFWP) has been involved in monitoring and researching selenium (Se) in Lake Koocanusa fish since 2008. MFWP has participated in the Lake Koocanusa Monitoring and Research Working Group since its inception in 2015, and has been an active member of the Monitoring and Research Committee (MRC), including the collection of fish and macro-invertebrate samples on the reservoir. To that end, MFWP has been actively involved in the effort to develop a site specific Se criteria for Lake Koocanusa and is fully supportive of the process led by British Columbia (BC) and MDEQ.

Like many large rivers in Montana, Lake Koocanusa is a complex system that has many anthropogenic stressors to fish. However, despite alterations to natural flow, temperature and sediment regimes, the reservoir still supports a largely native fish component. Burbot are a native species in the reservoir that are listed as a species of concern and have high cultural, sport-fish, and ecological values. Their populations have been declining steadily since 1989, however the reason for their decline is unclear. Burbot are difficult to capture, especially females with eggs, resulting in limited data for this species. Unfortunately, not much is known about burbot sensitivity to Se since there is no surrogate species to reference. These uncertainties warrant a conservative approach for the development of a Se criteria for Lake Koocanusa.

Due to the uncertainties surrounding the variability of the data used in the models to develop a site-specific criteria and unknown sensitivities of fish species, a conservative approach to criteria setting is imperative. The tissue concentrations of Se found in several fish species in Lake Koocanusa currently exceed EPA criteria and BC guidelines. With tissue exceedances occurring from waterborne concentration of ~1 ug/L, it is evident that a site-specific criterion would need to be less than 1 ug/L to be protective based on the best available science. Montana cannot wait any longer to adopt site specific criteria for Lake Koocanusa, and as more data becomes available this number could be revised (if needed) during triennial reviews. Waterborne concentration of Se downstream of Libby Dam have also

been observed ~1 ug/L. Therefore, applying a conservative approach for the Lake Koocanusa criteria would also provide protection of downstream fish populations where tissue exceedances have also been documented and where the most sensitive species to Se toxicity, the endangered white sturgeon, is present.

Thank you for the opportunity to comment on the adoption of site-specific criteria for Lake Koocanusa and for all the efforts MDEQ and BCMOE has made toward this process.

Trevor Selch

Fisheries Pollution Biologist
Montana Fish, Wildlife & Parks

Cc:

Lauren Sullivan (MDEQ)
Sheldon Reddekopp (BC MOE)
Tim Davis (MDEQ)
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August 31, 2020

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Re: Teck response to the USGS Sampling and Analysis Plan for the collection of selenium and associated water quality parameters in Lake Koocanusa

Dear Ms. Kelly and Ms. Epps,

In your roles as Co-Chairs of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG) and as requested during the working group meeting on August 26, 2020, I write to provide Teck's response to the recently published USGS Sampling and Analysis Plan and the process that will inform a draft selenium criteria for aquatic life in Koocanusa Reservoir. Given the nature of Teck's response, the Co-Chairs of the Selenium Technical Subcommittee (SeTSC) have been copied on this letter.

Teck has been operating for over 100 years to become a leading Canadian diversified natural resource company, committed to responsible mining and mineral development. Strong sustainability practices are core to how we do business and Teck's leadership in this area is recognized globally. For instance, Teck was named to the Dow Jones Sustainability Index in 2019 for our 10th straight year, and we were the top ranked mining company in the world on this index. Central to this is Teck's commitment to responsible management of water resources and to protecting water quality where we operate.

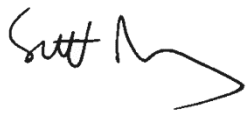
Over the last five years, Teck has made significant progress towards achieving the objectives of the Elk Valley Water Quality Plan (EVWQP), which is a long-term approach to addressing the management of selenium, nitrate and other substances released by current and historic mining activities in the Elk Valley. We forecast that our total investment in water quality management will be $1.2 billion through to 2024. This includes the water treatment facility at our Fording River Operations that is scheduled to complete in 2021. Additionally, Teck has more than 25 research and development projects underway, including the advancement of smaller, in-situ water treatment facilities that can be built closer to where treatment is needed. We are already seeing reductions in selenium and nitrate concentrations downstream of the Line Creek treatment facility, and we expect to see further significant reductions in other areas as future facilities come online. Details and information on our efforts can be found in the data and reports available online on Teck's website[1] and on the U.S. Environmental Protection Agency WQX Portal[2].

[1] Teck Resources Limited: "Responsible Mining in the Elk Valley".
[2] United States Environmental Protection Agency: "Water Quality Data (WQX)"

As members of the LKMRWG and observers to the SeTSC, we have made best efforts to understand and to provide input into the committee's work to inform a science-based and site-specific selenium recommended value for the Koocanusa Reservoir. This is a complex and multifaceted initiative and we appreciate the efforts that have gone into this work to date. It is our hope that the expertise and input of LKMRWG and SeTSC members and observers to this process is fully considered in your deliberations. We look forward to further engagement with the LKMRWG, SeTSC, Montana Department of Environmental Quality and the BC Ministry of Environment and Climate Change Strategy as this process continues to advance.

If you have any questions about our response to the USGS Sampling and Analysis Plan or Teck's approach to water quality management, please do not hesitate to contact me at Scott.Maloney@teck.com. We thank you for the level of engagement to date and for this opportunity to provide input directly to you for this process.

Sincerely,



Scott Maloney
Vice President, Environment

Cc: Tim Davis, Administrator, Water Quality Division, Montana Department of Environmental Quality
Lauren Sullivan, Water Quality Standards and Modeling, Montana Department of Environmental Quality
Kevin Jardine, Deputy Minister, Ministry of Environment and Climate Change Strategy
James Mack, Assistant Deputy Minister, Ministry of Environment and Climate Change Strategy
Sheldon Reddekopp, Sr. Environmental Assessment Biologist, Ministry of Environment and Climate Change Strategy
Katherine Gizikoff, General Manager, Environment and Social Responsibility, Teck Resources Limited
Carla Fraser, Manager, Water, Environment, Teck Resources Limited
Tom Syer, Head, Government Affairs, Teck Resources Limited
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Teck comments on the U.S. Geological Survey Lake Koocanusa Open-File Report 2020–1098

Overview

The comments below are Teck's response to the above-referenced open-file report developed for Lake Koocanusa by the U.S. Geological Survey (Presser and Naftz 2020). In summary, we believe there are a number of misleading and inaccurate statements within the report, and, despite the importance of site-specific fish tissue data, it was not used as a means to validate the model. As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the Koocanusa Reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion.

Misleading and inaccuracies within the report

The U.S. Geological Survey (USGS) fails to acknowledge that Lake Koocanusa has been listed under section 303(d) of the U.S. *Clean Water Act* as "impaired" since 2002 due to hydro-modifications, specifically Libby Dam[3]. By focusing on the 2012 reporting year, Presser and Naftz (2020) obscure this fact and create the potential for readers to inaccurately conclude that the listing is due to selenium concentrations. This is further exasperated by an incomplete analysis of data collected at the mouth of the Elk River by Environment and Climate Change Canada.

As noted by Presser and Naftz (2020), aqueous selenium concentrations measured at the mouth of the Elk River above Highway 93 (BC08NK0003)[4] have increased during the period of record, but incorrectly identify this trend as *continuing* to increase. Rather and as illustrated within Figure 1 below, selenium concentrations at the mouth of the Elk River have not increased since 2014. On the contrary, not only have selenium concentrations stabilized but a negative slope for the best-fit linear regression line suggests that concentrations are decreasing. This trend is wholly consistent with the goals and intentions of the Area Based Management Plan to "stabilize and reverse increasing trends in water contaminant concentrations". Furthermore, the suggestion that selenium loads are and will continue to increase is not supported by data.

[3] https://ofmpub.epa.gov/waters10/attains_waterbody.control?p_au_id=MT76D003_010&p_cycle=2002.

[4] http://aquatic.pyr.ec.gc.ca/webdataonlinenational/en/SiteDetails/BC08NK0003/Projects/PYLTM/Regions/0

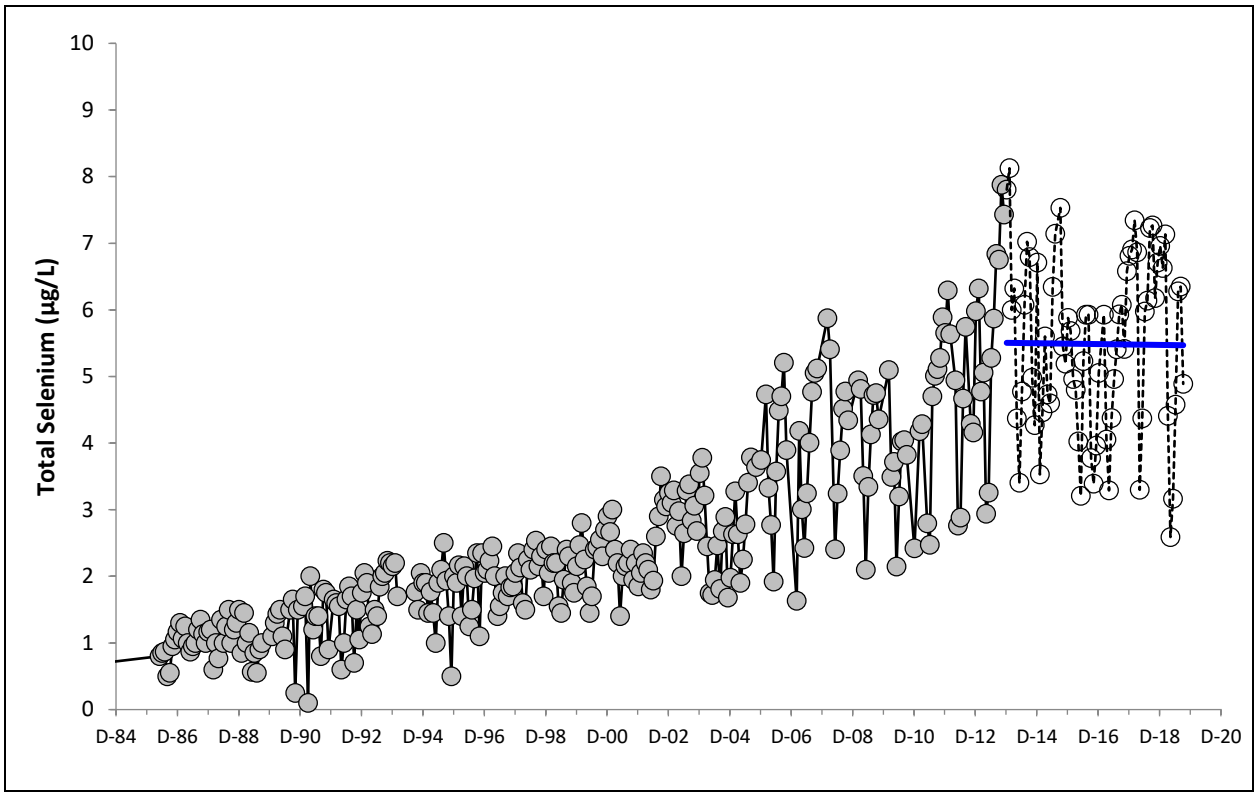

Figure 1. Monthly Average Total Selenium Concentrations as Recorded at the Mouth of the Elk River by Environment and Climate Change Canada.

Note: Data points are a monthly mean concentrations, while the blue line is a best-fit linear regression for data collected from 2014 to 2019 inclusive and has a slope of -1.7×10^{-5}.

Annual selenium loads from the Elk River into the reservoir have not been increasing and are inconsistent with gradient maps presented within the report. Presser and Naftz (2020) suggest that cumulative cross-sectional areas with selenium concentrations greater than 1 µg/L have increased three- (from 2017 to 2018) and four-fold (from 2017 to 2019) due to increasing selenium loads from the Elk River. Selenium concentrations (see Figure 1) and loads (see Figure 2) from the Elk River are inconsistent with the aforementioned suggestion.

Presser and Naftz (2020) correctly identify that the increase in proportional area of the reservoir containing selenium concentrations >1 µg/L suggests a system that is not in steady state, but fail to directly account for the significant role Libby Dam and its hydrodynamic cycles plays in this matter. Although Presser and Naftz (2020) identify Lake Koocanusa as a highly modified hydrological and ecological system, and that it experiences traditional problems associated with dam management (e.g., large elevation changes during drawdown and refilling operations), the report does not wholly consider the ramifications of such annual fluctuations particularly on the British Columbia side of the reservoir. As demonstrated by Dr. M. Sokal during the October 2016 Monitoring Research Committee meeting in Kalispell, Montana, Lake Koocanusa's hydrologic cycle significantly influences water quantity within the Canadian portion of the reservoir. During high water conditions (Figure 3, left panel) the reservoir is hydrologically like a "lake"; while under low water conditions (Figure 3, right panel) it is more like a "river" (Sokal 2016).

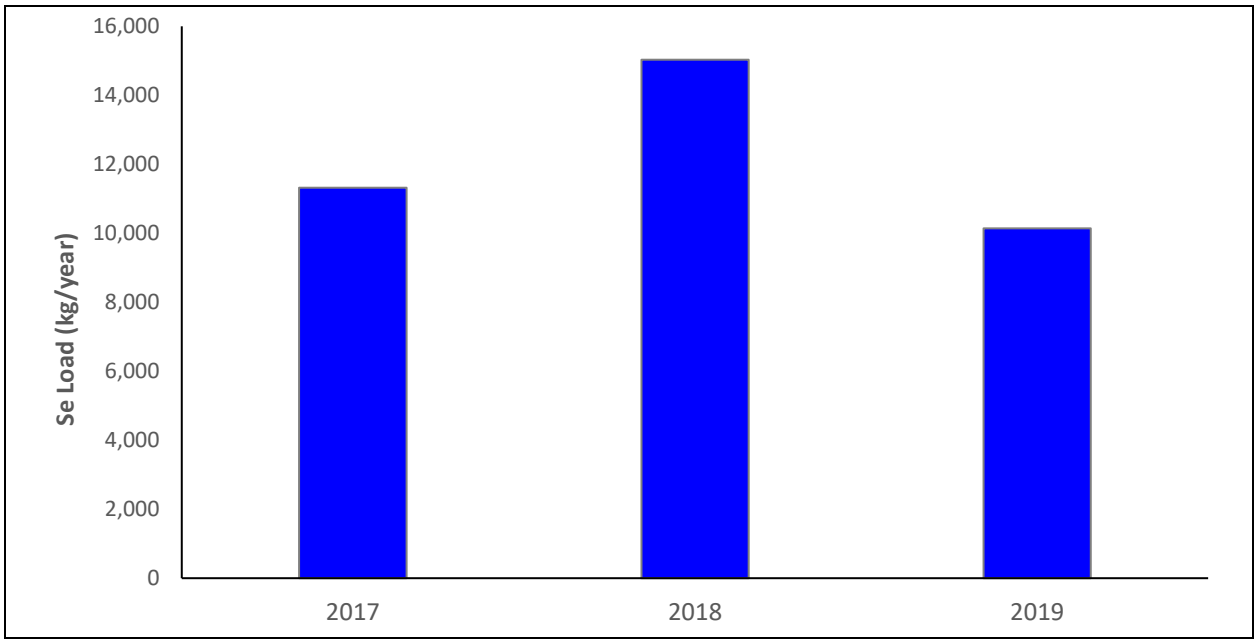

Figure 2. Selenium Loads associated with the Elk River into Lake Koocanusa.

Note: Selenium loads (2017 = 11,316 kg, 2018 = 15,035 kg, and 2019 = 10,144 kg) as determined from annual monitoring reports for the reservoir submitted to the ENV (Minnow, 2019 and 2020, and Teck 2018).

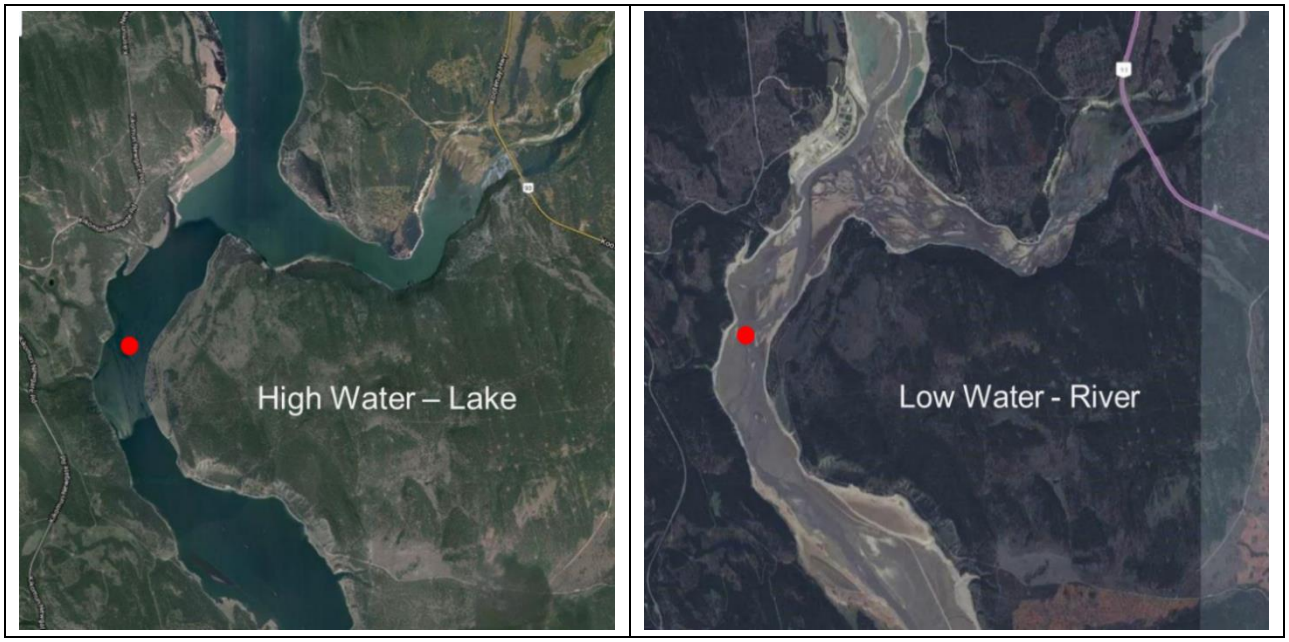

Figure 3. Aerial Imagery of Lake Koocanusa near the Confluence of the Elk River during High- (left panel) and Low-Water (right panel) Conditions.

Note: Imagery obtained from PowerPoint presentation made by Michael Sokal, PhD, Environmental Protection Division, BC ENV at the Lake Koocanusa Monitoring Research Working Group Meeting Kalispell, MT (October 17-18, 2016; Meeting No. 5). The red dot illustrated within the images reflects Order Station Environmental Monitoring System (EMS) E300230 (RG_DSELK).

Lack of model validation via fish tissue data

Model validation was limited to evaluating ranges of observed and predicted selenium concentrations in invertebrates (zooplankton and macroinvertebrates). Based on the qualitative evaluation presented within Presser and Naftz (2020), it is clear that unless an adjustment of selenium bioavailability is made, the model grossly over-predicts invertebrate concentrations. Even with a bioavailability assumption of 60 percent, the model over-estimates invertebrate concentrations. This over-estimation is carried through to the endpoint of primary concern (fish tissue).

For instance, assuming a bioavailability of 60 percent predicted whole-body fish tissue concentrations are two times greater than measured data (e.g., site-specific mean = 5.4 mg/kg dw, n = 22). Furthermore, if one considers fish muscle tissue concentrations relative to the U.S. Environmental Protection Agency's criterion which identifies a limit of 11.3 mg/kg dw for adult fish muscle this positive bias increases to a factor of three (e.g., site-specific mean = 3.8 mg/kg dw, n = 120). As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion. Therefore and consistent with recommendations made to Monitoring and Research Committee Co-Chairs on December 4, 2015, selenium criteria/objectives should focus on empirical data, specifically fish tissues, and be evaluated relative to the U.S. Environmental Protection Agency (2016) fish tissue-based selenium criteria which are protective of fish.

As a result, the question at hand for the Monitoring and Research Committee is better expressed in terms of environmental monitoring data within the reservoir, in relation to existing water quality criteria/guidelines. The presumption being that existing/pending water quality criteria/guidelines are, as intended, protective of aquatic life within the reservoir. This is different than what is currently being expressed which assumes that existing/pending water quality criteria/guidelines are not protective of aquatic life within the reservoir.

Process toward a recommended value

According to the BC-MT 2020 Work Plan, revised May 1, 2020, the Co-Chairs of the SeTSC will finalize a technical assessment report and recommendations of a draft selenium criteria for consideration by Montana Department of Environmental Quality and by the British Columbia Ministry of Environment and Climate Change. It is not clear whether the Co-Chairs will include responses to comments on the USGS report submitted by SeTSC members and observers. In the interest of transparency and in recognition of the deep technical expertise on the SeTSC, we recommend that the report include how SeTSC member and observer input was considered to inform the recommended value.

Appendix: references

Presser, T.S., and Naftz, D.L., 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020–1098, 40 p., https://doi.org/ 10.3133/ ofr20201098.

Minnow Environmental Inc. 2019. Koocanusa Reservoir Monitoring Program Annual Report, 2018. Prepared for: Teck Coal Limited. June 2019

Minnow Environmental Inc. 2020. Koocanusa Reservoir Monitoring Program Annual Report, 2019. Prepared for: Teck Coal Limited. June 2020

Teck Coal Limited. 2018. 2017 Summary Report of Monitoring Results in the Koocanusa Reservoir. June 28, 2018

U.S. Environmental Protection Agency. 2016. Aquatic life ambient water quality criterion for selenium – Freshwater 2016. Office of Water, Washington, D.C., USA. EPA 822-R-16-006.

wildsight

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Tim Davis, Montana DEQ
Sean Moore, BC MoE

SeTSC Co-chairs:
Sheldon Reddekopp, BC MoE
Lauren Sullivan, Montana DEQ

August 28, 2020

Thank you for the opportunity to provide input on the selection of the shared water column selenium limit to protect aquatic life for Lake Koocanusa.

Wildsight is strongly supportive of conservative, protective selenium limit for Lake Koocanusa, both for the protection of aquatic life in the reservoir and downstream in the Kootenai/Kootenay River and even in Kootenay Lake. As a general principle, we believe that modelling assumptions should be conservative and err on the side of caution in order to ensure protection for all species.

We recognize that ecological modelling will never be as precise or simple as we may wish it to be, nor will we ever have all the data we'd like; however, the USGS report and the SeTSC process that came before it have been open, transparent and comprehensive, involving detailed data collection over a number of years and peer-reviewed analysis from well-regarded experts on selenium toxicology. It is time to act on this data and modelling to set a protective selenium limit for Koocanusa, rather than allowing further increases in selenium with potentially disastrous effects on aquatic life. The USGS report makes it clear that selenium levels in Koocanusa must be reduced. Work to set a protective limit and to reduce selenium concentrations below that limit must begin immediately.

Specifically, a protective standard must be based on a protective whole-body selenium limit. We support the Ktunaxa Nation, KTOI and CSKT in calling for a 5.6 mg/kg dw whole-body limit, based on BC's 11 mg/kg egg/ovary Water Quality Guideline, which includes an uncertainty/safety factor of 2, and a conversion factor of 1.9 for rainbow trout. In this case, a median K_d of 4547 would be appropriate, as BC's Water Quality Guideline already includes an uncertainty/safety factor. However, if the limit is based on the EPA's limit of 8.5 mg/kg dw, we believe a more conservative approach is needed. In the Aquatic Life Ambient Water Quality

Criterion for Selenium, the EPA uses the 20th percentile of the translations of the egg-ovary criterion concentrations to water column concentrations to provide a "high probability of protection for most aquatic systems".[1] In this case, using a 80th percentile K_d of 5566 would be appropriate and provide an equivalent degree of protection to that intended in the EPA standard. Using a median or other lower K_d would not be appropriate if the EPA standard of 8.5 mg/kg is used.

With the very steep curve relating selenium egg/ovary concentrations to mortality, and relatively widely distributed data for selenium concentrations for individuals of the same species found in Koocanusa, it is clearly necessary to take a cautious, protective approach, which favours the lower BC whole-body limit with its uncertainty/safety factor.

We also support the 5.6 mg/kg whole-body limit to allow for preferred Ktunaxa fish consumption rates without exceeding human health limits.

We share the concerns expressed by some SeTSC members about lag times for biological effects from increasing selenium concentrations in Koocanusa. Lag times for selenium moving from water to sediment and at the various trophic levels could lead to an underestimation of the risk to aquatic life from the measured selenium concentrations. This concern should further strengthen the case for a standard with a reasonable safety margin to ensure protection.

We support the use of the W6 model (TFM w TL3 100% aquatic insects) to protect burbot and mountain whitefish. Using this model with the 5.6 mg/kg limit and a median K_d leads to a 0.6µg/L water limit, while using the model with a 8.5 mg/kg limit and a 80th percentile K_d leads to a 0.8µg/L water limit.

Additionally, a conservative approach is needed to protect downstream species in the Kootenai/Kootenay River and Kootenay Lake. Of particular concern are white sturgeon, endangered in both Canada and the US, and burbot. We note that elevated selenium levels in water above 1µg/L have been found in both the US and Canadian portions of the river and recent KTOI data shows significant selenium levels in fish tissue. With the Canadian portion of the Kootenay River between the US border and Kootenay Lake, as well as the Kootenay River delta into Kootenay Lake, designated as critical habitat for white sturgeon under Canadian SARA, it is crucial that a protective standard be set for Koocanusa that ensures protection for white sturgeon in this critical habitat. While we are not aware of any current tissue data available for sturgeon in Canada, we note the sensitivity of the species in the EPA Criterion, the recent troubling KTOI data that showed selenium in fish tissue above EPA and BC standards, and BC water quality data showing selenium exceeding 1.0µg/L in the Kootenay River at Creston and exceeding 0.6µg/L in the southern portion of Kootenay Lake[2]. Clearly, more study is needed, but these pieces of evidence already available should be highly concerning. A conservative

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[1] Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf, p. 92.

[2] Data from BC EMS, Locations IDs E206587 and E216952

standard should be set immediately in Koocanusa, the only significant source of downstream selenium, while work is done to quantify the effects of selenium concentrations in the Kootenai/Kootenay River and Kootenay Lake on sturgeon, burbot and other species.

Comparing the USGS model to the EVWQP model

We have heard concerns about the validity of the USGS model and the assumptions made for inputs to the model. In this context, we believe it is constructive to compare the USGS modelling to the modelling done to support the selenium limits set under Teck's EMA discharge permit in the Elk Valley, based on the 2014 Elk Valley Water Quality Plan.

While converting from the established thresholds for egg/ovary selenium to whole body values may not be ideal, it is necessary to use the existing, available data and to accept that obtaining reliable egg/ovary data is difficult. Here we compare the USGS approach to Teck's approach for the EVWQP modelling in 2014[3]. While Teck's approach uses egg/ovary data directly, the study on which the EVWQP modelling is based gives a large range for the 95% confidence interval of 12-31µg/L for WCT reproductive EC_{10} in eggs/ovaries, with a result that the derived selenium concentrations in water for 10% effect size should be subject to large uncertainties, though no uncertainties are given. Consequently, the calculated water concentration for 10% effect size and the water limit based on this concentration should be subject to a large degree of uncertainty.

We believe the USGS approach, based on established egg-ovary criteria, is far superior to the species-specific approach taken by Teck. The conversion to whole body values may increase uncertainty to a small degree, but far less than relying on one study with eggs from a few fish to determine thresholds.

We also have heard significant concern about widely varying values used in the USGS model, especially K_d values. In Teck's EVWQP modelling, we also observe very wide ranges of values in matched samples for water to invertebrates and invertebrates to fish eggs. We believe the USGS conclusions are far better supported by the data than the conclusions in the EVWQP modelling, because the data used by the USGS is mostly in the relevant ranges of selenium concentrations, while Teck's data is largely outside the relevant ranges. The overwhelming majority of the data used by Teck is significantly below or significantly above the relevant egg/ovary and water concentrations. Teck's data is also from a mix of lentic and lotic environments.

Overall, if BC is willing to rely on the relatively weak modelling behind the EVWQP to protect fish in the Elk River Watershed, they should also be willing to immediately adopt limits based on the modelling in the stronger USGS study. In any case, uncertainty in the data or the model should

[3] This modelling work was done by Golder Associates.

lead to a more protective limit in order to keep aquatic life safe, rather than further delay while selenium in the reservoir is already above safe limits in water and in fish tissue.

Timeline for adoption

We support Montana's plan to adopt a protective limit in 2020 and urge BC to also adopt the same limit this year. Increasing selenium concentrations in recent years have already put fish and other aquatic life at risk. We cannot afford to continue to allow selenium concentrations to increase. BC must provide a regulatory framework to give strong incentives for Teck to immediately reduce selenium loadings entering Lake Koocanusa to safe levels. We cannot continue to rely on promises of future selenium reductions, while mining and selenium-leaching waste rock dumps expand daily.

Additionally, BC needs to take immediate action to characterize the full timescale of the selenium leaching problem and to evaluate proposed solutions such as active water treatment or saturated rock fills in that context. BC needs to ensure that Teck meets this Koocanusa limit not only in the immediate future, but also in the long term, after mining ends and for the centuries or longer that selenium leaching will continue. BC must not allow a situation where $100 million for operating and capital costs is required annually to maintain water treatment in perpetuity in order to meet the Koocanusa limit.[4]

With Teck's plans to nearly double the amount of selenium-leaching waste rock over the next 20 years per the 2019 Implementation Plan Adjustment, it is essential that BC provide a clear and strong regulatory signal to Teck immediately, in order to ensure the full costs of mitigating pollution from that waste rock, if such mitigation is possible, are included in Teck's plans—and selenium levels are not allowed to increase past the point where Teck is able to reduce selenium below the Koocanusa limit.

The adoption of the conservative, protective limit for Koocanusa this year would also enable effective environmental assessments over the next two years, both federally and provincially, for Teck's Castle Project and North Coal's Michel Project.

Geographic application of the limit

We urge BC to adopt the shared Koocanusa limit in concert with Montana and to apply that limit to the entire Canadian portion of Lake Koocanusa. Fish may be resident in certain areas of

[4] Teck 2019 Annual Report, p. 13 indicates an estimated long-term operating cost for water treatment of $3 per tonne and annual production of roughly 24 million tonnes, leading to an estimate of $72 million annually. Including capital costs for replacement of treatment facilities would increase this estimate significantly. However, the report states that current operating costs are $31 million ($1.30 per tonne) with only two small facilities in operation and 14 or more additional facilities are planned. This estimate appears to be highly optimistic and the true cost may be much higher.

the reservoir, especially smaller species like redside shiner and peamouth chub, which have both shown tissue concentrations well above BC and EPA limits. We oppose Teck's proposal in their 2019 EVWQP Implementation Plan Adjustment to measure selenium concentrations in Lake Koocanusa as an average of four sampling sites distributed from the Elk River to the border.

In particular, BC should adopt the Koocanusa limit at the current LK2 (South of the Elk River) permit order station. If there are concerns about full mixing at this location, BC should allow an average concentration of a reservoir transect, at various depths, to measure compliance with the limit. The limit should also apply at the border, in the Forebay, and at any other sampling locations in BC or Montana.

Sincerely,

Lars Sander-Green
Mining Lead
Wildsight

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

Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016

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

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NOTICE

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

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

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
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ACKNOWLEDGEMENTS

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

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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

Technical support was provided by Great Lakes Environmental Center

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).

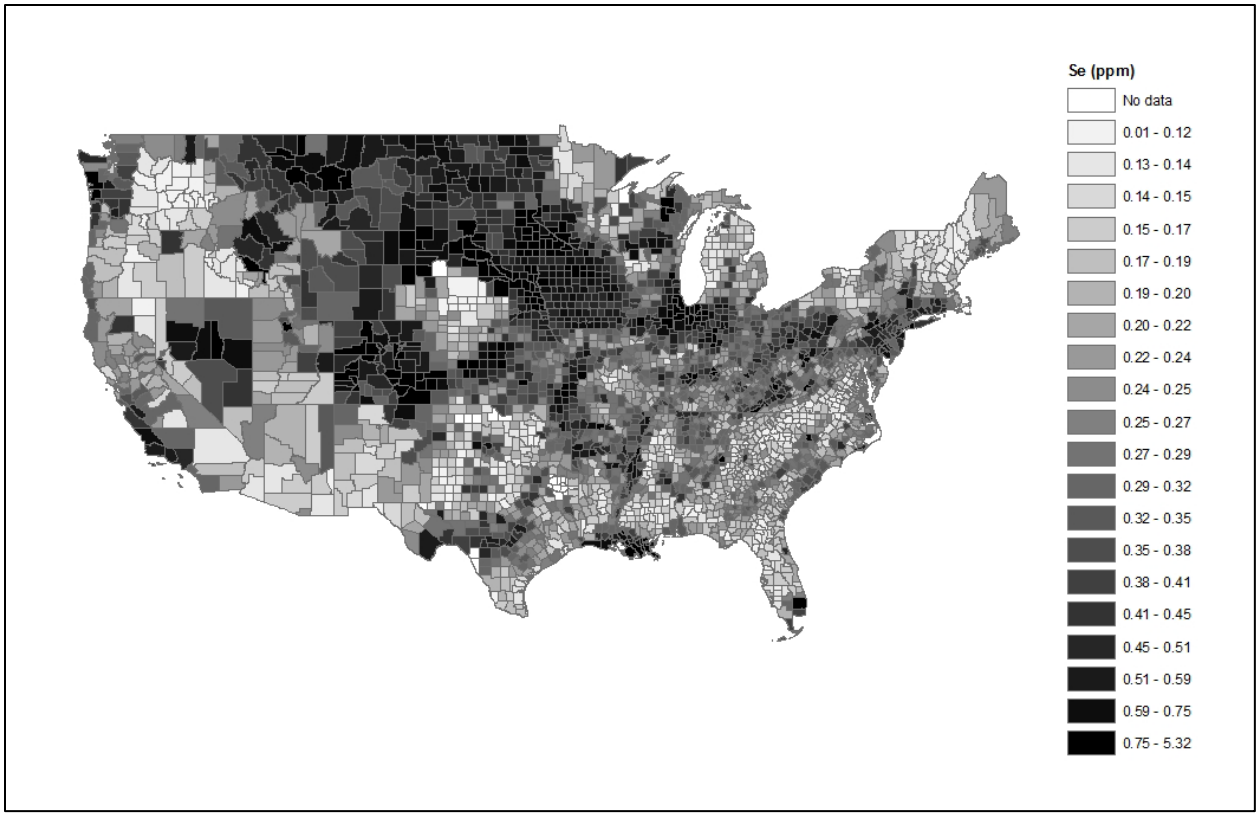


Figure 2.1. Selenium in Surficial Soils and Aquatic Sediments in counties of the Conterminous United States.
U.S. Geological Survey Open-File Report 2004-1001. URL: http://mrdata.usgs.gov/geochem/doc/averages/countydata.htm. Data are available from: http://mrdata.usgs.gov/geochem/doc/groups-cats.htm.

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

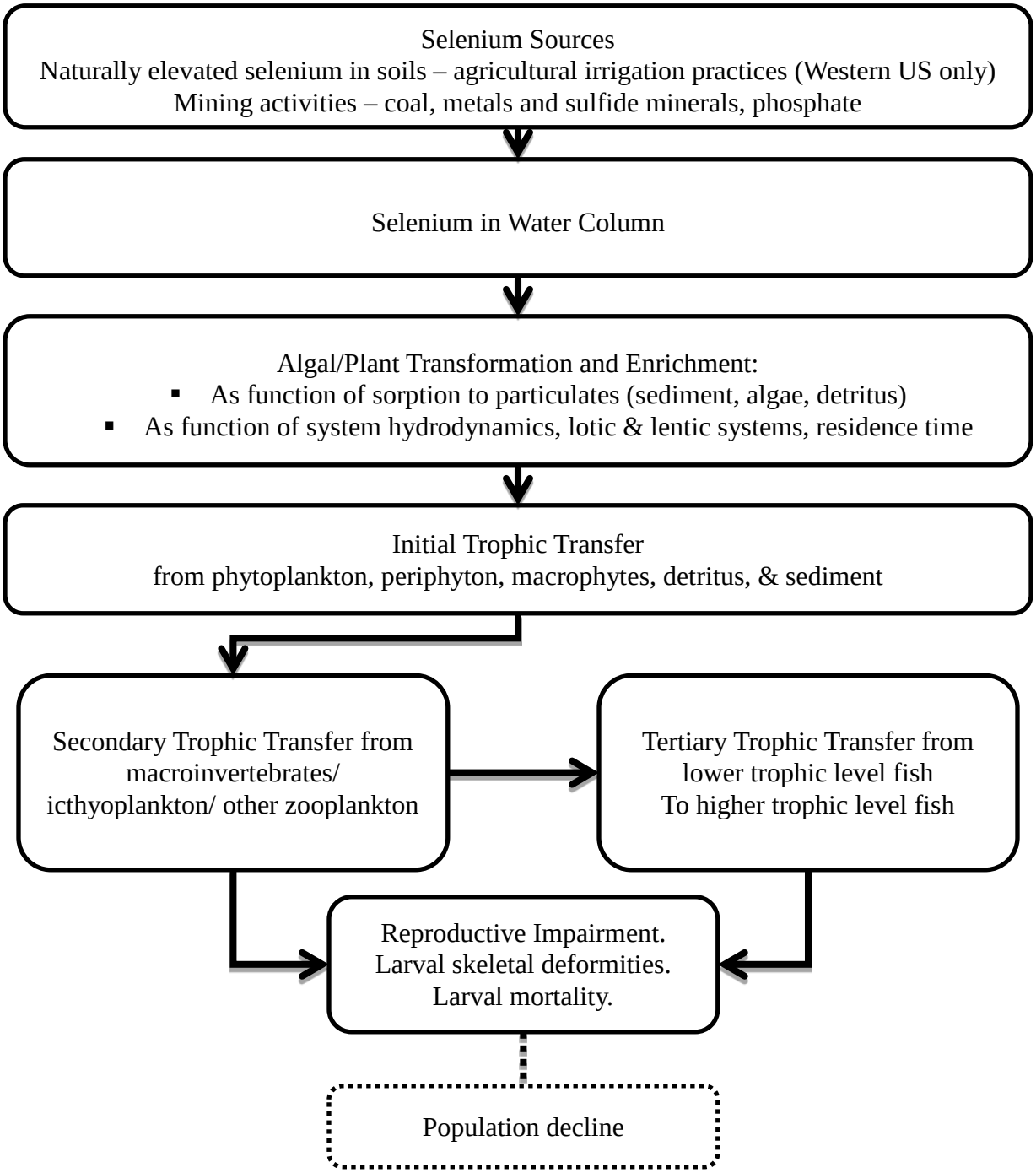


Figure 2.2. Diagram of Selenium Partitioning, Bioaccumulation, and Effects in the Aquatic Environment.

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.

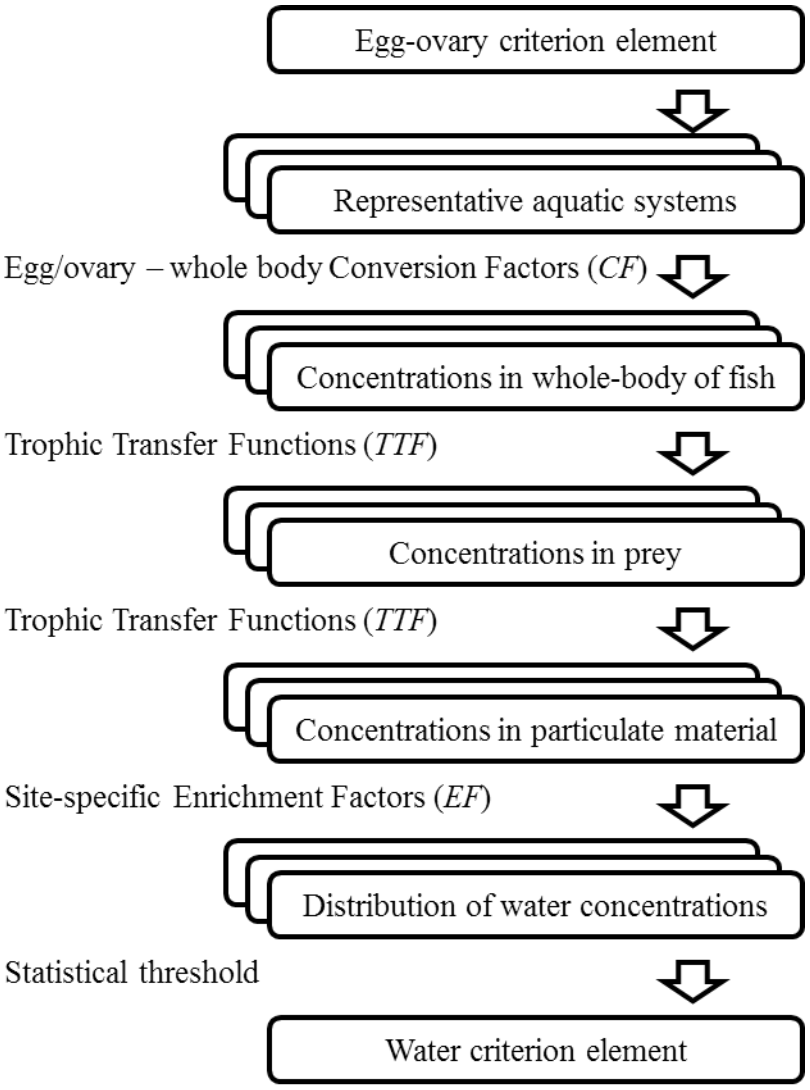


Figure 2.3. Conceptual Model for Translating the Selenium Egg-Ovary Concentration to a Water Column Concentration.

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**.

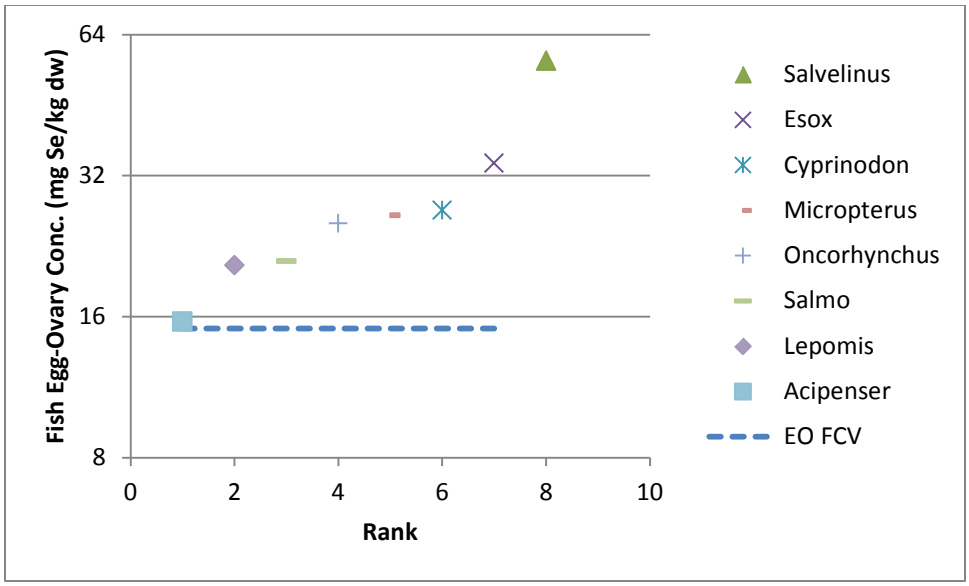


Figure 3.1. Distribution of Reproductive-Effect GMCVs for Fish Measured as Egg-Ovary Concentrations.

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**.

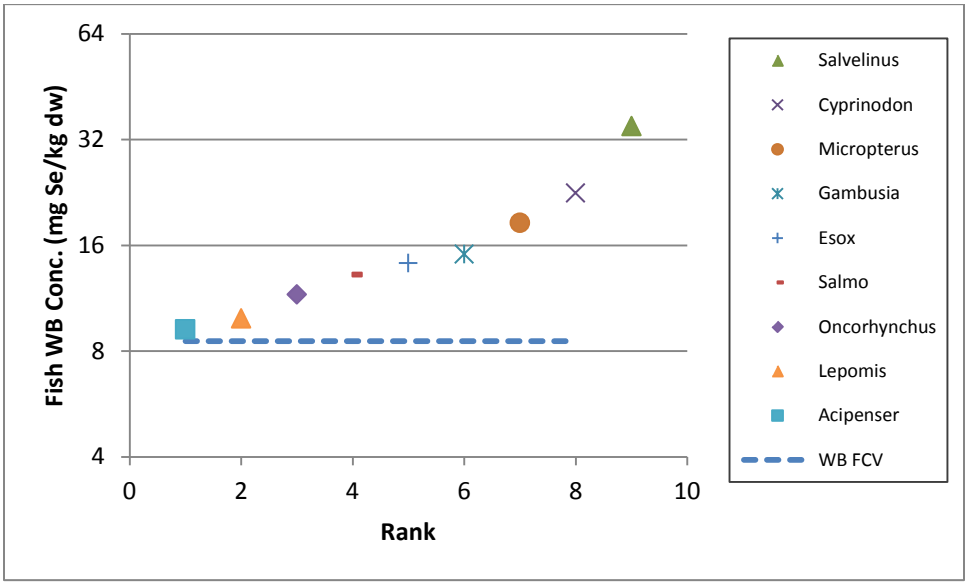


Figure 3.2. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Whole-Body Concentrations in the Original Tests, or Measured as Egg-Ovary Concentrations but Converted to Whole-Body.
(As shown in **Table 3.4**).

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**.

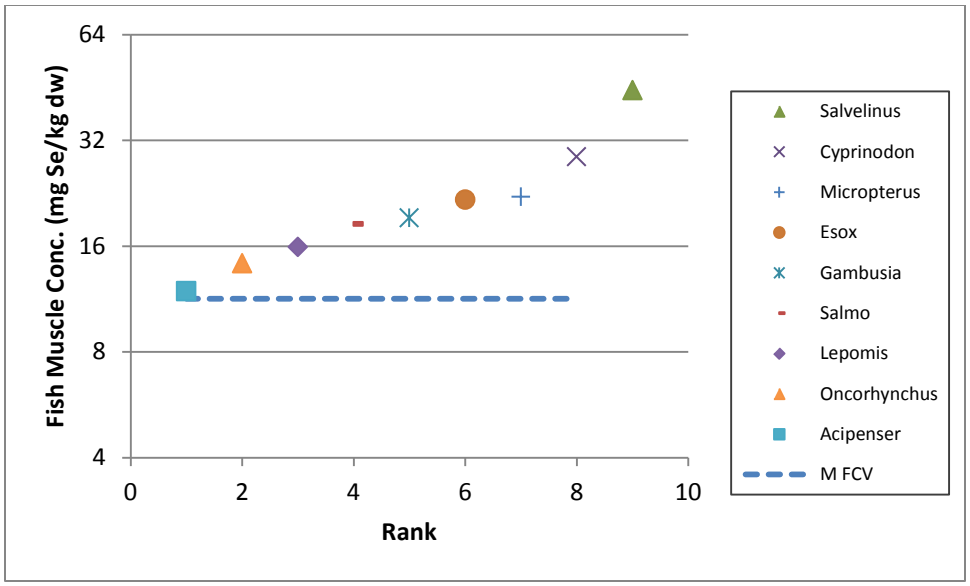


Figure 3.3. Distribution of Reproductive-Effect GMCVs for Fish, either Measured as Muscle in the Original tests, or Measured as Egg-Ovary Concentrations but Converted to Muscle Concentrations.
(As shown in **Table 3.6**). (Live-bearer *Gambusia* was converted from WB to muscle).

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 5^{th} percentile calculation methods for the FCV use actual numeric values for the GMCVs of the four most sensitive (fish) genera in the selenium dataset, it is only necessary to know that the more tolerant genera have GMCVs that are greater than those of the lowest four. A recommendation in the draft white paper on Aquatic Life Criteria for Contaminants of Emerging Concern Part I (U.S. EPA 2008b), which was supported by the Science Advisory Board, states "because only the four most sensitive genus mean chronic values (GMCVs) are used in the criterion calculations, chronic testing requirements for a taxon needed to meet an MDR should be waived if there is sufficient information to conclude that this taxon is more tolerant than the four most sensitive genera."

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

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

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

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

3.2 Chronic Water Column-based Selenium Criterion Element

3.2.1 Translation from Fish Tissue Concentration to Water Column Concentration

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

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

Where:

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

3.2.1.1 Simplifying the Bioaccumulation Model

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

Growth Rate

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

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

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

Uptake Rate

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

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

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

Derivation of the Translation Equation

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

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

or:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

where:

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

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

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

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

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

where:

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

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

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

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

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

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

where:

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

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

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

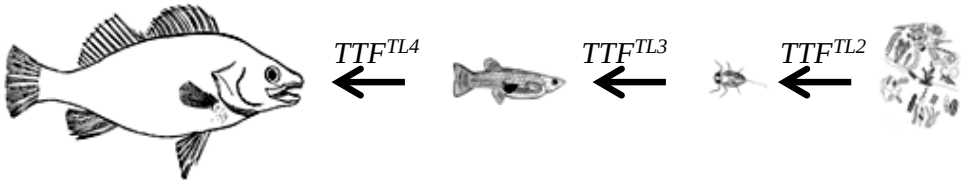
The parameter $TTF^{composite}$ quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system, w_i, the proportion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

A) Three trophic levels (simple):

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

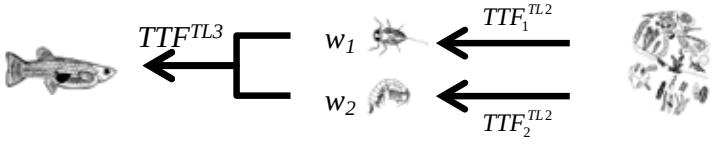
B) Four trophic levels (simple):

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



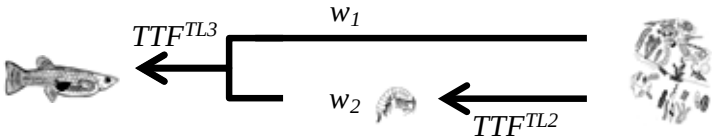
C) Three trophic levels (mix within trophic levels):

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



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

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



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

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

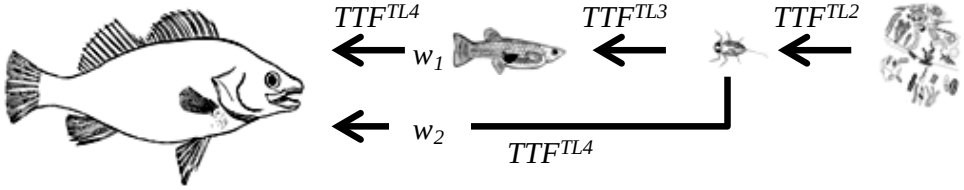


Figure 3.4. Example Aquatic System Scenarios and the Derivation of the Equation Parameter $TTF^{composite}$.

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

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

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

Where:

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

Rearranging the terms of Equation 12:

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

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

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

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

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

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

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

Where:

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

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

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

Rearranging the terms of Equation 16 yields:

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

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

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

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

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

3.2.2 Equation Parameters

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

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

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

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

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

Where:

k_e = Elimination rate constant (/d)

AE = Assimilation efficiency (%)

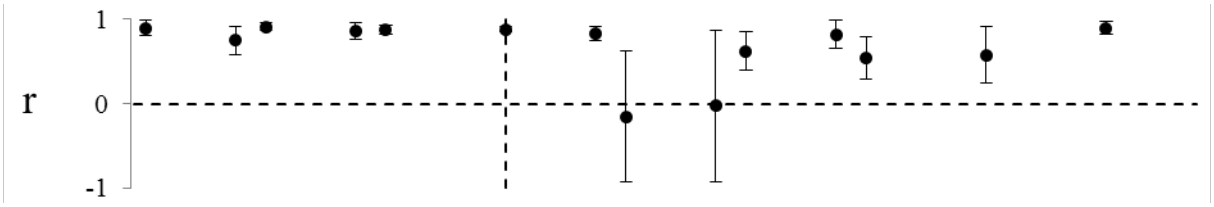
IR = Ingestion rate (g/g-d)

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

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

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

Particulate versus invertebrate



Invertebrate versus fish

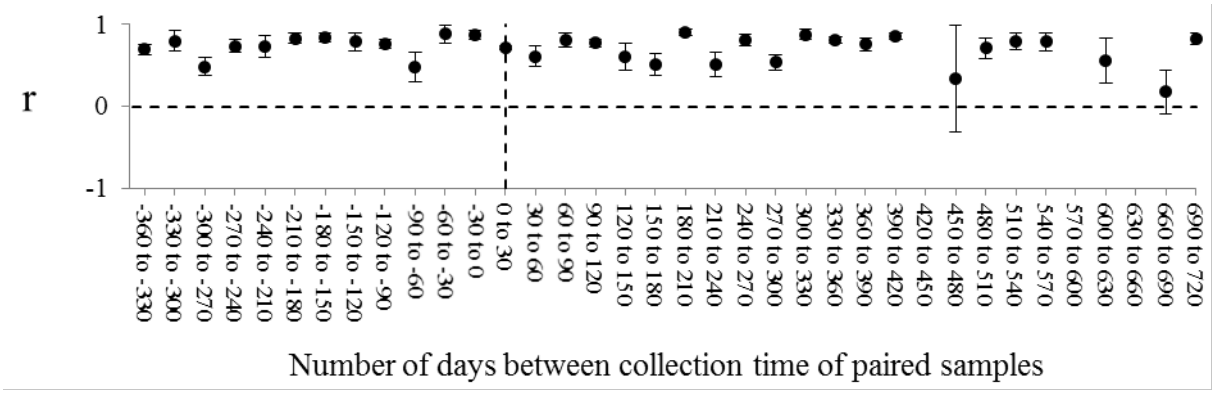


Figure 3.5. Effect of Relative Sample Collection Time on Correlation Coefficients of Selenium Measurements in Particulate Material, and Invertebrate and Fish Tissue. Error bars indicate the 95% confidence interval of r calculated using Fisher's r to z transformation. Horizontal dashed line indicates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

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

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

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

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

Rearranging the terms of Equation 4 yields Equation 19:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.2.3 Food-Web Models

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

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

3.2.4 Classifying Categories of Aquatic Systems

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

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

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

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

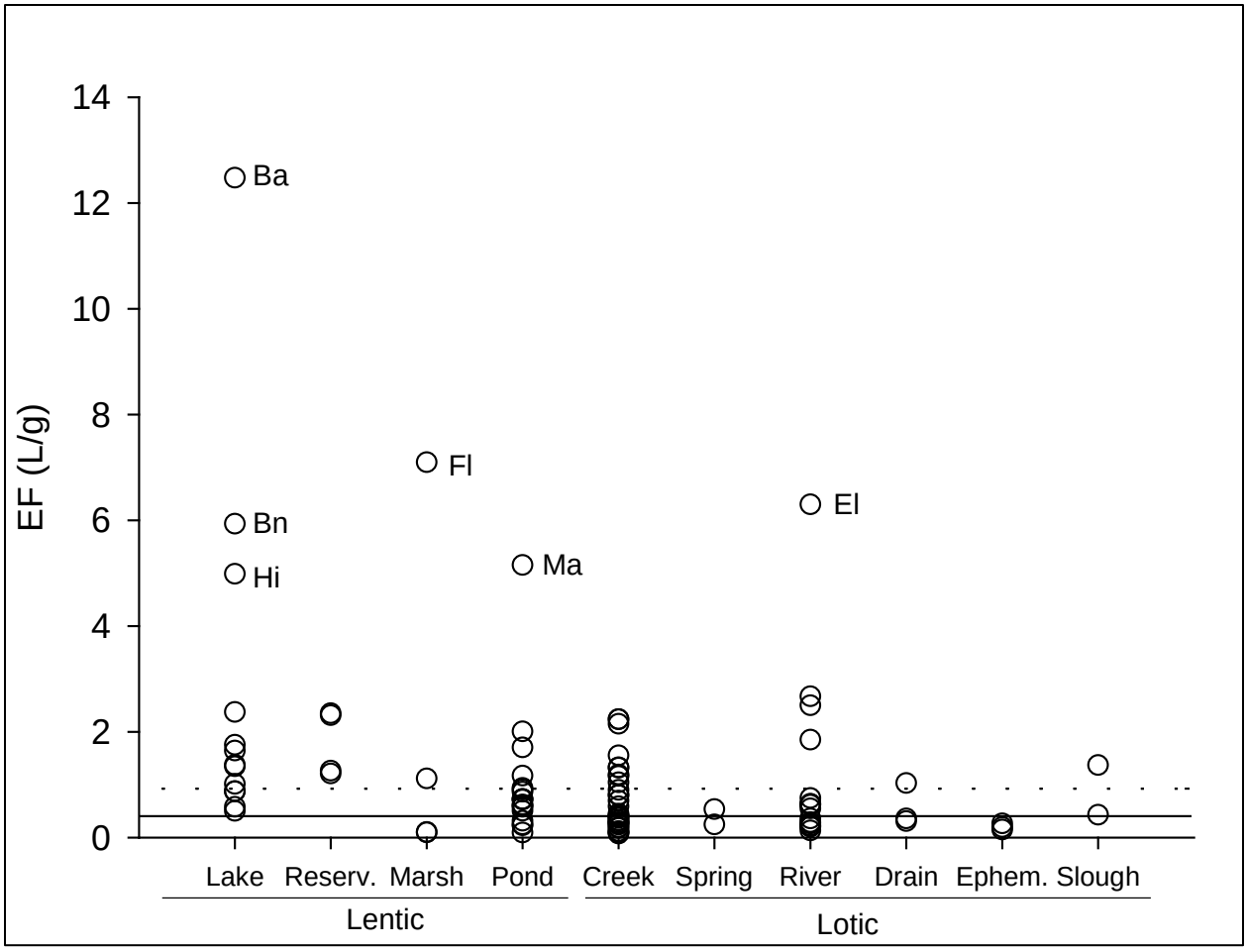


Figure 3.6. Enrichment Factors (*EF*) for 96 Aquatic Sites Derived from Published Studies and Organized by Waterbody Type.
The dashed line represents the median *EF* for the 39 lentic sites (0.9 L/g), and the solid line represents the median *EF* for the 57 lotic sites (0.4 L/g). See text for information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

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

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

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

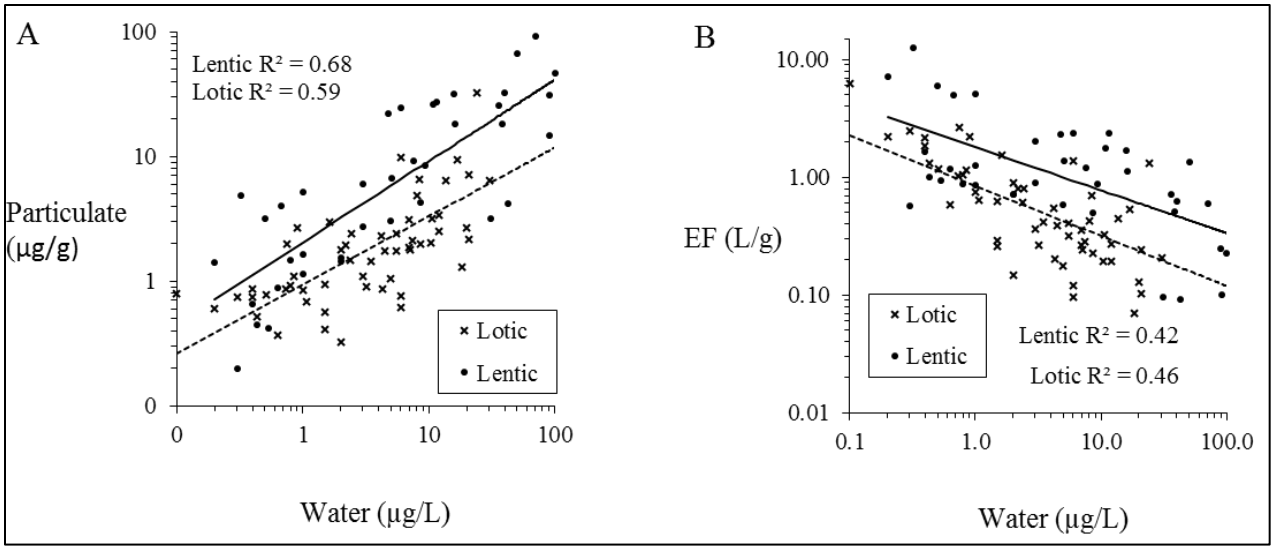


Figure 3.7. The Relationship between C_{water} and $C_{particulate}$, and C_{water} and *EF* for the 39 Lentic and 57 Lotic Aquatic Systems.
A: Relationship between C_{water} and $C_{particulate}$ by site category.
B: Relationship between C_{water} and *EF* by site category.
Solid line, ordinary least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

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

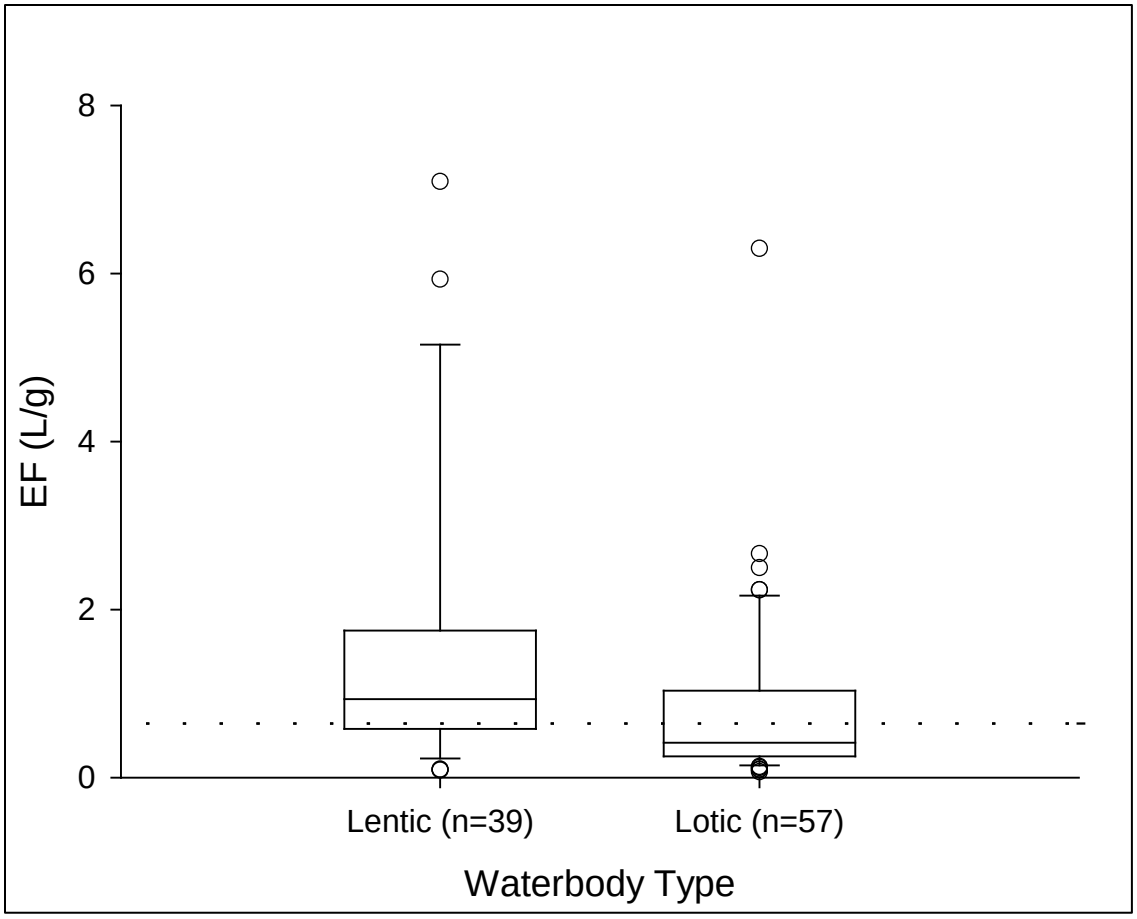


Figure 3.8. Distribution of *EF* Values for the Same 96 Aquatic Systems.
(As shown in **Figure 3.6** and **Figure 3.7** grouped by lentic and lotic aquatic system categories). Boxes show the 25th centile, median, and 75th centile *EF* values; whiskers show the 10th and 90th centiles. Circles represent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

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

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

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

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

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

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

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

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

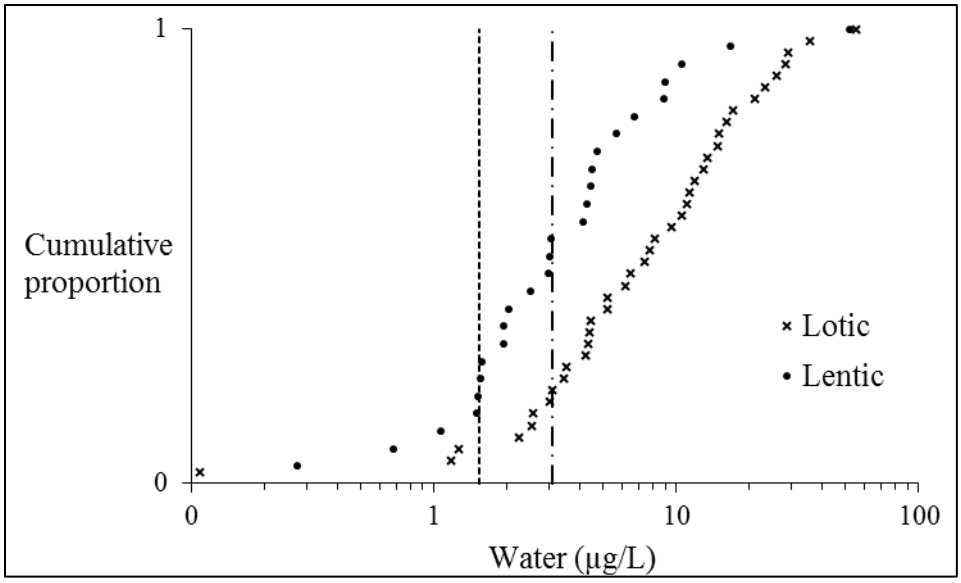


Figure 3.9. Probability Distribution of the Water Column Concentrations Translated from the Egg-Ovary Criterion Element at 26 Lentic and 39 Lotic Aquatic Sites.
Dashed and dash-dot lines show the 20th percentiles of the lentic and lotic distributions, respectively.

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

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

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

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

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

3.2.6 Derivation of Averaging Period for Chronic Water Criterion Element

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

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

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

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

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

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

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

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

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

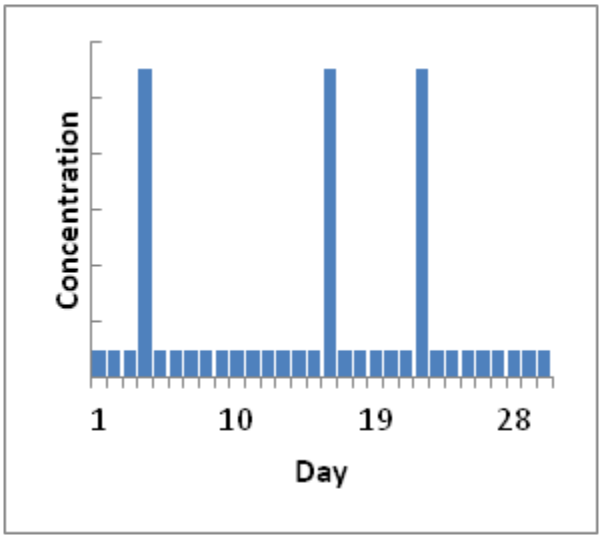


Figure 3.10. Illustration of Intermittent Spike Exposure Occurring for a Certain Percentage of Time (e.g., 10%) Over a 30-Day Period, and Background Exposure Occurring for the Remaining Percentage of Time (e.g., 90%).

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

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

Where:

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

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

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

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

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

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

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

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

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

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

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

4 NATIONAL CRITERION FOR SELENIUM IN FRESH WATERS

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

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

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

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

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured.
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.

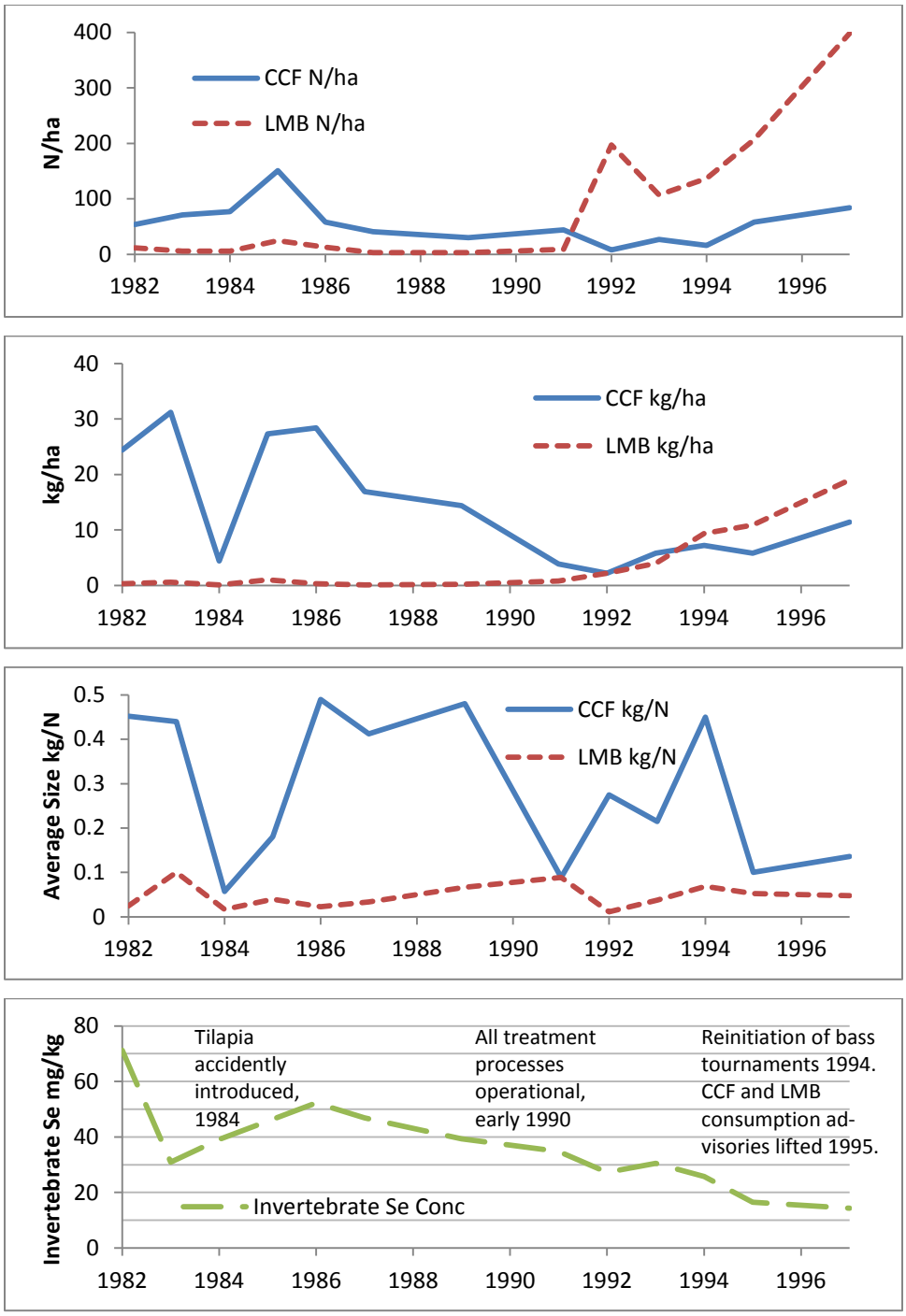


Figure 6.1. Crutchfield (2000) Observations of Channel Catfish (CCF) and Largemouth Bass (LMB) in Hyco Reservoir Beginning a Few Years after Populations of Largemouth Bass had been Reduced by Se Contamination.
(A) number of individuals/ha, (B) mass/ha, (C) mass/ha divided by number/ha, yielding average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

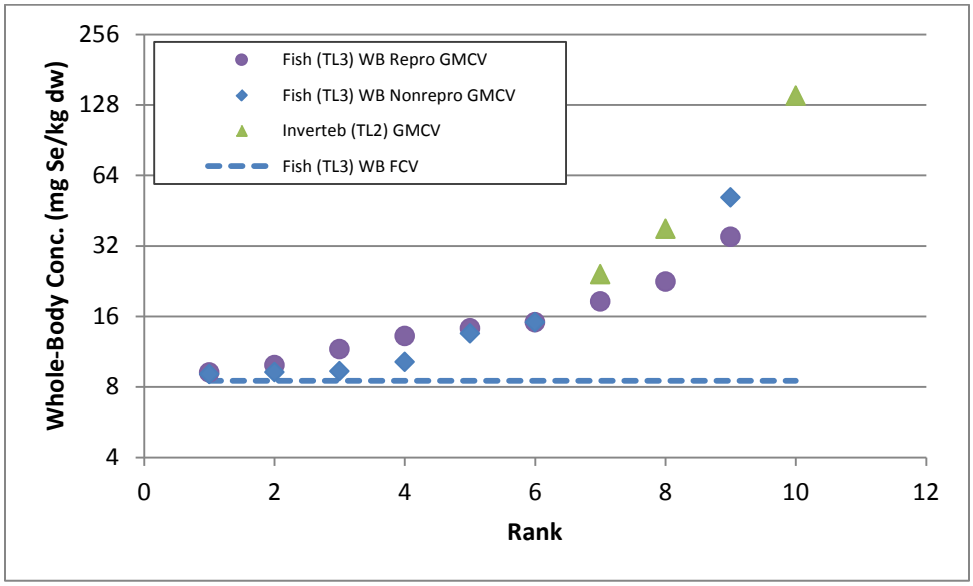


Figure 6.2. Distribution of Fish Reproductive Effect GMCVs from Figure 3.2 and Distribution of Fish Nonreproductive Effect GMCVs and Invertebrate GMCVs.

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.

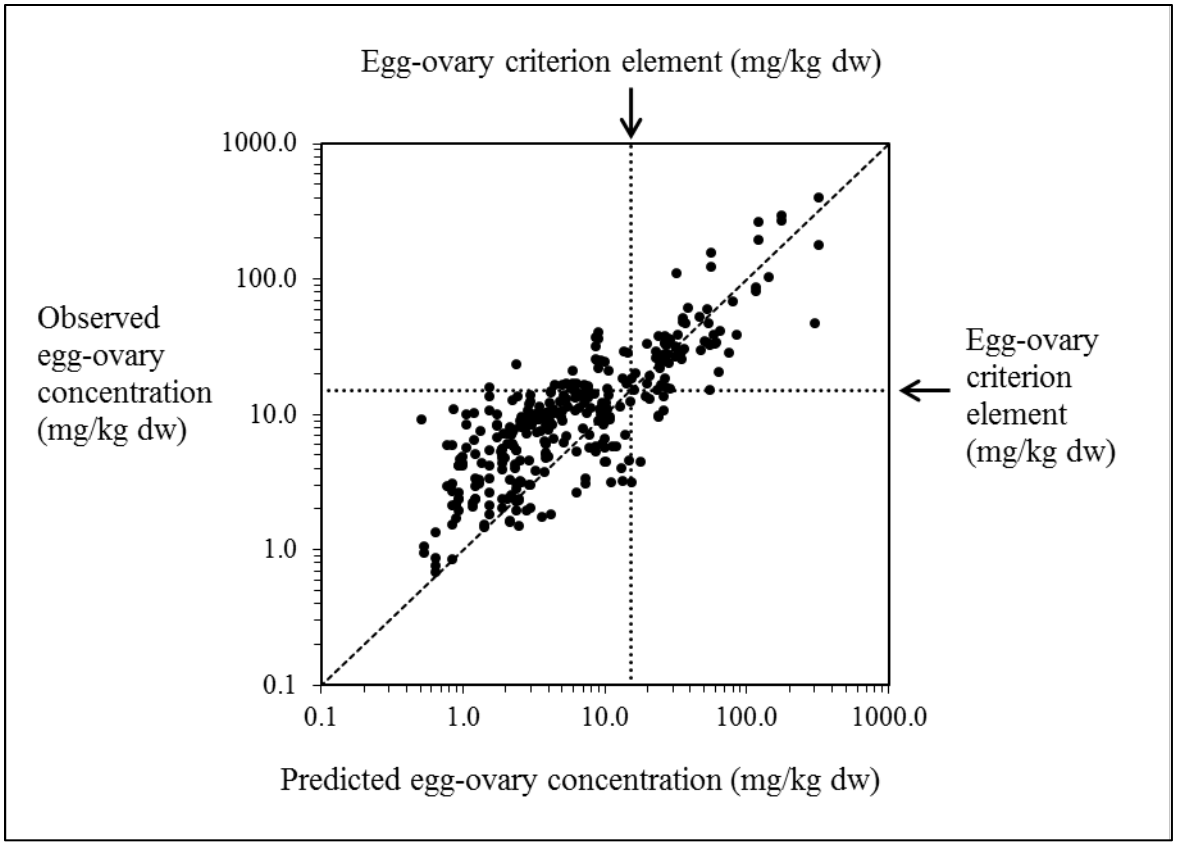


Figure 6.3. Scatter Plot of Predicted Versus Measured Concentrations of Selenium in Fish. Solid line shows unity y = x line; dashed lines show the egg-ovary criterion element value.

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.

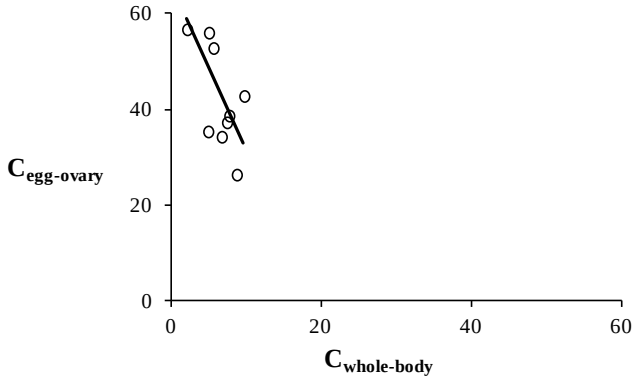
2.2 CF values calculated directly from whole-body and egg-ovary selenium measurements

$C_{whole\text{-}body}$ = 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 $\left(\frac{C_{egg} + C_{ovary}}{2}\right)$

Ratio = $\frac{C_{egg-ovary}}{C_{whole-body}}$

Black bullhead (*Ameiurus melas*)

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



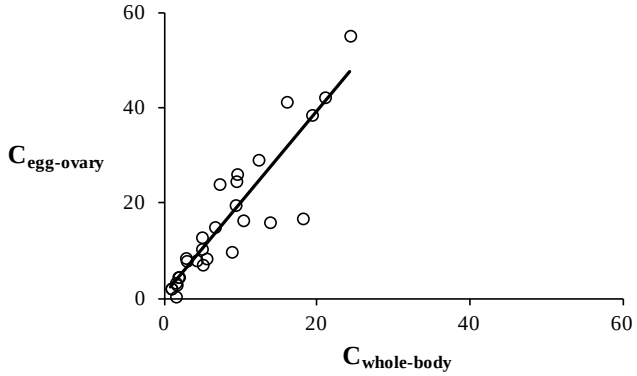
Median ratio: 6.29

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

Not used because negative slope.

Bluegill (*Lepomis macrochirus*)

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

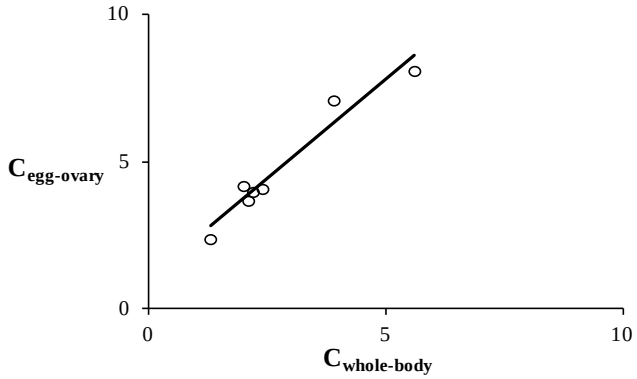


Median ratio: 2.13

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

Bluehead sucker (*Catostomus discobolus*)

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



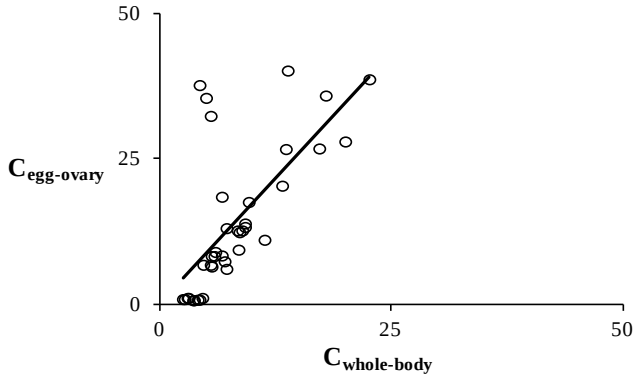
Median ratio: 1.82

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

Brown trout (*Salmo trutta*)

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

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

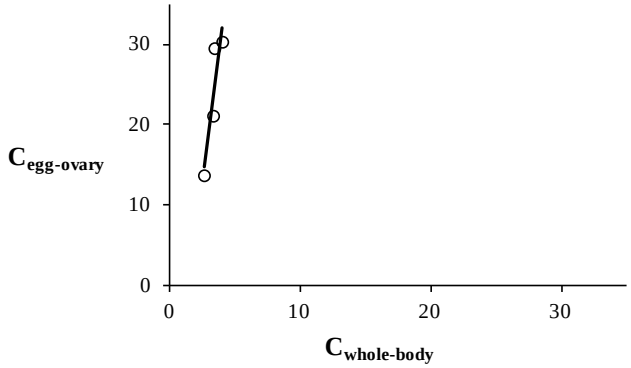


Median ratio: 1.45

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

Channel catfish (*Ictalurus punctatus*)

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



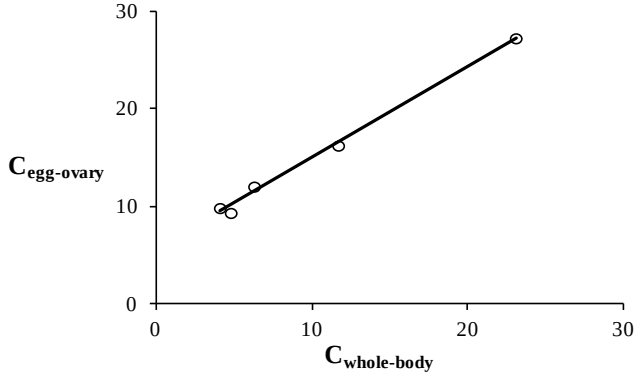
Median ratio: 6.98

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

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

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

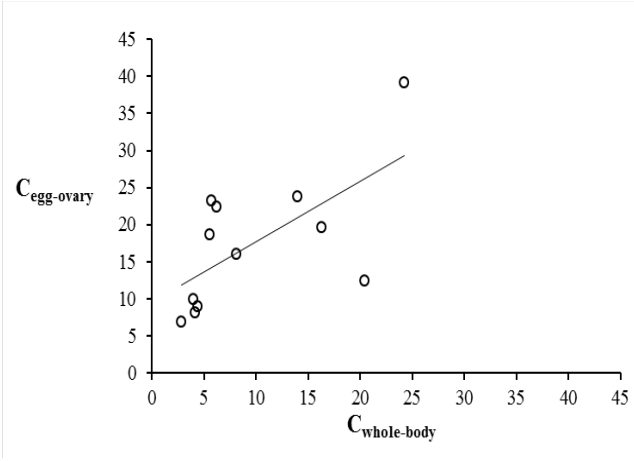


Median ratio: 1.92

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

Creek chub (*Semotilus atromaculatus*)

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



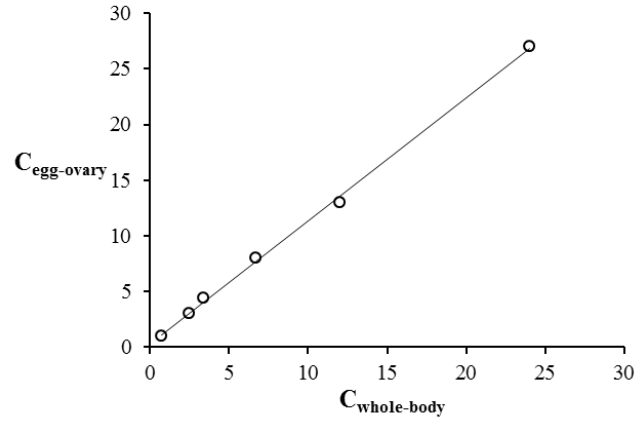
Median ratio: 1.99

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

Desert pupfish (*Cyprinodon macularius*)

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

Desert pupfish (*Cyprinodon macularius*)



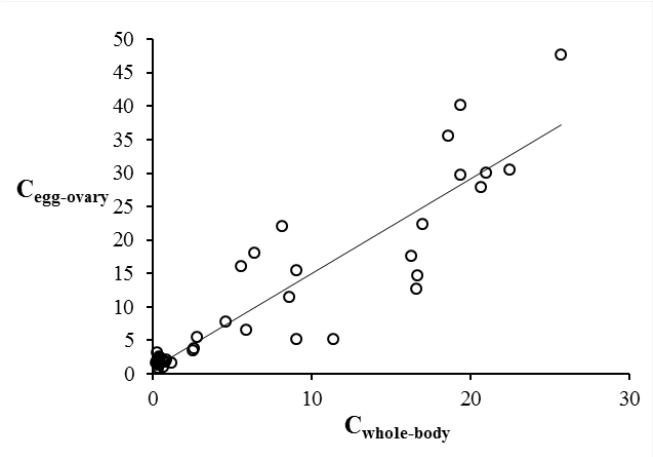
Median ratio: 1.20

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

Cutthroat trout (*Oncorhynchus clarkii*)

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

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



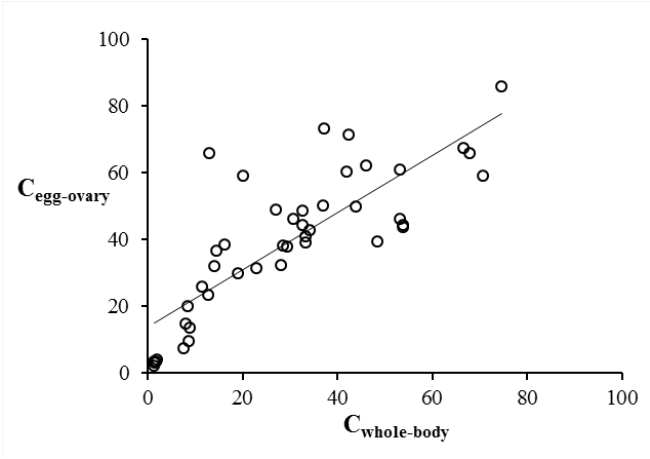
Median ratio: 1.96

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

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

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

Fathead minnow (*Pimephales promelas*)

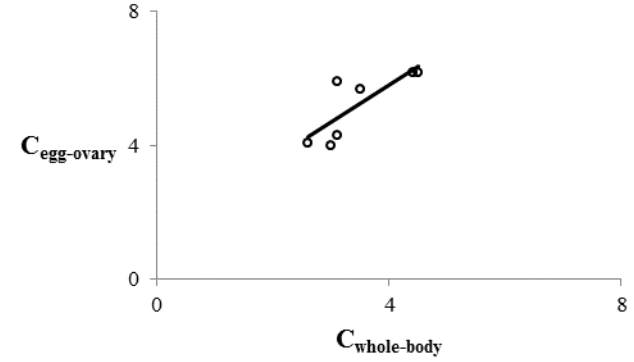


Median ratio: 1.40

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

Flannelmouth sucker (*Catostomus latipinnis*)

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



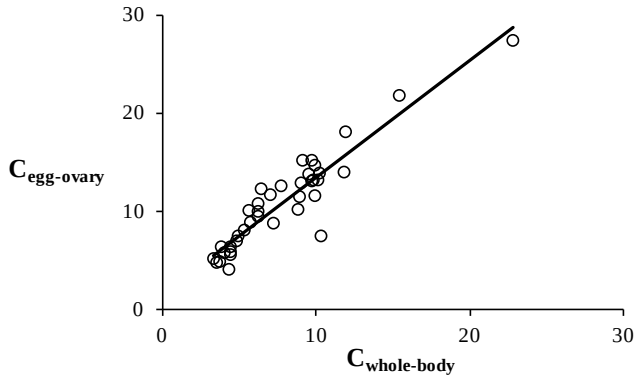
Median ratio: 1.41

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

Green sunfish (*Lepomis cyanellus*)

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

Green sunfish (*Lepomis cyanellus*)

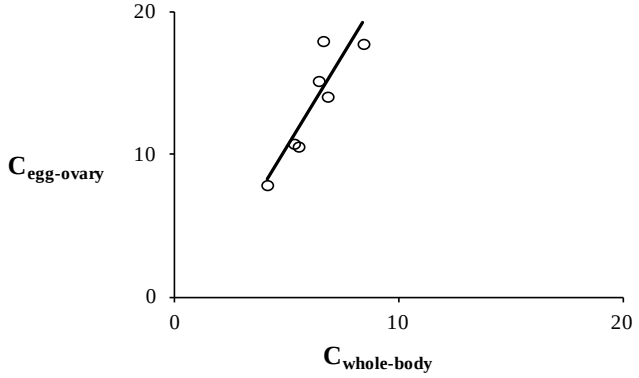


Median ratio: 1.45

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

Roundtail chub (*Gila robusta*)

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

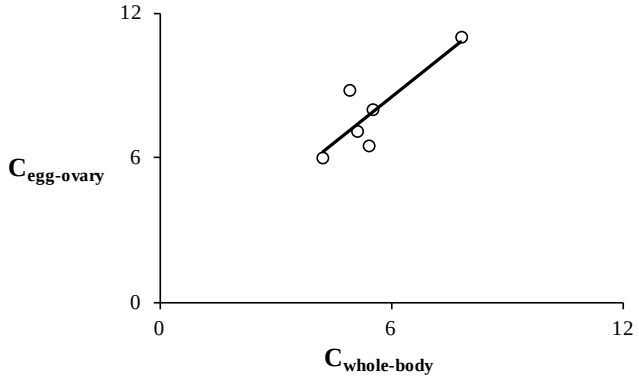


Median ratio: 2.07

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

Smallmouth bass (*Micropterus dolomieu*)

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



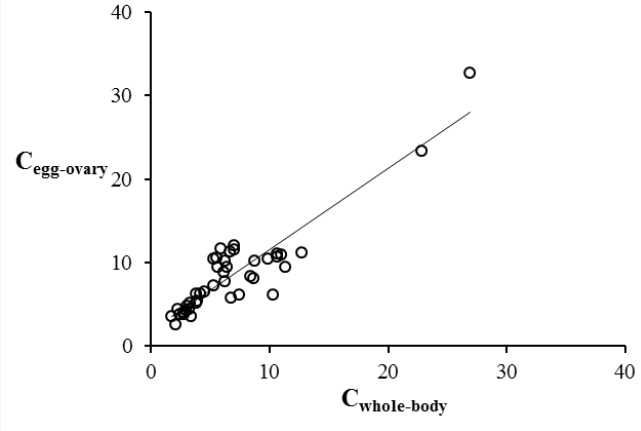
Median ratio: 1.42

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

White sucker (*Catostomus commersonii*)

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

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



Median ratio: 1.38

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

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

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

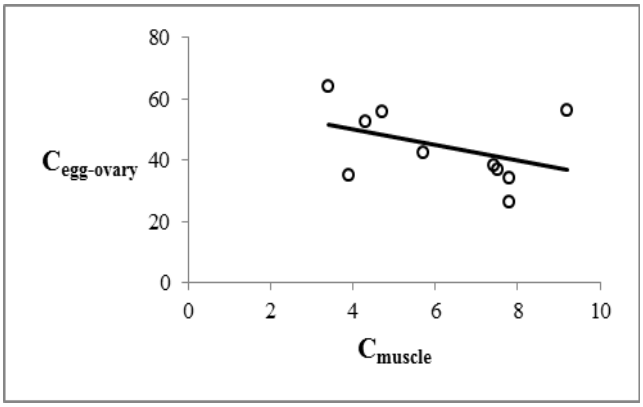
2.3 Muscle to egg-ovary conversion factors

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

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

Black bullhead (*Ameiurus melas*)

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



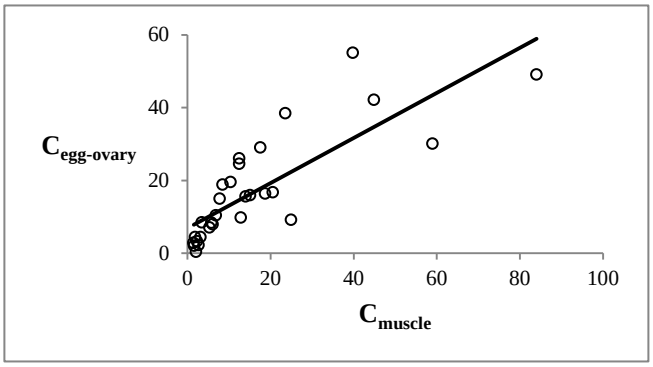
Median ratio: 6.84

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

Not used because P > 0.05 and negative slope.

Bluegill (*Lepomis macrochirus*)

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

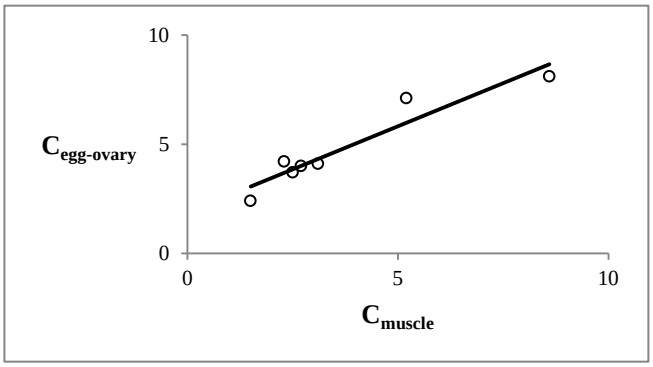


Median ratio: 1.38

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

Bluehead sucker (*Catostomus discobolus*)

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



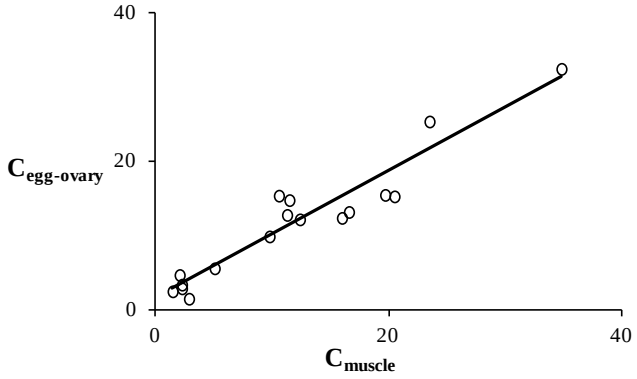
Median ratio: 1.48

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

Brook trout (*Salvelinus fontinalis*)

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

Brook trout (*Salvelinus fontinalis*)

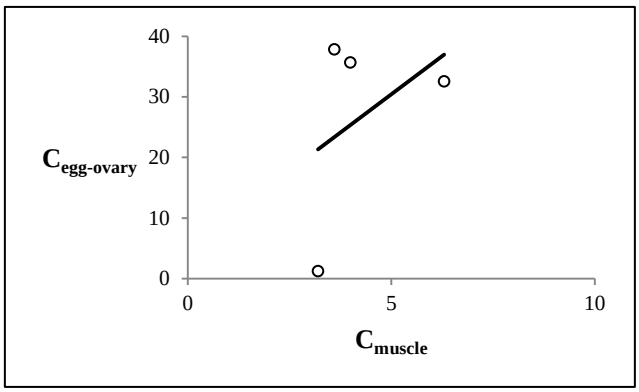


Median ratio: 1.09

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

Brown trout (*Salmo trutta*)

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



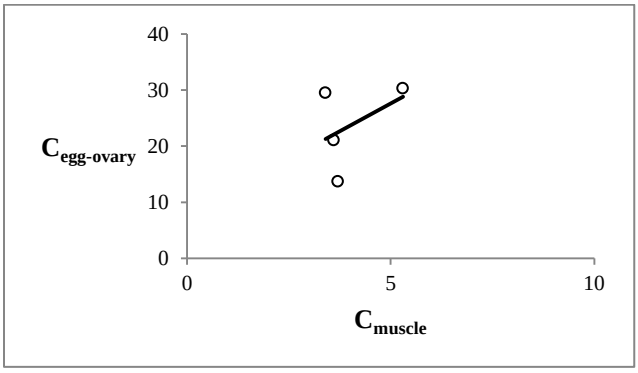
Median ratio: 7.03

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

Not used because P > 0.05.

Channel catfish (*Ictaluris punctatus*)

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



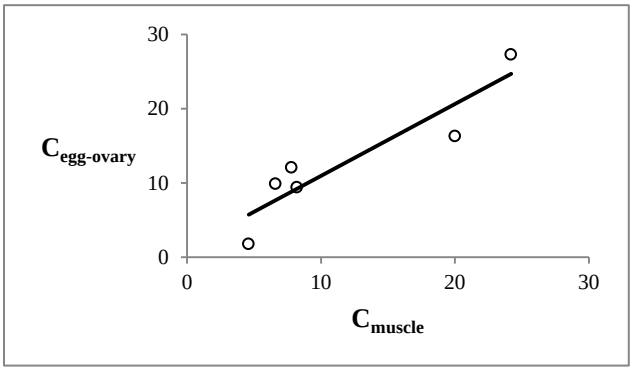
Median ratio: 5.79

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

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

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



Median ratio: 1.14

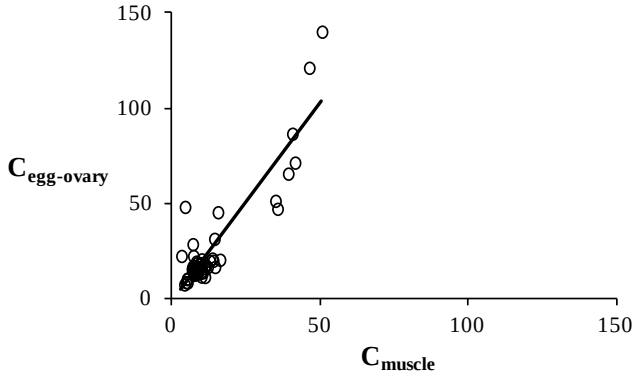
R^2: 0.84
F: 21.7
df: 4
P: 0.007

Cutthroat trout (*Oncorhynchus clarkii*)

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

Cutthroat trout (*Oncorhynchus clarkii*)

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

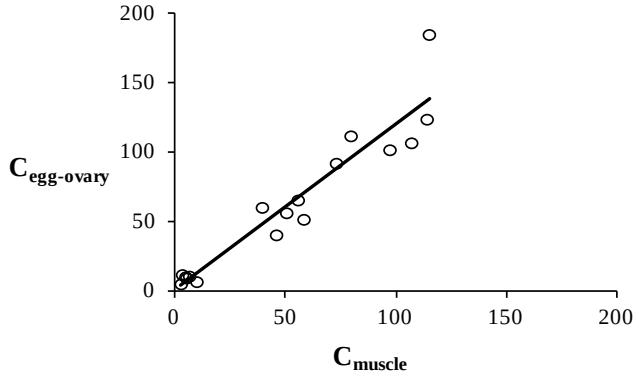


Median ratio: 1.81

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

Dolly Varden (*Salvelinus malma*)

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

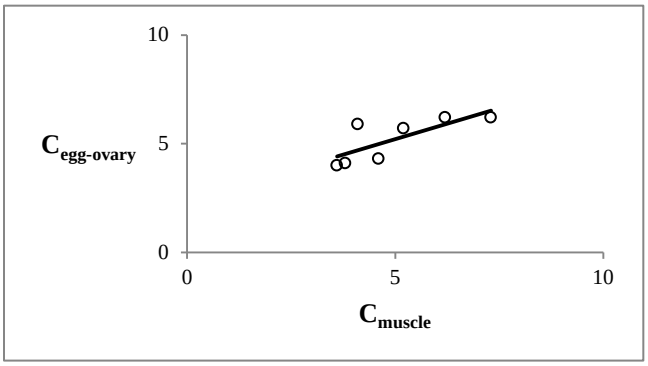


Median ratio: 1.26

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

Flannelmouth sucker (*Catostomus latipinnis*)

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



Median ratio: 1.08

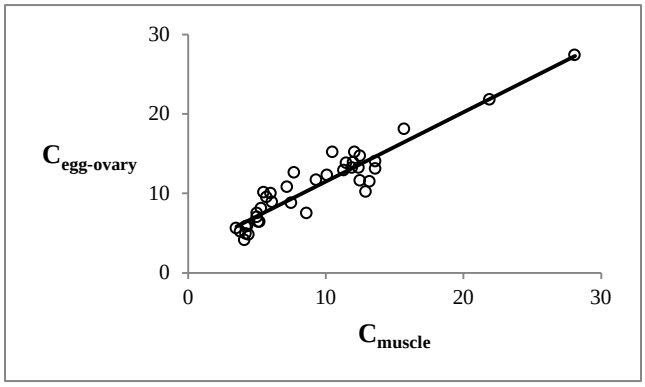
R^2: 0.58
F: 6.92
df: 5
P: 0.036

Green sunfish (*Lepomis cyanellus*)

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

Green sunfish (*Lepomis cyanellus*)

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

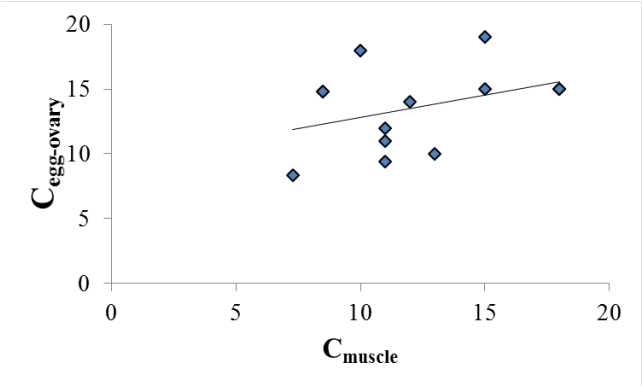


Median ratio: 1.21

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

Largemouth bass (*Micropterus salmoides*)

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

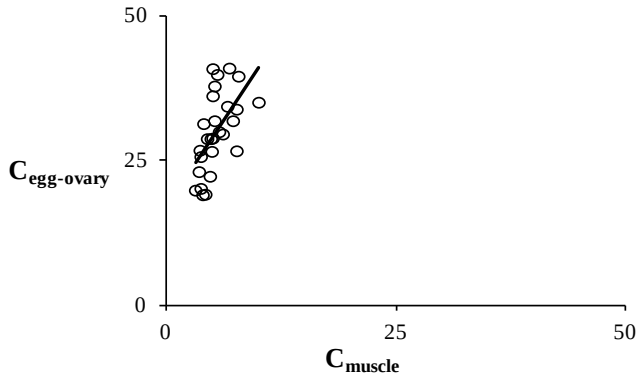


Median ratio: 1.09

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

Mountain whitefish (*Prosopium williamsoni*)

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

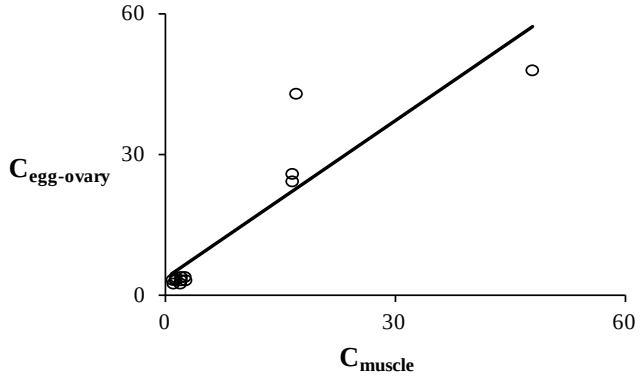


Median ratio: 5.80

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

Northern pike (*Esox lucius*)

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



Median ratio: 1.88

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

Rainbow trout (*Oncorhynchus mykiss*)

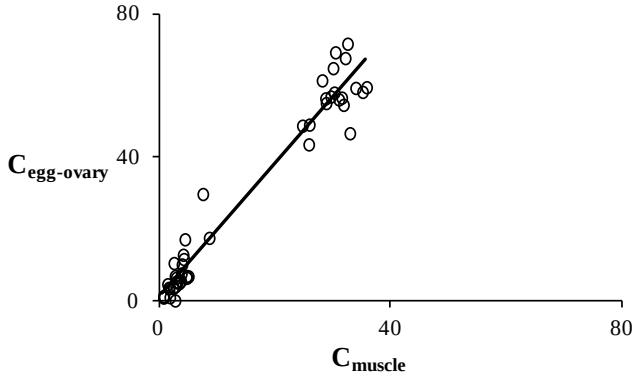
Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Casey and Siwik 2000	4.10	11.60	-	11.60	2.83
Casey and Siwik 2000	3.80	10.10	-	10.10	2.66
Casey and Siwik 2000	2.60	0.10	-	0.10	0.04
Casey and Siwik 2000	3.30	4.90	-	4.90	1.48
Casey and Siwik 2000	2.30	3.60	-	3.60	1.57
Casey and Siwik 2000	2.80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

Rainbow trout (*Oncorhynchus mykiss*)

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

Rainbow trout (*Oncorhynchus mykiss*)

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



Median ratio: 1.92

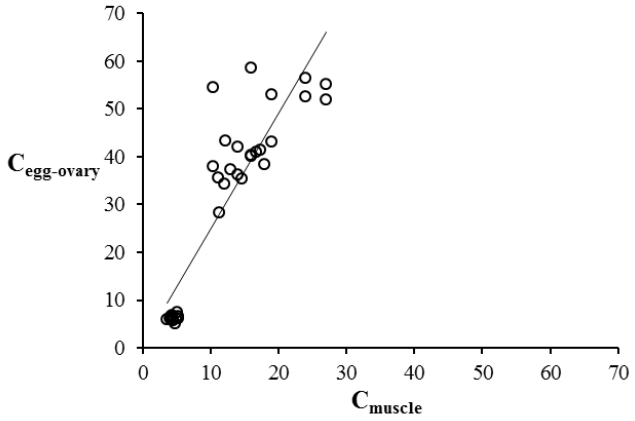
R^2: 0.96
F: 990.0
df: 45
P: <0. 001

Razorback sucker (*Xyrauchen texanus*)

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

Razorback sucker (*Xyrauchen texanus*)

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



Median ratio: 2.31

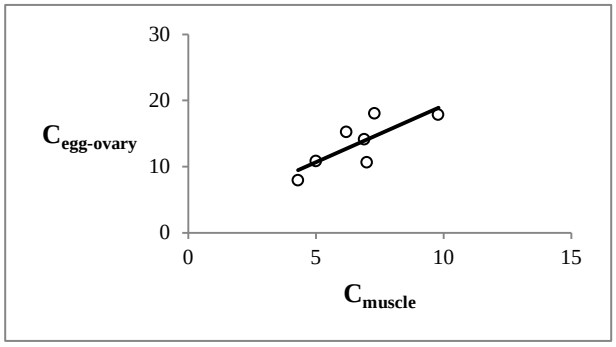
R^2: 0.80
F: 125.6
df: 32
P: <0.001

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

Roundtail chub (*Gila robusta*)

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

Roundtail chub (*Gila robusta*)

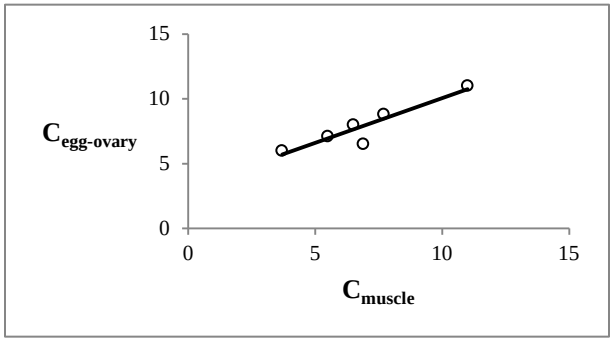


Median ratio: 2.04

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

Smallmouth bass (*Micropterus dolomieu*)

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

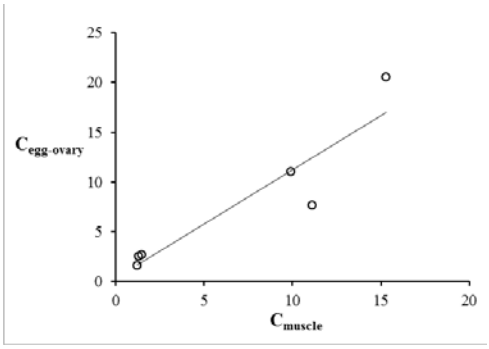


Median ratio: 1.19

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

White Sturgeon (*Acipenser transmontanus*)

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



Median ratio: 1.33

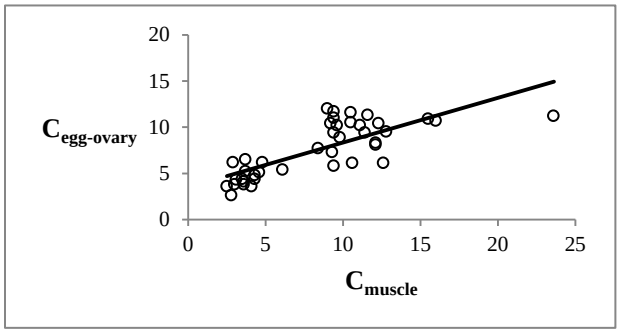
R^2: 0.86
F: 24.96
df: 4
P: 0.006

White Sucker (*Catostomus commersonii*)

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

White Sucker (*Catostomus commersonii*)

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



Median ratio: 1.00

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

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

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

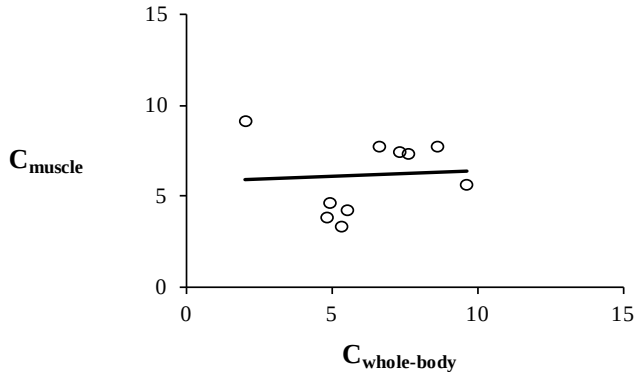
2.4 Muscle to whole-body conversion factors

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

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

Black bullhead (*Ameiurus melas*)

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



Median ratio: 0.93

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

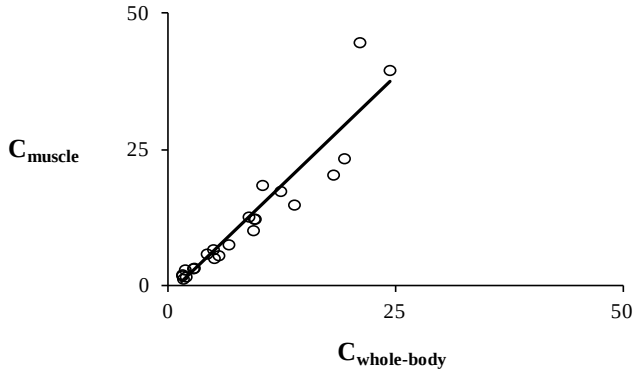
Not used because P > 0.05.

Bluegill (*Lepomis macrochirus*)

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

Bluegill (*Lepomis macrochirus*)

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

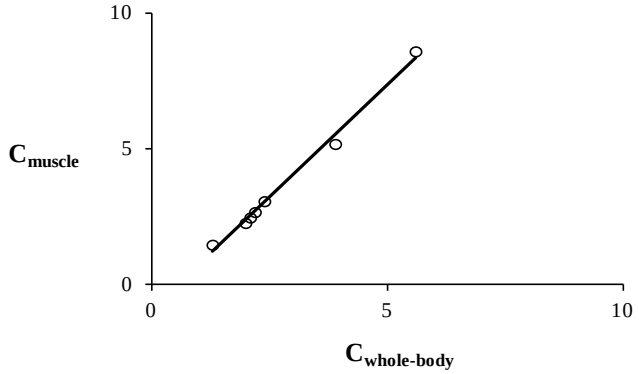


Median ratio: 1.32

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

Bluehead sucker (*Catostomus discobolus*)

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

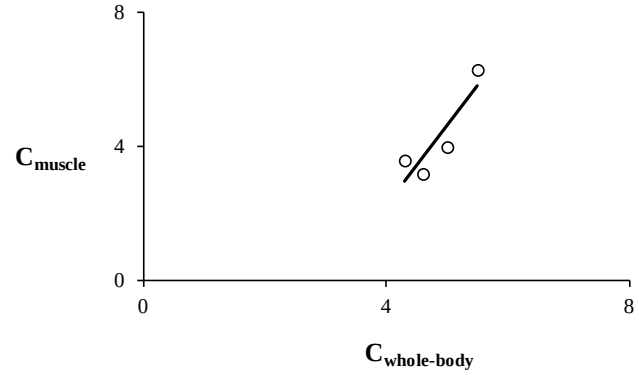


Median ratio: 1.23

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

Brown trout (*Salmo trutta*)

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



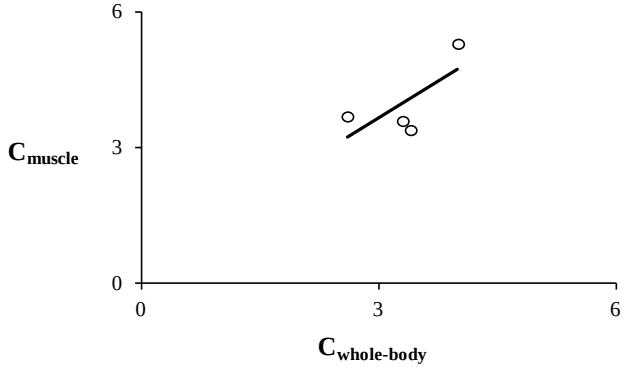
Median ratio: 0.82

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

Not used because P > 0.05.

Channel catfish (*Ictalurus punctatus*)

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



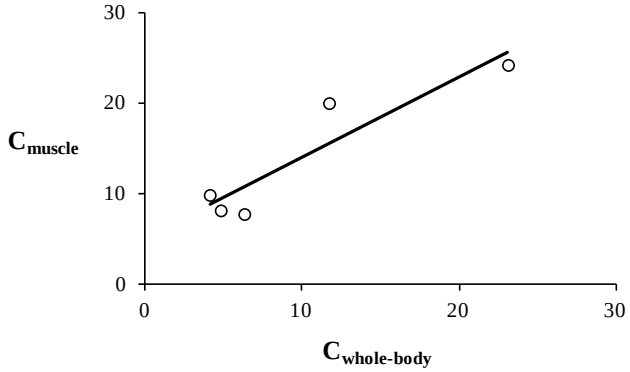
Median ratio: 1.21

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

Not used because P > 0.05.

Common carp (*Cyprinus carpio*)

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

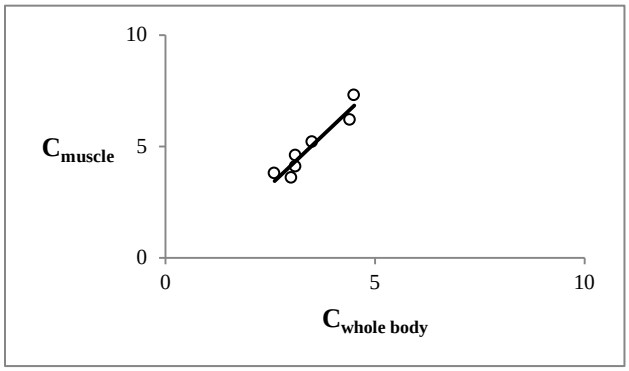


Median ratio: 1.61

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

Flannelmouth sucker (*Catostomus latipinnis*)

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



Median ratio: 1.46

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

Green sunfish (*Lepomis cyanellus*)

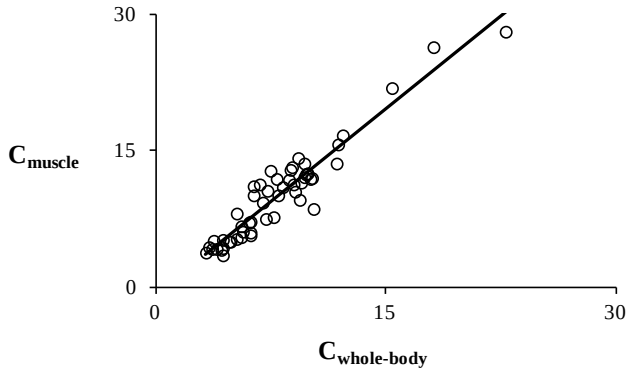
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	22.80	28.10	1.23
Osmundson et al. 2007	8.80	12.90	1.47
Osmundson et al. 2007	15.40	21.90	1.42
Osmundson et al. 2007	4.80	5.00	1.04
Osmundson et al. 2007	5.70	6.10	1.07
Osmundson et al. 2007	4.40	5.20	1.18
Osmundson et al. 2007	3.80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

Green sunfish (*Lepomis cyanellus*)

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

Green sunfish (*Lepomis cyanellus*)

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

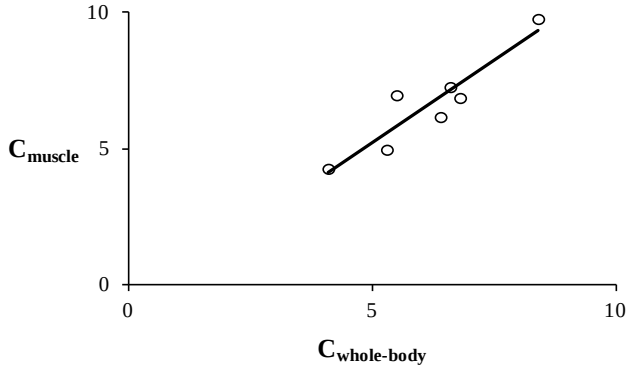


Median ratio: 1.23

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

Roundtail chub (*Gila robusta*)

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

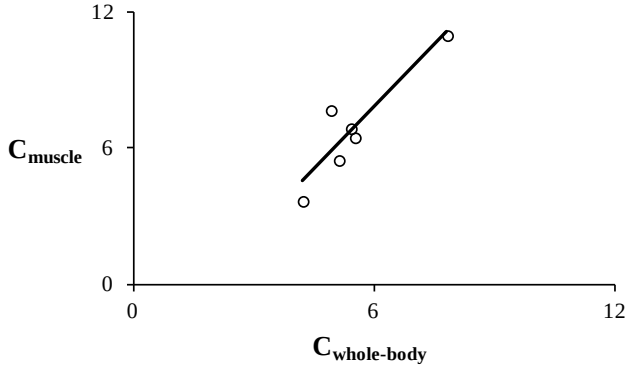


Median ratio: 1.05

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

Smallmouth bass (*Micropterus dolomieu*)

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



Median ratio: 1.23

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

White sucker (*Catostomus commersonii*)

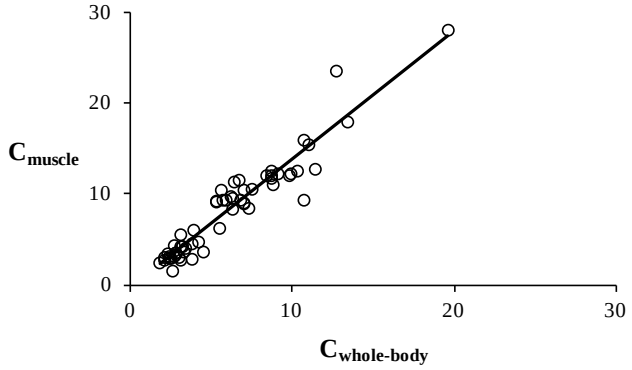
Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	3.80	2.90	0.76
Osmundson et al. 2007	4.20	4.80	1.14
Osmundson et al. 2007	3.30	3.70	1.12
Osmundson et al. 2007	4.50	3.70	0.82
Osmundson et al. 2007	6.30	8.40	1.33
Osmundson et al. 2007	6.80	9.40	1.38
Osmundson et al. 2007	11.00	15.50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

White sucker (*Catostomus commersonii*)

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

White sucker (*Catostomus commersonii*)

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



Median ratio: 1.34

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

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

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

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

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

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

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

Table B-6. All EPA-derived egg-ovary to whole body (CF), egg-ovary to muscle, and muscle to whole body conversion factors directly calculated or estimated using taxonomic classification. (See main text for explanation of the taxonomic classification approach).

		Direct calculation			Values based on taxonomic classification								Final E-O / WB	
Common name	Scientific name	E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
alligator gar	Atractosteus spatula				Lepistosteiformes	Lepisosteidae	Atractosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bigmouth buffalo	Ictiobus cyprinellus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
black bullhead	Ameiurus melas				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
black crappie	Pomoxis nigromaculatus				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
black redhorse	Moxostoma duquesnei				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
blacknose dace	Rhinichthys atratulus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
blue catfish	Ictalurus furcatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bluegill	Lepomis macrochirus	2.13	1.38	1.32	Perciformes	Centrarchidae	Lepomis	2.13	Exact match	1.38	Exact match	1.32	Exact match	2.13	Exact match
bluehead sucker	Catostomus discobolus	1.82	1.48	1.23	Cypriniformes	Catostomidae	Catostomus	1.82	Exact match	1.48	Exact match	1.23	Exact match	1.82	Exact match
brassy minnow	Hybognathus hankinsoni				Cypriniformes	Cyprinidae	Hybognathus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
brook stickleback	Culaea inconstans				Gasterosteiformes	Gasterosteidae	Culaea	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brook trout	Salvelinus fontinalis		1.09		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.09	Exact match	1.27	All fish	1.38	E-O/WB * M/WB
brown bullhead	Ameiurus nebulosus				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
brown trout	Salmo trutta	1.45			Salmoniformes	Salmonidae	Salmo	1.45	Exact match	1.81	Family Salmonidae	1.27	All fish	1.45	Exact match
burbot	Lota lota				Gadiformes	Lotidae	Lota	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
bullhead					Siluriformes	Ictaluridae		1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
chain pickerel	Esox				Esociformes	Esocidae	Esox	1.45	All fish	1.88	Genus Esox	1.27	All fish	2.39	E-O/WB * M/WB
channel catfish	Ictalurus punctatus				Siluriformes	Ictaluridae	Ictalurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
common carp	Cyprinus carpio	1.92	1.14	1.61	Cypriniformes	Cyprinidae	Cyprinus	1.92	Exact match	1.14	Exact match	1.61	Exact match	1.92	Exact match
common snook	Centropomus undecimalis				Perciformes	Centropomidae	Centropomus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
crappie	Pomoxis sp.				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
creek chub	Semotilus atromaculatus	1.99			Cypriniformes	Cyprinidae	Semotilus	1.99	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.99	Family Cyprinidae
cutthroat trout	Oncorhynchus clarkii	1.96	1.81		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Exact match	1.81	Exact match	1.27	All fish	1.96	Exact match

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
desert pupfish	Cyprinodon macularius	1.20			Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.20	Exact match	1.35	All fish	1.27	All fish	1.20	Exact match
Dolly Varden	Salvelinus malma		1.26		Salmoniformes	Salmonidae	Salvelinus	1.71	Family Salmonidae	1.26	Exact match	1.27	All fish	1.61	E-O/WB * M/WB
fathead minnow	Pimephales promelas	1.40			Cypriniformes	Cyprinidae	Pimephales	1.40	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.40	Family Cyprinidae
flannelmouth sucker	Catostomus latipinnis	1.41	1.08	1.46	Cypriniformes	Catostomidae	Catostomus	1.41	Exact match	1.08	Exact match	1.46	Exact match	1.41	Exact match
flathead catfish	Pylodictis				Siluriformes	Ictaluridae	Pylodictus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
flathead chub	Platygobio gracilis				Cypriniformes	Cyprinidae	Platygobio	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
freshwater drum	Aplodinotus grunniens				Perciformes	Sciaenidae	Aplodinotus	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
goldeye	Hiodon alosoides				Hiodontiformes	Hiodontidae	Hiodon	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
gizzard shad	Dorosoma cepedianum				Clupeiformes	Clupeidae	Dorosoma	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
green sunfish	Lepomis cyanellus	1.45	1.21	1.23	Perciformes	Centrarchidae	Lepomis	1.45	Exact match	1.21	Exact match	1.23	Exact match	1.45	Exact match
iowa darter	Etheostoma exile				Perciformes	Percidae	Etheostoma	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
Japanese medaka	Oryzias latipes				Beloniformes	Adrianichthyidae	Oryzias	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
kokanee salmon	Oncorhynchus nerka				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
largemouth bass	Micropterus salmoides				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
largescale sucker	Catostomus macrocheilus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
longnose dace	Rhinichthys cataractae				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
longnose sucker	Catostomus catostomus				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
mosquitofish	Gambusia sp.				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
mottled sculpin	Cottus bairdi				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
mountain whitefish	Prosopium williamsoni		5.80		Salmoniformes	Salmonidae	Prosopium	1.71	Family Salmonidae	5.80	Exact match	1.27	All fish	7.39	E-O/WB * M/WB
ninespine stickleback	Pungitius pungitius				Gasterosteiformes	Gasterosteidae	Pungitius	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
northern pike	Esox lucius		1.88		Esociformes	Esocidae	Esox	1.45	All fish	1.88	Exact match	1.27	All fish	2.39	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
northern pikeminnow	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern plains killifish	Fundulus kansae				Cyprinodontiformes	Fundulidae	Fundulus	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
northern redbelly dace	Chrosomus eos				Cypriniformes	Cyprinidae	Chrosomus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
northern squawfish	Ptychocheilus oregonensis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
quillback	Carpiodes cyprinus				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rainbow trout	Oncorhynchus mykiss		1.92		Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.92	Exact match	1.27	All fish	2.44	E-O/WB * M/WB
razorback sucker	Xyrauchen texanus		2.31		Cypriniformes	Catostomidae	Xyrauchen	1.41	Family Catostomidae	2.31	Exact match	1.34	Family Catostomidae	3.11	E-O/WB * M/WB
red shiner	Cyprinella lutrensis				Cypriniformes	Cyprinidae	Cyprinella	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
redbreast sunfish	Lepomis auritus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redear sunfish	Lepomis microlophus				Perciformes	Centrarchidae	Lepomis	1.79	Genus Lepomis	1.29	Genus Lepomis	1.27	Genus Lepomis	1.79	Genus Lepomis
redside shiner	Richardsonius balteatus				Cypriniformes	Cyprinidae	Richardsonius	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
river carpsucker	Carpiodes carpio				Cypriniformes	Catostomidae	Carpiodes	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
river redhorse	Moxostoma carinatum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
rock bass	Ambloplites rupestris				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
roundtail chub	Gila robusta	2.07	2.04	1.05	Cypriniformes	Cyprinidae	Gila	2.07	Exact match	2.04	Exact match	1.05	Exact match	2.07	Exact match
sacramento perch	Archoplites interruptus				Perciformes	Centrarchidae	Archoplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
sacramento pikeminnow	Ptychocheilus grandis				Cypriniformes	Cyprinidae	Ptychocheilus	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sailfin molly	Poecilia latipinna				Cyprinodontiformes	Poeciliidae	Poecilia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
sand shiner	Notropis stramineus				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
sauger	Sander canadensis				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
sculpin	Cottus sp.				Scorpaeniformes	Cottidae	Cottus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
shadow bass	Ambloplites ariommus				Perciformes	Centrarchidae	Ambloplites	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
shorthead redhorse	Moxostoma macrolepidotum				Cypriniformes	Catostomidae	Moxostoma	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
silver carp	Hypophthalmichthys molitrix				Cypriniformes	Cyprinidae	Hypophthalmichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
smallmouth bass	Micropterus dolomieu	1.42	1.19	1.23	Perciformes	Centrarchidae	Micropterus	1.42	Exact match	1.19	Exact match	1.23	Exact match	1.42	Exact match
smallmouth buffalo	Ictiobus bubalus				Cypriniformes	Catostomidae	Ictiobus	1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
speckled dace	Rhinichthys osculus				Cypriniformes	Cyprinidae	Rhinichthys	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spottail shiner	Notropis hudsonius				Cypriniformes	Cyprinidae	Notropis	1.95	Family Cyprinidae	1.59	Family Cyprinidae	1.33	Family Cyprinidae	1.95	Family Cyprinidae
spotted bass	Micropterus punctulatus				Perciformes	Centrarchidae	Micropterus	1.42	Genus Micropterus	1.19	Genus Micropterus	1.23	Genus Micropterus	1.42	Genus Micropterus
spotted gar	Lepisosteus oculatus				Lepistosteiformes	Lepisosteidae	Lepisosteus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
stonecat	Noturus flavus				Siluriformes	Ictaluridae	Noturus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
striped bass	Morone saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
striped mullet	Mugil cephalus				Mugiliformes	Mugilidae	Mugil	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
sucker					Cypriniformes	Catostomidae		1.41	Family Catostomidae	1.28	Family Catostomidae	1.34	Family Catostomidae	1.41	Family Catostomidae
tilapia					Perciformes	Cichlidae		1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
trout species	Oncorhynchus sp.				Salmoniformes	Salmonidae	Oncorhynchus	1.96	Genus Oncorhynchus	1.86	Genus Oncorhynchus	1.27	All fish	1.96	Genus Oncorhynchus
tui chub	Gila bicolor				Cypriniformes	Cyprinidae	Gila	2.07	Genus Gila	2.04	Genus Gila	1.05	Genus Gila	2.07	Genus Gila
utah sucker	Catostomus ardens				Cypriniformes	Catostomidae	Catostomus	1.41	Genus Catostomus	1.08	Genus Catostomus	1.34	Genus Catostomus	1.41	Genus Catostomus
walleye	Sander vitreus				Perciformes	Percidae	Sander	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
western mosquitofish	Gambusia affinis				Cyprinodontiformes	Poeciliidae	Gambusia	1.20	Order Cyprinodontiformes	1.35	All fish	1.27	All fish	1.20	Order Cyprinodontiformes
white bass	Morone chrysops				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
white crappie	Pomoxis annularis				Perciformes	Centrarchidae	Pomoxis	1.45	Family Centrarchidae	1.21	Family Centrarchidae	1.23	Family Centrarchidae	1.45	Family Centrarchidae
white sturgeon	Acipenser transmontanus		1.33		Acipenseriformes	Acipenseridae	Acipenser	1.45	All fish	1.33	Exact match	1.27	All fish	1.69	E-O/WB * M/WB

Common name	Scientific name	Direct calculation			Values based on taxonomic classification									Final E-O / WB	
		E-O / WB	E-O / M	M / WB	Order	Family	Genus	E-O / WB	E-O / WB source	E-O / M	E-O / M source	M / WB	M / WB source	Final E-O / WB	Final E-O / WB source
white sucker	Catostomus commersonii	1.38	1.00	1.34	Cypriniformes	Catostomidae	Catostomus	1.38	Exact match	1.00	Exact match	1.34	Exact match	1.38	Exact match
wiper	Morone chrysops x Moron saxatilis				Perciformes	Moronidae	Morone	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes
yellow bullhead	Ameiurus natalis				Siluriformes	Ictaluridae	Ameiurus	1.45	All fish	1.35	All fish	1.27	All fish	1.45	All fish
yellow perch	Perca flavescens				Perciformes	Percidae	Perca	1.45	Order Perciformes	1.21	Order Perciformes	1.23	Order Perciformes	1.45	Order Perciformes

3.0 DERIVATION OF TROPHIC TRANSFER FACTOR VALUES

3.1 Methodology

Taxa specific trophic transfer factors (TTF) to quantify the degree of biomagnification across a given trophic level were calculated from either physiological parameters measured in laboratory studies or from field measurements of paired selenium concentrations in consumer species and their food. TTFs from both approaches were used to calculate translated water concentrations; however, when TTF data of similar quality are available from both approached, as was the case with bluegill, field-derived TTF data are used.

Physiological data consisted of assimilation efficiencies (AE), measured as either a percentage or a proportion, ingestion rates (IR), measured as grams of Se per grams of food consumed per day, and efflux rate constant (k_e), measured as 1/day. All available data were collected for a particular species, and then the TTF for that species was calculated using the equation:

$$\text{TTF} = \frac{AE \; x \; IR}{k_e}$$

Where AE, IR, and K_e were estimated as the median value of all available data for that parameter for that species.

The majority of TTF were calculated using paired whole-body Se measurements from organisms collected at the same site in the field. TTFs for trophic level 2 organisms were determined using the equation:

$$TTF^{TL2} = \frac{C_{tissue}^{TL2}}{C_{food}^{TL2}}$$

Where C_{food}^{TL2} equals the average Se concentration in particulate matter, defined as the average of C_{algae}, $C_{detritus}$, and $C_{sediment}$. Of the three types of particulate matter potentially assumed by TL2 organisms (e.g., the majority of invertebrates), $C_{sediment}$ correlated relatively poorly to C_{tissue}^{TL2}, when compared to C_{algae} and $C_{detritus}$. In order to minimize potentially erroneous TTF calculations based solely on sediment Se concentrations, while note completely discounting the importance of organic matter in sediments as a potential food source, $C_{sediment}$ was included in $C_{particulate}$ calculations only when either C_{algae} or $C_{detritus}$ data were also available.

TTFs for trophic level 3 organisms were determined using the equation:

$$TTF^{TL3} = \frac{C_{tissue}^{TL3}}{C_{food}^{TL3}}$$

Where C_{food}^{TL3} equals the average whole-body Se concentration in invertebrates collected at the same site as their potential predator species. The majority of trophic level 3 organisms are fish species, but damselflies and dragonflies of the order Odonata are also trophic level 3 organisms, and TTF^{TL3} values were calculated for those species as well.

For all field derived data used to determine TTFs, EPA first confirmed a statistical relationship between whole-body selenium concentrations for each species and its food using OLS linear regression. If the regression resulted in a statistically significant ($P<0.05$) positive slope, EPA calculated the TTF as the median ratio of the paired concentration data.

3.2 TTF values from physiological coefficients

AE (%) = Assimilation efficiency
IR ($g\ g^{-1}\ d^{-1}$) = Ingestion rate
k_e (d^{-1}) = Efflux rate constant
TTF = $\frac{AE\ x\ IR}{K_e}$

3.2.1 Invertebrates

Baltic macoma (*Macoma balthica*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
22.5				Luoma et al. 1992
91.0				Luoma et al. 1992
84.0				Luoma et al. 1992
95.0				Luoma et al. 1992
78.0		0.03		Reinfelder et al. 1997
74.0		0.03		Reinfelder et al. 1997
92.3				Schleckat et al. 2002
58.0				Schleckat et al. 2002
85.8				Schleckat et al. 2002
64.9				Schleckat et al. 2002
90.4				Schleckat et al. 2002
Median Values and TTF				
84.0	0.27[a]	0.03	7.56	

[a] Value taken from *Mytilus edulis*

Short-necked clam (*Ruditapes philippinarum*)

Physiological Parameters				
AE (%)	**IR($g\ g^{-1}\ d^{-1}$)**	**k_e (d^{-1})**	**TTF**	**Study**
70.0		0.013		Zhang et al. 1990
52.0		0.013		Zhang et al. 1990
Median Values and TTF				
61.0	0.27[a]	0.013	12.67	

[a] Value taken from *Mytilus edulis*

Quahog (*Mercenaria mercenaria*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
100.1				Reinfelder and Fisher 1994
92.0		0.01		Reinfelder et al. 1997
Median Values and TTF				
96.1	0.27[a]	0.01	25.93	

[a] Value taken from *Mytilus edulis*

Eastern Oyster (*Crassostrea virginica*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.005		Okazaki and Panietz 1981
105.4				Reinfelder and Fisher 1994
70.0		0.070		Reinfelder et al. 1997
Median Values and TTF				
87.7	0.27a	0.038	6.31	

[a] Value taken from *Mytilus edulis*

Common mussel (*Mytilus edulis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
86.0		0.02		Reinfelder et al. 1997
75.0		0.05		Reinfelder et al. 1997
60.7				Wang and Fisher 1996
48.0				Wang and Fisher 1996
13.7				Wang and Fisher 1996
55.1				Wang and Fisher 1996
55.8				Wang and Fisher 1996
71.9				Wang and Fisher 1996
71.5				Wang and Fisher 1996
27.9				Wang and Fisher 1996
84.4				Wang and Fisher 1996
81.0				Wang and Fisher 1996
79.4				Wang and Fisher 1996

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
63.0		0.037		Wang and Fisher 1996
61.5		0.05		Wang and Fisher 1996
69.0		0.027		Wang and Fisher 1996
81.0		0.022		Wang and Fisher 1997
82.0		0.020		Wang and Fisher 1997
72.0		0.018		Wang and Fisher 1997
78.0		0.055		Wang et al. 1995
76.0		0.065		Wang et al. 1995
71.0		0.058		Wang et al. 1995
33.9				Wang et al. 1996
27.5				Wang et al. 1996
				Wang et al. 1996
				Wang et al. 1996
	0.27	0.022		Wang et al. 1996
		0.026		Wang et al. 1996
		0.019		Wang et al. 1996
Median Values and TTF				
71.3	0.27	0.026	7.30	

Asian clam (*Corbicula fluminea*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.05	0.006		Lee et al. 2006
Median Values and TTF				
55.0	0.05	0.006	4.58	

Zebra mussel (*Dreissena polymorpha*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
18.0				Roditi and Fisher 1999
24.0				Roditi and Fisher 1999
46.0				Roditi and Fisher 1999
40.0				Roditi and Fisher 1999
41.0				Roditi and Fisher 1999
7.7				Roditi and Fisher 1999
23.0				Roditi and Fisher 1999
28.0				Roditi and Fisher 1999
	0.40			Roditi and Fisher 1999
		0.026		Roditi and Fisher 1999
Median Values and TTF				
26.0	0.40	0.026	4.00	

Water flea (*Daphnia magna*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
	0.08			Goulet et al. 2007
	0.34			Goulet et al. 2007
57.9				Yu and Wang 2002b
43.0				Yu and Wang 2002b
39.8				Yu and Wang 2002b
33.0				Yu and Wang 2002b
41.4				Yu and Wang 2002b
41.5				Yu and Wang 2002b
38.0				Yu and Wang 2002b
24.5				Yu and Wang 2002b
		0.101		Yu and Wang 2002b
		0.12		Yu and Wang 2002b
		0.131		Yu and Wang 2002b
		0.134		Yu and Wang 2002b
		0.108		Yu and Wang 2002b
		0.112		Yu and Wang 2002b
Median Values and TTF				
40.6	0.21	0.12	0.74	

Copepod (*Temora longicornis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
55.0	0.42	0.115		Wang and Fisher 1998
Median Values and TTF				
55.0	0.42	0.115	2.01	

Copepod (Small, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.35	

Copepod (Large, unidentified)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
52.0	0.42	0.155		Schlekat et al. 2004
Median Values and TTF				
50.0	0.42	0.155	1.41	

Blackworm (*Lumbriculus variegatus*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
		0.009		Riedel and Cole 2001
		0.006		Riedel and Cole 2001
24.0	0.067	0.013		Riedel and Cole 2001
9.0	0.067	0.009		Riedel and Cole 2001
Median Values and TTF				
16.5	0.067	0.0086	1.29	

Mayfly (*Centroptilum triangulifer*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
38.0	0.72	0.25		Riedel and Cole 2001
40.0	0.72	0.19		Riedel and Cole 2001
Median Values and TTF				
39.0	0.72	0.22	1.28	

a – not used because field TTF data available

3.2.2 Vertebrates

Bluegill (*Lepomis macrochirus*)[a]

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
34.0				Besser et al. 1993
22.0				Besser et al. 1993
24.0				Besser et al. 1993
36.0				Besser et al. 1993
30.0				Besser et al. 1993
32.0				Besser et al. 1993
43.0				Besser et al. 1993
40.0				Besser et al. 1993
37.0		0.041		Besser et al. 1993
		0.031		Besser et al. 1993
		0.034		Besser et al. 1993
36.0		0.031		Besser et al. 1993
		0.038		Besser et al. 1993
		0.038		Besser et al. 1993
	0.008			Whitledge and Haywood 2000
	0.042			Whitledge and Haywood 2000
Median Values and TTF				
35.0	0.025	0.036	1.156[a]	

[a] Not used because of availability of acceptable field-based TTF data

Fathead Minnow (*Pimephales promelas*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
50.0				Presser and Luoma 2010
	0.050			Bertram and Brooks 1986
		0.029		Bertram and Brooks 1986
		0.019		Bertram and Brooks 1986
		0.3		Bertram and Brooks 1986
		0.014		Bertram and Brooks 1986
		0.013		Bertram and Brooks 1986
		0.016		Bertram and Brooks 1986
		0.012		Bertram and Brooks 1986
		0.026		Bertram and Brooks 1986
		0.018		Bertram and Brooks 1986
		0.025		Bertram and Brooks 1986
Median Values and TTF				
50.0	0.050	0.0185	1.35	

Striped Bass (*Morone saxatilis*)

Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
33	0.17	0.09		Baines et al. 2002
42	0.5	0.08		Baines et al. 2002
	0.12			Buckel and Stoner 2004
	0.16			Buckel and Stoner 2004
	0.11			Buckel and Stoner 2004
	0.08			Buckel and Stoner 2004
Median Values and TTF				
37.5	0.335	0.085	1.48	

TTF calculated from only Baines et al. (2002) because it had complete data.

3.3 TTF values from field data

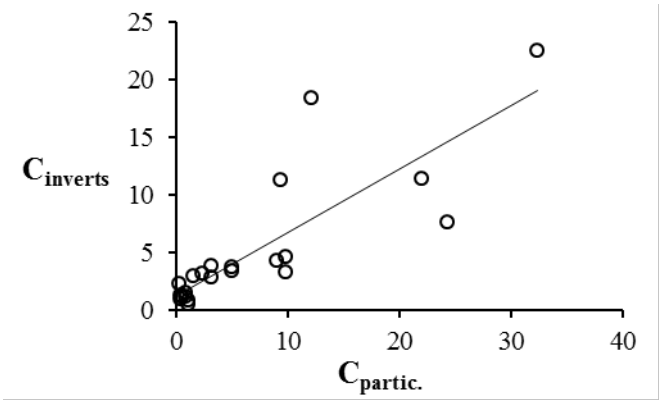
3.3.1 Invertebrates

C_{alg} = Selenium concentration in algae (mg/kg)
C_{det} = Selenium concentration in detritus (mg/kg)
C_{sed} = Selenium concentration in sediment (mg/kg)
C_{invert} = Selenium concentration in invertebrate tissue (mg/kg)
C_{part} = Average selenium concentration in particulate material $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$
Ratio = $\frac{C_{invert}}{C_{part}}$

Scuds (Amphipoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	18.40	1.52
Birkner 1978	20	3.00		41.00	22.00	11.40	0.52
Birkner 1978	7	0.18		2.80	1.49	2.90	1.95
Birkner 1978	19	16.80		1.20	9.00	4.30	0.48
Birkner 1978	30	17.30		47.30	32.30	22.50	0.70
Birkner 1978	3	0.10		0.30	0.20	2.30	11.50
Birkner 1978	22	4.60		44.00	24.30	7.60	0.31
Birkner 1978	23	7.80		10.80	9.30	11.30	1.22
Lambing et al. 1994	S46	2.30			2.30	3.20	1.39
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.44	0.40
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.86	0.79
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.60	0.47
Saiki et al. 1993	GT5	4.50	14.95		9.73	3.30	0.34
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.40	0.69
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.70	0.76
Saiki et al. 1993	SJR2	1.25	5.00		3.13	3.80	1.22
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.80	0.90
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.10	1.30
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.89	2.47
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42

Scuds (Amphipoda)

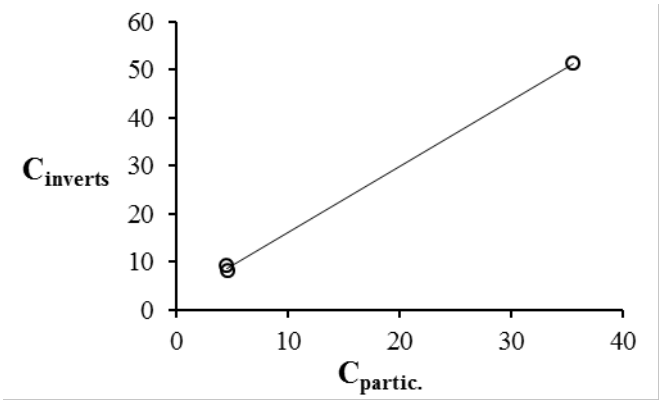


Median ratio: 1.22

R^2: 0.69
F: 46.9
df: 21
P: < 0.001

Earthworms and Leeches (Annelida)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Lemly 1985	Badin Lake	8.20		0.91	4.56	8.10	1.78
Lemly 1985	Belews Lake	62.70		8.27	35.49	51.15	1.44
Lemly 1985	High Rock Lake	8.25		0.79	4.52	9.05	2.00



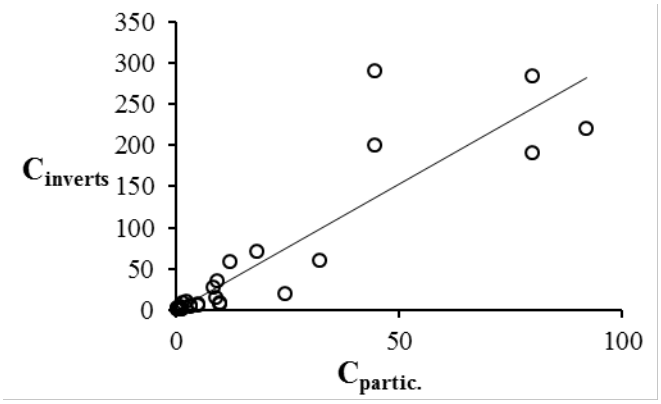
Median ratio: 1.78

R^2: 1.00
F: 2426
df: 1
P: < 0.001

Midges (Chironomidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	58.20	4.81
Birkner 1978	19	16.80		1.20	9.00	15.30	1.70
Birkner 1978	30	17.30		47.30	32.30	59.30	1.84
Birkner 1978	3	0.10		0.30	0.20	2.50	12.50
Birkner 1978	22	4.60		44.00	24.30	18.80	0.77
Birkner 1978	27	10.35		6.50	8.43	26.70	3.17
Birkner 1978	12	2.30		0.30	1.30	7.70	5.92
Birkner 1978	23	7.80		10.80	9.30	34.20	3.68
Grasso et al. 1995	17	1.87		0.40	1.14	2.07	1.82
Lambing et al. 1994	S46	2.30			2.30	9.70	4.22
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	71.00	3.91
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	200.0	4.48
Saiki and Lowe 1987	Kesterson Pond 2	152.7	44.65	34.82	44.65	290.0	6.49
Saiki and Lowe 1987	Kesterson Pond 8	136.5	92.00	6.05	92.00	220.0	2.39
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	190.0	2.38
Saiki and Lowe 1987	San Luis Drain	67.00	275.0	79.90	79.90	284.0	3.55
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.74	4.18
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.30	3.13
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	3.00	3.37
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.30	1.46
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.58	0.53
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.00	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	8.90	0.92
Saiki et al. 1993	GT5	4.50	14.95		9.73	7.20	0.74
Saiki et al. 1993	GT4	1.39	8.40		4.90	5.40	1.10
Saiki et al. 1993	GT4	1.39	8.40		4.90	6.90	1.41
Saiki et al. 1993	SJR2	1.25	5.00		3.13	6.00	1.92
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.10	1.31
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.50	1.77
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.47	1.31
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.00	2.78
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.53	1.16
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.84	1.85

Midges (Chironomidae)

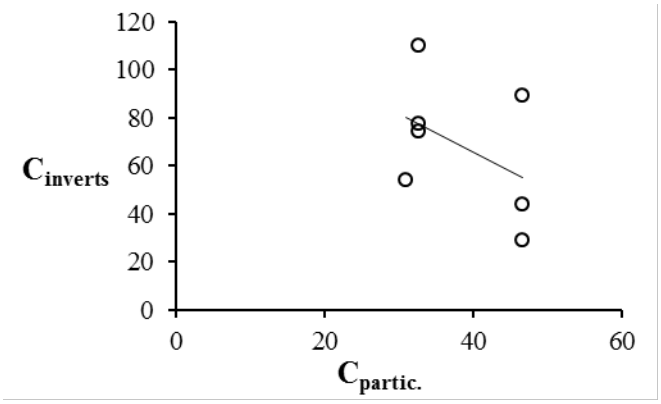


Median ratio: 1.90

R^2: 0.82
F: 144.0
df: 32
P: < 0.001

Beetles (Coleoptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	77.60	2.38
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	74.10	2.27
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	110.00	3.37
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	54.00	1.75
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	89.10	1.92
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	28.80	0.62
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	43.70	0.94



Median ratio: 1.92

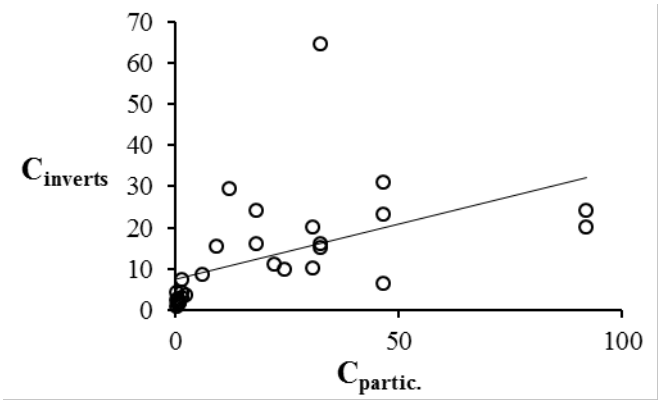
R^2: 0.20
F: 1.24
df: 5
P: 0.36
Not used because P > 0.05 and negative slope.

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	18	7.60		4.30	5.95	8.40	1.41
Birkner 1978	29	8.80		15.40	12.10	29.40	2.43

Water boatmen (Corixidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	4.20	2.82
Birkner 1978	3	0.10		0.30	0.20	4.20	21.00
Birkner 1978	22	4.60		44.00	24.30	9.90	0.41
Birkner 1978	12	2.30		0.30	1.30	7.30	5.62
Birkner 1978	23	7.80		10.80	9.30	15.50	1.67
Lambing et al. 1994	S46	2.30			2.30	3.40	1.48
Rinella et al. 1994	G	0.84		0.50	0.67	1.38	2.06
Rinella et al. 1994	A	2.21		0.40	1.31	2.98	2.28
Rinella et al. 1994	Q	1.42		0.50	0.96	2.00	2.08
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	24.00	1.32
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	16.00	0.88
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	20.00	0.22
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	24.00	0.26
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	2.15	5.17
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	0.87	2.10
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.76	1.98
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	1.53	1.72
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.90	0.49
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	64.60	1.98
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	15.10	0.46
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	20.00	0.65
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	10.00	0.32
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	23.00	0.49
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	30.90	0.66
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	6.46	0.14
Rinella and Schuler 1992	18	0.59			0.59	2.70	4.58



Median ratio: 1.48

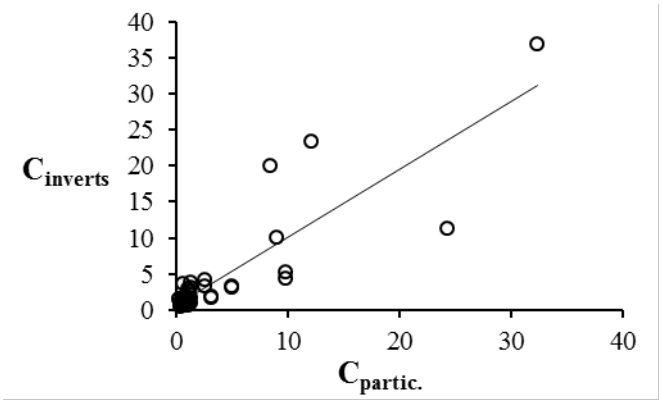
R^2: 0.25
F: 9.17
df: 27
P: < 0.001

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	23.30	1.93
Birkner 1978	19	16.80		1.20	9.00	10.10	1.12
Birkner 1978	30	17.30		47.30	32.30	36.80	1.14
Birkner 1978	22	4.60		44.00	24.30	11.30	0.47
Birkner 1978	27	10.35		6.50	8.43	20.00	2.37
Butler et al. 1993	SP2	1.60		0.50	1.05	2.60	2.48
Butler et al. 1993	SP2	1.60		0.50	1.05	2.90	2.76
Butler et al. 1995	AK	0.45		0.20	0.33	0.76	2.34
Butler et al. 1995	AK	0.45		0.20	0.33	0.79	2.43
Butler et al. 1995	DD	0.88		0.70	0.79	0.62	0.78
Butler et al. 1995	DD	0.88		0.70	0.79	1.10	1.39
Butler et al. 1995	HD1	0.59			0.59	0.86	1.46
Butler et al. 1995	HD1	0.59			0.59	0.79	1.34
Butler et al. 1995	HD2	0.45		0.20	0.32	0.96	2.98
Butler et al. 1995	HD2	0.45		0.20	0.32	1.00	3.10
Butler et al. 1995	ME2	1.11		1.10	1.10	1.10	1.00
Butler et al. 1995	ME2	1.11		1.10	1.10	1.40	1.27
Butler et al. 1995	ME4	1.04		0.50	0.77	1.30	1.69
Butler et al. 1995	ME4	1.04		0.50	0.77	1.80	2.35
Butler et al. 1995	ME3	0.82		0.40	0.61	1.40	2.30
Butler et al. 1995	ME3	0.82		0.40	0.61	3.70	6.07
Butler et al. 1995	NW	3.45		1.60	2.53	4.20	1.66
Butler et al. 1995	NW	3.45		1.60	2.53	3.30	1.31
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.40	6.83
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.50	7.32
Butler et al. 1997	CHK	1.19			1.19	0.90	0.76
Butler et al. 1997	MN2	0.79			0.79	0.83	1.06
Butler et al. 1997	MUD2	1.30			1.30	3.10	2.38
Butler et al. 1997	MUD2	1.30			1.30	3.80	2.92
Butler et al. 1997	TRH	1.25			1.25	0.98	0.78
Butler et al. 1997	TRH	1.25			1.25	1.60	1.28
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.67	0.62
Saiki et al. 1993	ET6	1.03	1.15		1.09	0.83	0.76
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.20	0.53
Saiki et al. 1993	GT5	4.50	14.95		9.73	4.40	0.45
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.10	0.63
Saiki et al. 1993	GT4	1.39	8.40		4.90	3.20	0.65
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.70	0.54
Saiki et al. 1993	SJR2	1.25	5.00		3.13	1.90	0.61

Crayfish (Astacidae)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	SJR3	0.45	1.25		0.85	0.77	0.91
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.30	1.53
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.50	1.39
Saiki et al. 1993	SJR1	0.22	0.50		0.36	0.74	2.06
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.87	1.91
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.85	1.87

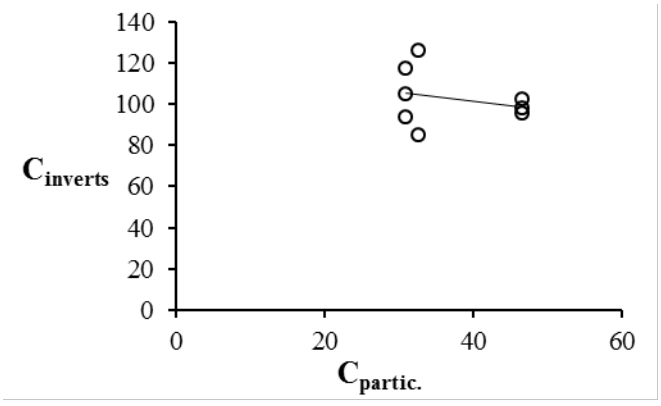


Median ratio: 1.46

R^2: 0.74
F: 130.8
df: 45
P: < 0.001

True flies (Diptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	126.00	3.87
Schuler et al. 1990	Kesterson Pond 11	53.70		11.50	32.60	85.10	2.61
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	117.00	3.79
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	93.30	3.02
Schuler et al. 1990	Kesterson Pond 2	52.50		9.30	30.90	105.00	3.40
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	95.50	2.05
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	97.70	2.10
Schuler et al. 1990	Kesterson Pond 7	87.10		5.90	46.50	102.00	2.19

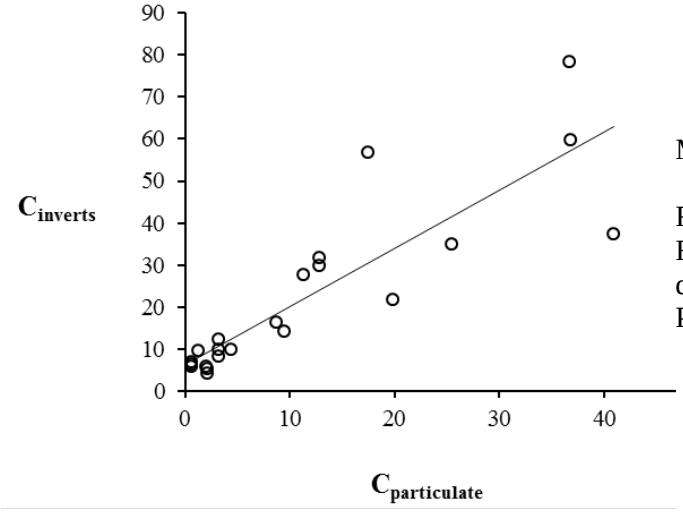


Median ratio: 2.81

R^2: 0.07
F: 0.46
df: 6
P: 0.65
Not used because P > 0.05 and negative slope.

Mayflies (Ephemeroptera)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Rinella et al. 1994	A	2.21		0.40	1.31	9.65	7.39
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.40	10.67
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	8.20	2.56
Casey 2005	Deerlick Creek		1.00	0.20	0.60	5.70	9.50
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	9.70	3.03
Casey 2005	Deerlick Creek		1.00	0.20	0.60	6.80	11.33
Casey 2005	Luscar Creek	5.50	3.20	2.40	3.20	12.30	3.84
Conley et al. 2009	Plate 10A	4.40			4.40	9.70	2.20
Conley et al. 2009	Plate 20A	25.50			25.50	34.80	1.36
Conley et al. 2009	Plate 20B	17.50			17.50	56.70	3.24
Conley et al. 2009	Plate 20C	8.70			8.70	16.20	1.86
Conley et al. 2009	Plate 20D	11.30			11.30	27.50	2.43
Conley et al. 2009	Plate 5A	2.20			2.20	4.20	1.91
Conley et al. 2009	Plate 5B	2.00			2.00	5.70	2.85
Conley et al. 2011	2x-High	40.90			40.90	37.30	0.91
Conley et al. 2011	2x-Low	9.50			9.50	14.10	1.48
Conley et al. 2011	2x-Medium	19.90			19.90	21.60	1.09
Conley et al. 2013	Control	2.20			2.20	5.10	2.32
Conley et al. 2013	Selenate-high	36.80			36.80	59.80	1.63
Conley et al. 2013	Selenate-low	12.80			12.80	31.70	2.48
Conley et al. 2013	Selenite-high	36.70			36.70	78.40	2.14
Conley et al. 2013	Selenite-low	12.80			12.80	29.80	2.33

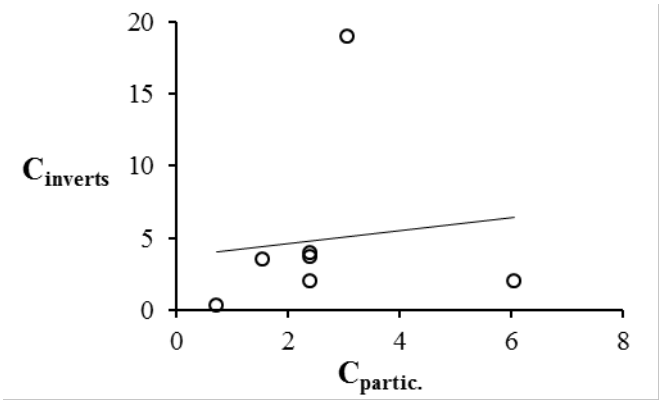


Median ratio: 2.38

R^2: 0.75
F: 59.19
df: 20
P: <0.001

Snails (Gastropoda)

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Butler et al. 1995	WC	3.30		1.50	2.40	3.70	1.54
Butler et al. 1995	WC	3.30		1.50	2.40	3.90	1.63
Butler et al. 1995	WC	3.30		1.50	2.40	2.00	0.83
Butler et al. 1997	DCP1	1.00		2.10	1.55	3.50	2.26
Butler et al. 1997	MNP2	5.40		6.70	6.05	2.00	0.33
Butler et al. 1997	CHP	4.00		2.10	3.05	19.00	6.23
Butler et al. 1997	LCHP1	0.33		1.10	0.72	0.32	0.45



Median ratio: 1.54

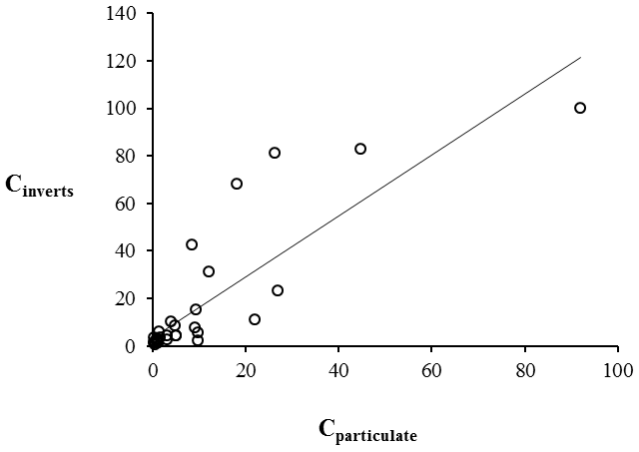
R^2: 0.01
F: 0.07
df: 5
P: 0.93
Not used because P > 0.05.

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Birkner 1978	29	8.80		15.40	12.10	31.30	2.59
Birkner 1978	20	3.00		41.00	22.00	11.00	0.50
Birkner 1978	7	0.18		2.80	1.49	3.30	2.22
Birkner 1978	19	16.80		1.20	9.00	7.70	0.86
Birkner 1978	3	0.10		0.30	0.20	3.40	17.00
Birkner 1978	27	10.35		6.50	8.43	42.50	5.04
Birkner 1978	12	2.30		0.30	1.30	5.80	4.46
Birkner 1978	23	7.80		10.80	9.30	15.40	1.66
Bowie et al. 1996	Hyco Reservoir	27.0			27.0	23.0	0.85
Lambing et al. 1988	12	1.40		0.30	0.85	2.60	3.06
Saiki and Lowe 1987	Kesterson Pond 11	18.15	47.95	8.56	18.15	68.30	3.76
Saiki and Lowe 1987	Kesterson Pond 2	152.70	44.65	34.82	44.65	83.00	1.86
Saiki and Lowe 1987	Kesterson Pond 8	136.50	92.00	6.05	92.00	100.00	1.09
Saiki and Lowe 1987	Volta Pond 26	0.42	1.01	0.29	0.42	1.46	3.51
Saiki and Lowe 1987	Volta Pond 7		1.39	0.39	0.89	2.90	3.26
Saiki and Lowe 1987	Volta Wasteway	0.87	2.03	0.24	0.87	2.80	3.21
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.20	1.10
Saiki et al. 1993	ET6	1.03	1.15		1.09	1.50	1.38

Zooplankton

Study	Site	C_{alg}	C_{det}	C_{sed}	C_{part}	C_{invert}	Ratio
Saiki et al. 1993	GT5	4.50	14.95		9.73	2.40	0.25
Saiki et al. 1993	GT5	4.50	14.95		9.73	5.40	0.56
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.50	0.92
Saiki et al. 1993	GT4	1.39	8.40		4.90	4.40	0.90
Saiki et al. 1993	SJR2	1.25	5.00		3.13	2.60	0.83
Saiki et al. 1993	SJR2	1.25	5.00		3.13	4.30	1.38
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.60	1.89
Saiki et al. 1993	SJR3	0.45	1.25		0.85	1.80	2.12
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.40	3.89
Saiki et al. 1993	SJR1	0.22	0.50		0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.63	1.38
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.40	3.08



Median ratio: 1.89

R^2: 0.71
F: 76.3
df: 31
P: < 0.001

Special case of Odonates (Damselflies and Dragonflies) consuming invertebrates

n = Number of invertebrate food species co-occurring with an Odonate species.

C_{part} = Average selenium concentration in particulate material (mg/kg): $\left(\frac{C_{alg}+C_{det}+C_{sed}}{3}\right)$

C_{food} = Median selenium concentration in all invertebrate tissues that co-occur with an Odonate species (mg/kg)

C_{damsel} = Selenium concentration in damselfly tissue (mg/kg)

C_{dragon} = Selenium concentration in dragonfly tissue (mg/kg)

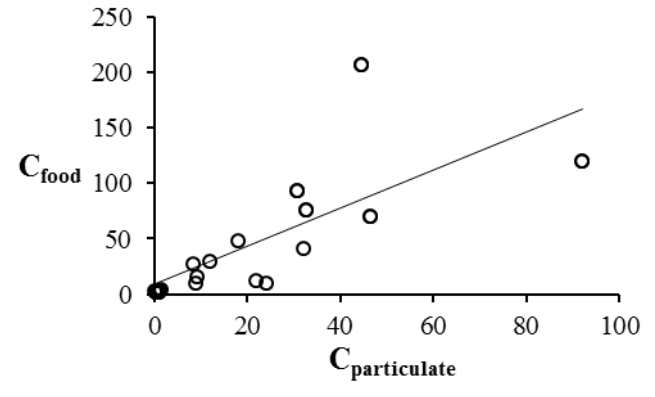
Ratio = $\frac{C_{food}}{C_{part}}$, $\frac{C_{damsel}}{C_{food}}$, or $\frac{C_{dragon}}{C_{food}}$

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Saiki and Lowe 1987	Kesterson Pond 11	dragonflies	4	18.15	47.5	2.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 2	dragonflies	4	44.65	206.5	4.62
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Kesterson Pond 8	dragonflies	5	92.00	120	1.30
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 26	dragonflies	4	0.42	1.52	3.65
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Pond 7	dragonflies	5	0.89	1.53	1.72
Saiki and Lowe 1987	Volta Wasteway	dragonflies	2	0.87	1.83	2.10
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 11	dragonflies	10	32.60	75.85	2.33
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02
Schuler et al. 1990	Kesterson Pond 2	dragonflies	8	30.90	93.3	3.02

Co-occurring potential food species of damselflies and dragonflies (Odonata)

Study	Site	Co-occurs with:	n	C_{part}	C_{food}	Ratio
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Schuler et al. 1990	Kesterson Pond 7	dragonflies	11	46.50	69.2	1.49
Birkner 1978	29	damselflies	3	12.10	29.4	2.43
Birkner 1978	20	damselflies	2	22.00	11.2	0.51
Birkner 1978	7	damselflies	2	1.49	3.55	2.39
Birkner 1978	19	damselflies	2	9.00	9.8	1.09
Birkner 1978	30	damselflies	2	32.30	40.9	1.27
Birkner 1978	3	damselflies	3	0.20	2.5	12.50
Birkner 1978	22	damselflies	3	24.30	9.9	0.41
Birkner 1978	27	damselflies	1	8.43	26.7	3.17
Birkner 1978	23	damselflies	3	9.30	15.5	1.67
Grasso et al. 1995	17	damselflies	1	1.14	2.07	1.82



Median ratio: 2.21

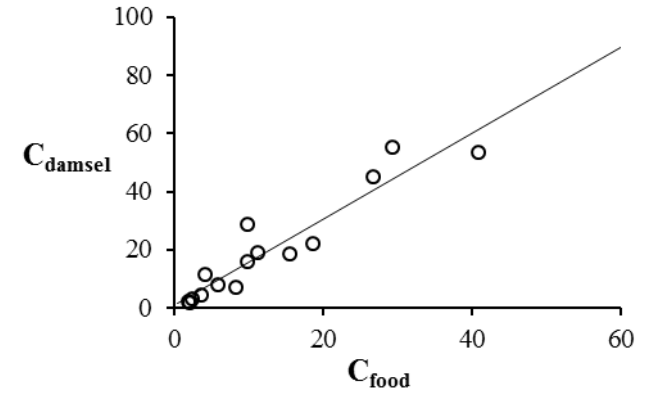
R^2: 0.54
F: 28.7
df: 24
P: < 0.001

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	29	29.4	55	1.87
Birkner 1978	4	1.95	1.8	0.92
Birkner 1978	25	18.7	21.9	1.17
Birkner 1978	20	11.2	18.7	1.67
Birkner 1978	7	3.55	4.4	1.24
Birkner 1978	19	9.8	28.4	2.90
Birkner 1978	6	4.2	11.1	2.64
Birkner 1978	30	40.9	53.3	1.30
Birkner 1978	3	2.5	3.1	1.24
Birkner 1978	22	9.9	15.8	1.60

Damselflies (Anisoptera)

Study	Site	C_{food}	C_{damsel}	Ratio
Birkner 1978	27	26.7	45.1	1.69
Birkner 1978	23	15.5	18.4	1.19
Birkner 1978	11	5.9	7.7	1.31
Grasso et al. 1995	17	2.07	1.75	0.85
Grasso et al. 1995	9	8.2	6.98	0.85



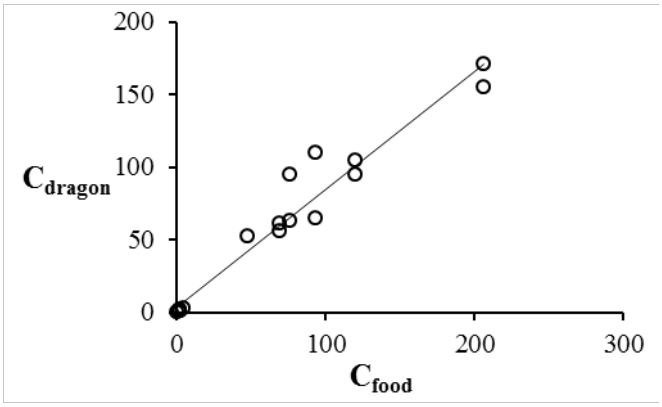
Median ratio: 1.30 x 2.21
(damselfly food to particulate) = 2.88

R^2: 0.89
F: 104.4
df: 13
P: <0.001

Dragonflies (Zygoptera)

Study	Site	C_{food}	C_{dragon}	Ratio
Mason et al. 2000	BK	1.845	1.665	0.90
Mason et al. 2000	HCRT	4.305	2.81	0.65
Saiki and Lowe 1987	Kesterson Pond 11	47.5	53	1.12
Saiki and Lowe 1987	Kesterson Pond 2	206.5	155	0.75
Saiki and Lowe 1987	Kesterson Pond 2	206.5	171	0.83
Saiki and Lowe 1987	Kesterson Pond 8	120	95.5	0.80
Saiki and Lowe 1987	Kesterson Pond 8	120	105	0.88
Saiki and Lowe 1987	Volta Pond 26	1.52	1.4	0.92
Saiki and Lowe 1987	Volta Pond 26	1.52	1.42	0.93
Saiki and Lowe 1987	Volta Pond 7	1.53	1.2	0.78
Saiki and Lowe 1987	Volta Pond 7	1.53	1.4	0.92
Saiki and Lowe 1987	Volta Wasteway	1.83	2.5	1.37
Schuler et al. 1990	Kesterson Pond 11	75.85	63.1	0.83
Schuler et al. 1990	Kesterson Pond 11	75.85	95.5	1.26
Schuler et al. 1990	Kesterson Pond 2	93.3	110	1.18
Schuler et al. 1990	Kesterson Pond 2	93.3	65	0.70
Schuler et al. 1990	Kesterson Pond 7	69.2	61.7	0.89
Schuler et al. 1990	Kesterson Pond 7	69.2	56.2	0.81
Sorenson & Schwarzbach 1991	5	0.42	0.49	1.17

Dragonflies (Zygoptera)



Median ratio: 0.89 x 2.21
(damselfly food to particulate) = 1.97

R^2: 0.95
F: 343.5
df: 17
P: <0.001

3.3.2 Vertebrates

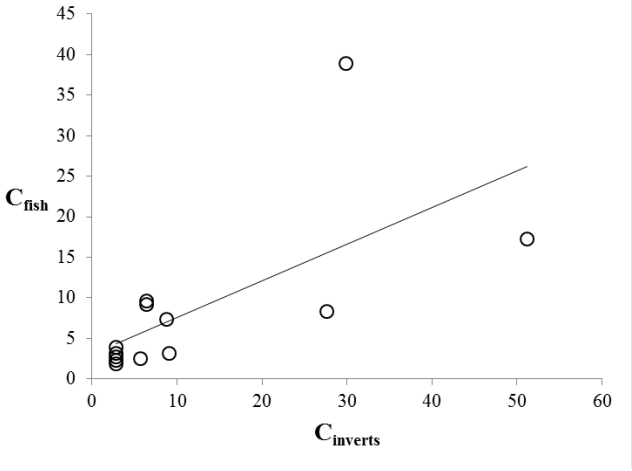
C_{invert} = Selenium concentration in invertebrate tissue (µg/g)
C_{fish} = Average selenium concentration in the whole-body of fish (µg/g)

Ratio = $\frac{C_{fish}}{C_{invert}}$

Black bullhead (*Ameiurus melas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	39.00	1.31
GEI 2013	SWA1	2.81	2.37	0.84
GEI 2013	SWA1	2.81	2.73	0.97
GEI 2013	SWA1	2.81	3.96	1.41
GEI 2013	SWA1	2.81	1.95	0.70
GEI 2013	SWA1	2.81	3.21	1.14
Mueller et al. 1991	R2	6.40	9.70	1.52
Mueller et al. 1991	R2	6.40	9.20	1.44
Mueller et al. 1991	R1	8.70	7.40	0.85
Lemly 1985	Badin Lake	5.70	2.58	0.45
Lemly 1985	Belews Lake	51.15	17.32	0.34
Lemly 1985	High Rock Lake	9.05	3.24	0.36
GEI 2014	SC-6	27.54	8.42	0.31

Black bullhead (*Ameiurus melas*)



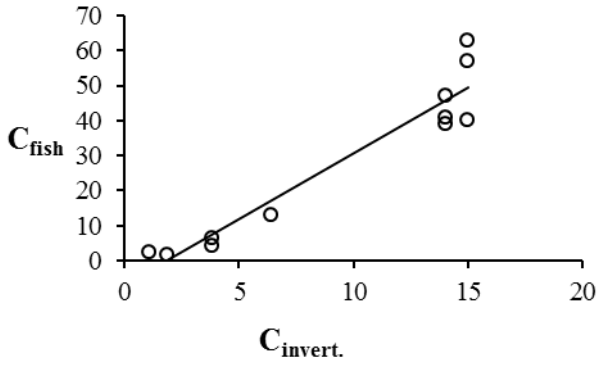
Median ratio: 0.85

R^2: 0.44
F: 8.52
df: 11
P: 0.006

Black crappie (*Pomoxis nigromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	Totten Reservoir	1.07	2.50	2.35
Butler et al. 1995	Summit Reservoir	1.85	1.70	0.92
Peterson et al. 1991	Ocean Lake, west side	3.83	4.20	1.10
Peterson et al. 1991	Ocean Lake, west side	3.83	6.32	1.65
Mueller et al. 1991	Lake Meredith near Ordway, CO	6.40	13.00	2.03
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	39.00	2.79
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	41.00	2.93
Lambing et al. 1994	Priest Butte Lakes near Choteau	14.00	47.00	3.36
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	40.00	2.67
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	57.00	3.80
Lambing et al. 1994	Priest Butte Lakes near Choteau	15.00	63.00	4.20

Black crappie (*Pomoxis nigromaculatus*)



Median ratio: 2.67

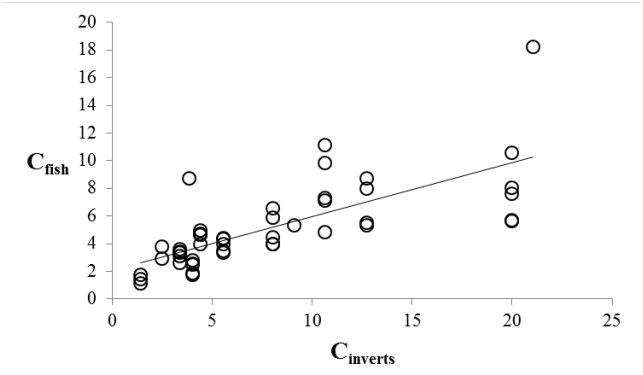
R^2: 0.92
F: 97.9
df: 9
P: < 0.001

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Cabin Creek, C-CC1	4.40	4.65	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	4.74	1.08
GEI 2014	Cabin Creek, C-CC1	4.40	4.95	1.12
GEI 2014	Cabin Creek, C-CC1	4.40	4.69	1.06
GEI 2014	Cabin Creek, C-CC1	4.40	3.98	0.90
GEI 2014	Cabin Creek, C-CC2	5.56	3.46	0.62
GEI 2014	Cabin Creek, C-CC2	5.56	3.38	0.61
GEI 2014	Cabin Creek, C-CC2	5.56	3.95	0.71
GEI 2014	Cabin Creek, C-CC2	5.56	4.36	0.78
GEI 2014	Cabin Creek, C-CC2	5.56	4.39	0.79
GEI 2014	Coal Fork, C-CF1	3.39	3.58	1.06
GEI 2014	Coal Fork, C-CF1	3.39	3.09	0.91
GEI 2014	Coal Fork, C-CF1	3.39	3.37	0.99
GEI 2014	Coal Fork, C-CF1	3.39	2.64	0.78
GEI 2014	Coal Fork, C-CF1	3.39	3.42	1.01
GEI 2014	Hazy Creek, C-HC1	8.03	3.99	0.50
GEI 2014	Hazy Creek, C-HC1	8.03	5.88	0.73
GEI 2014	Hazy Creek, C-HC1	8.03	4.46	0.56
GEI 2014	Hazy Creek, C-HC1	8.03	6.55	0.82
GEI 2014	Hazy Creek, C-HC1	8.03	3.98	0.50
GEI 2014	Laurel Fork, C-LF1	12.73	5.36	0.42
GEI 2014	Laurel Fork, C-LF1	12.73	7.99	0.63
GEI 2014	Laurel Fork, C-LF1	12.73	8.72	0.68
GEI 2014	Laurel Fork, C-LF1	12.73	5.49	0.43
GEI 2014	Tenmile Fork, C-TF1	20.00	7.62	0.38
GEI 2014	Tenmile Fork, C-TF1	20.00	10.56	0.53

Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Tenmile Fork, C-TF1	20.00	8.02	0.40
GEI 2014	Tenmile Fork, C-TF1	20.00	5.63	0.28
GEI 2014	Tenmile Fork, C-TF1	20.00	5.68	0.28
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.81	0.70
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.86	0.46
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.78	0.44
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.47	0.61
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.55	0.63
GEI 2014	Lukey Fork, H-LF1	9.09	5.32	0.59
GEI 2014	Mud River, H-MR3	3.86	8.72	2.26
GEI 2014	Mud River, H-MR6	2.49	3.80	1.53
GEI 2014	Mud River, H-MR6	2.49	2.93	1.18
GEI 2014	Sugartree Branch, H-SB1	10.62	9.82	0.92
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	11.14	1.05
GEI 2014	Sugartree Branch, H-SB1	10.62	4.85	0.46
GEI 2014	Sugartree Branch, H-SB1	10.62	7.16	0.67
GEI 2014	Stanley Fork, H-SF1	21.05	18.21	0.87



Median ratio: 0.71

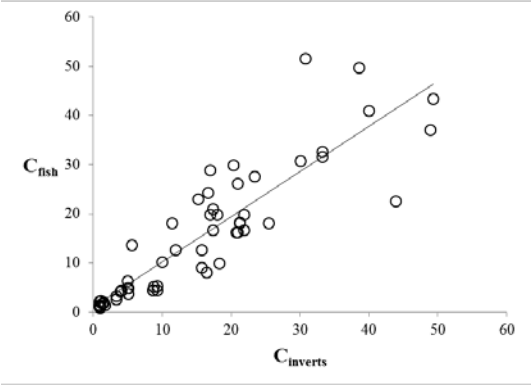
R^2: 0.52
F: 48.97
df: 45
P: < 0.001

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.30	2.16
Hermanutz et al. 1996	MSO II	16.63	24.29	1.46
Hermanutz et al. 1996	MSO III	5.55	13.77	2.48
Hermanutz et al. 1996	MSO I	21.19	18.28	0.86
Hermanutz et al. 1996	MSO I	21.19	18.13	0.86
Hermanutz et al. 1996	MSO II	17.30	20.99	1.21
Hermanutz et al. 1996	MSO II	5.05	4.88	0.97
Hermanutz et al. 1996	MSO I	0.87	1.55	1.78
Hermanutz et al. 1996	MSO II	1.70	1.55	0.91
Hermanutz et al. 1996	MSO III	1.20	1.83	1.52
Hermanutz et al. 1996	MSO III	10.00	10.32	1.03
Hermanutz et al. 1996	MSO III	3.95	4.21	1.06
Hermanutz et al. 1996	MSO II	17.30	16.76	0.97
Hermanutz et al. 1996	MSO II	5.05	3.86	0.76
Mueller et al. 1991	R1	8.70	5.20	0.60
Saiki et al. 1993	ET6	0.85	2.20	2.60
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.40	1.31
Saiki et al. 1993	GT5	4.90	5.00	1.02
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.30	1.06
Saiki et al. 1993	SJR2	3.30	3.30	1.00
Saiki et al. 1993	SJR2	3.30	2.70	0.82
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR3	1.50	1.90	1.27
Saiki et al. 1993	SJR1	0.95	0.87	0.92
Saiki et al. 1993	SJR1	0.95	1.40	1.48
Saiki et al. 1993	ET7	0.86	1.20	1.40
Saiki et al. 1993	ET7	0.86	1.20	1.40
Crutchfield 2000	transect 3	21.80	19.91	0.91
Crutchfield 2000	transect 3	21.80	16.72	0.77
Crutchfield 2000	transect 3	17.90	19.91	1.11
Crutchfield 2000	transect 3	20.70	16.26	0.79
Crutchfield 2000	transect 3	20.35	29.87	1.47
Crutchfield 2000	transect 3	23.40	27.59	1.18
Crutchfield 2000	transect 3	15.20	23.10	1.52
Crutchfield 2000	transect 3	16.95	28.96	1.71
Crutchfield 2000	transect 3	16.95	19.91	1.17
Crutchfield 2000	transect 3	11.95	12.69	1.06
Crutchfield 2000	transect 3	11.40	18.09	1.59
Crutchfield 2000	transect 3	9.25	4.56	0.49
Crutchfield 2000	transect 3	9.25	5.40	0.58

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 3	8.60	4.56	0.53
Crutchfield 2000	transect 4	30.70	51.60	1.68
Crutchfield 2000	transect 4	30.00	30.78	1.03
Crutchfield 2000	transect 4	33.20	31.69	0.95
Crutchfield 2000	transect 4	48.90	37.09	0.76
Crutchfield 2000	transect 4	38.55	49.78	1.29
Crutchfield 2000	transect 4	49.30	43.40	0.88
Crutchfield 2000	transect 4	43.90	22.65	0.52
Crutchfield 2000	transect 4	33.25	32.60	0.98
Crutchfield 2000	transect 4	25.40	18.09	0.71
Crutchfield 2000	transect 4	20.90	16.26	0.78
Crutchfield 2000	transect 4	20.90	26.22	1.25
Crutchfield 2000	transect 4	15.70	12.69	0.81
Crutchfield 2000	transect 4	15.70	9.04	0.58
Crutchfield 2000	transect 4	16.45	8.13	0.49
Crutchfield 2000	transect 4	18.25	9.96	0.55
Bowie et al. 1996	Hyco Reservoir	40.00	41.00	1.03



Median ratio: 1.03

R^2: 0.80
F: 226.0
df: 58
P: < 0.001

Bluehead sucker (*Catostomus discobolus*)

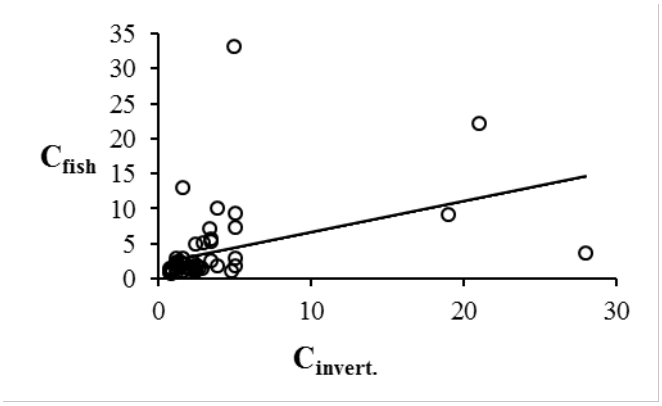
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	AK	0.78	0.94	1.21
Butler et al. 1995	HD1	0.83	0.83	1.01
Butler et al. 1995	HD1	0.83	0.86	1.04
Butler et al. 1995	HD1	0.83	1.20	1.45
Butler et al. 1995	HD1	0.83	1.40	1.70
Butler et al. 1995	DD	0.86	0.64	0.74
Butler et al. 1995	DD	0.86	0.88	1.02
Butler et al. 1995	DD	0.86	1.30	1.51

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	D1	1.20	2.80	2.33
Butler et al. 1993	B1	1.25	1.90	1.52
Butler et al. 1993	B1	1.25	2.20	1.76
Butler et al. 1995	ME2	1.25	0.83	0.66
Butler et al. 1995	ME2	1.25	1.30	1.04
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	SD	1.40	1.50	1.07
Butler et al. 1995	SD	1.40	1.80	1.29
Butler et al. 1993	D2	1.45	1.60	1.10
Butler et al. 1993	D2	1.45	2.30	1.59
Butler et al. 1993	P1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	RB3	1.60	13.00	8.13
Butler et al. 1995	YJ2	1.65	0.96	0.58
Butler et al. 1995	YJ2	1.65	2.80	1.70
Butler et al. 1994	NFK3	2.00	1.40	0.70
Butler et al. 1997	MN2	2.20	1.20	0.55
Butler et al. 1997	MUD	2.30	1.80	0.78
Butler et al. 1997	MUD	2.30	2.30	1.00
Butler et al. 1997	CHK	2.40	1.20	0.50
Butler et al. 1997	CHK	2.40	1.60	0.67
Butler et al. 1993	U1	2.45	4.80	1.96
Butler et al. 1995	SJ1	2.50	0.94	0.38
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	SJ1	2.50	1.20	0.48
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.80	0.71
Butler et al. 1997	MN3	2.70	1.50	0.56
Butler et al. 1997	MN1	2.90	1.40	0.48
Butler et al. 1993	SP1	2.95	5.10	1.73
Butler et al. 1993	SP2	3.40	7.10	2.09
Butler et al. 1997	MUD2	3.45	2.50	0.72
Butler et al. 1997	MUD2	3.45	5.20	1.51
Butler et al. 1997	MUD2	3.45	5.60	1.62
Butler et al. 1993	F2	3.90	10.00	2.56
Butler et al. 1991	4	3.90	1.80	0.46
Butler et al. 1993	F2	4.80	0.94	0.20
Butler et al. 1994	BSW1	5.00	33.00	6.60
Butler et al. 1997	WBR	5.05	1.80	0.36
Butler et al. 1997	WBR	5.05	2.80	0.55
Butler et al. 1995	NW	5.10	7.20	1.41
Butler et al. 1995	NW	5.10	9.30	1.82

Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	LZA1	19.00	9.00	0.47
Butler et al. 1994	RB1	21.00	22.00	1.05
Butler et al. 1994	GUN2	28.00	3.60	0.13



Median ratio: 1.04

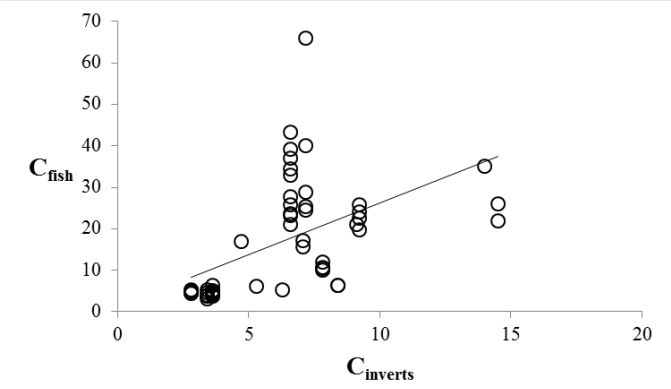
R^2: 0.16
F: 9.6
df: 51
P: < 0.001

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWA1	2.81	4.40	1.57
GEI 2013	SWA1	2.81	4.59	1.64
GEI 2013	SWA1	2.81	4.66	1.66
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.21	1.86
GEI 2013	SWA1	3.64	3.69	1.02
GEI 2013	SWA1	3.64	4.16	1.14
GEI 2013	SWA1	3.64	4.21	1.16
GEI 2013	SWA1	3.64	4.62	1.27
GEI 2013	SWA1	3.64	4.78	1.31
GEI 2013	SWA1	3.64	4.98	1.37
GEI 2013	SWA1	3.64	5.06	1.39
GEI 2013	SWA1	3.64	6.28	1.73
Lambing et al. 1994	S38	4.70	17.00	3.62
Lambing et al. 1994	S37	5.30	6.10	1.15
Lambing et al. 1994	S36	6.30	5.30	0.84
GEI 2014	Dry Creek, DC-2	3	3.14	0.92
GEI 2014	Dry Creek, DC-2	3	4.03	1.18
GEI 2014	Dry Creek, DC-2	3	3.76	1.10
GEI 2014	Dry Creek, DC-2	3	5.31	1.55
GEI 2014	Dry Creek, DC-2	3	4.59	1.34
GEI 2014	Dry Creek, DC-3	7	28.89	4.02
GEI 2014	Dry Creek, DC-3	7	66.07	9.20

Brook stickleback (*Culaea inconstans*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7	24.43	3.40
GEI 2014	Dry Creek, DC-3	7	25.36	3.53
GEI 2014	Dry Creek, DC-3	7	40.17	5.59
GEI 2014	Dry Creek, DC-3	9	25.80	2.80
GEI 2014	Dry Creek, DC-3	9	24.14	2.62
GEI 2014	Dry Creek, DC-3	9	22.46	2.43
GEI 2014	Dry Creek, DC-3	9	19.86	2.15
GEI 2013	SW2-1	6.60	21.14	3.21
GEI 2013	SW2-1	6.60	23.21	3.52
GEI 2013	SW2-1	6.60	23.64	3.58
GEI 2013	SW2-1	6.60	25.89	3.93
GEI 2013	SW2-1	6.60	27.71	4.20
GEI 2013	SW2-1	6.60	32.97	5.00
GEI 2013	SW2-1	6.60	34.54	5.24
GEI 2013	SW2-1	6.60	37.05	5.62
GEI 2013	SW2-1	6.60	39.26	5.95
GEI 2013	SW2-1	6.60	43.38	6.58
GEI 2013	SWB	7.06	15.74	2.23
GEI 2013	SWB	7.06	17.15	2.43
GEI 2013	SW1	7.82	9.96	1.27
GEI 2013	SW1	7.82	10.38	1.33
GEI 2013	SW1	7.82	10.58	1.35
GEI 2013	SW1	7.82	11.98	1.53
GEI 2013	SW11	8.41	6.36	0.76
GEI 2013	SW11	8.41	6.45	0.77
GEI 2013	SW2-1	9.14	21.09	2.31
Lambing et al. 1994	S34	14.00	35.00	2.50
Lambing et al. 1994	S11	14.50	22.00	1.52
Lambing et al. 1994	S11	14.50	26.00	1.79

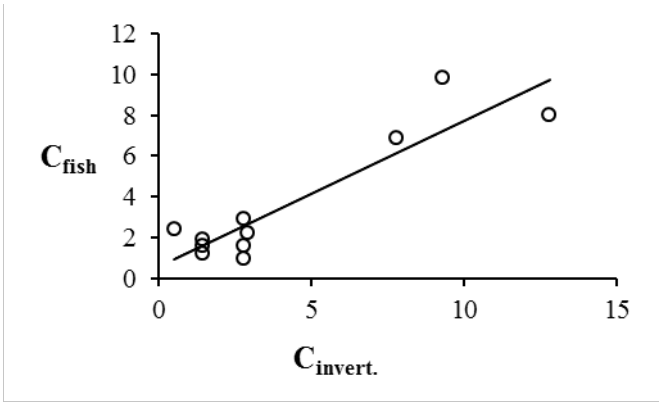


Median ratio: 1.79

R^2: 0.27
F: 18.48
df: 50
P: < 0.001

Brook trout (*Salvelinus fontinalis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	2.40	4.80
Mason et al. 2000	BK	1.43	1.21	0.84
Mason et al. 2000	BK	1.43	1.57	1.10
Mason et al. 2000	BK	1.43	1.90	1.33
Mason et al. 2000	HCRT	2.81	0.99	0.35
Mason et al. 2000	HCRT	2.81	1.59	0.57
Mason et al. 2000	HCRT	2.81	2.95	1.05
Butler et al. 1997	MN1	2.90	2.20	0.76
Hamilton and Buhl 2005	LGC	7.80	6.90	0.88
Hamilton and Buhl 2005	UGC	9.30	9.80	1.05
Hamilton and Buhl 2004	DVC	12.80	8.00	0.63
Hamilton and Buhl 2004	USC	0.50	2.40	4.80

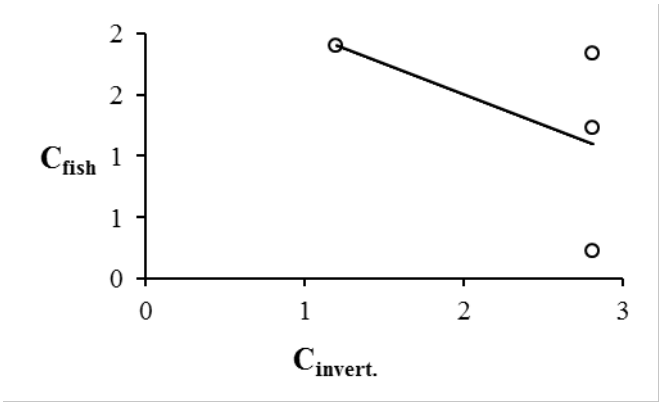


Median ratio: 0.88

R^2: 0.83
F: 43.6
df: 9
P: < 0.001

Brown bullhead (*Ameiurus nebulosus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Rinella and Schuler 1992		1.20	1.90	1.58
Mason et al. 2000	HCRT	2.81	0.22	0.08
Mason et al. 2000	HCRT	2.81	1.23	0.44
Mason et al. 2000	HCRT	2.81	1.83	0.65



Median ratio: 0.55

R^2: 0.27
F: 0.73
df: 2
P: 0.58
Not used because P > 0.05 and negative slope

Brown trout (*Salmo trutta*)

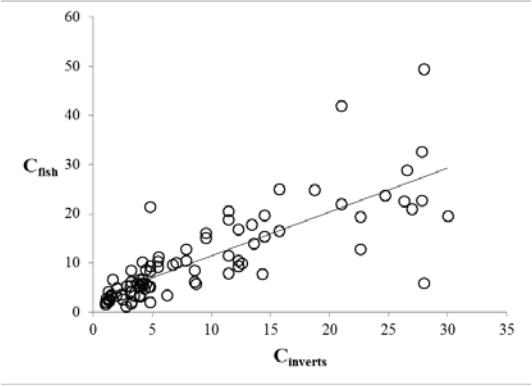
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.00	0.42
Butler et al. 1991	12	2.80	5.40	1.93
Butler et al. 1991	4	3.90	3.30	0.85
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1991	3	6.20	3.50	0.56
Butler et al. 1993	SP2	3.40	3.40	1.00
Butler et al. 1993	SP2	2.75	1.20	0.44
Butler et al. 1993	B2	1.35	2.40	1.78
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B2	1.35	2.70	2.00
Butler et al. 1993	B1	1.25	4.20	3.36
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.20	2.21
Butler et al. 1993	P1	1.95	3.30	1.69
Butler et al. 1993	LP2	1.00	1.70	1.70
Butler et al. 1993	LP2	1.00	2.10	2.10
Butler et al. 1993	LP2	1.00	1.60	1.60
Butler et al. 1993	LP3	1.12	2.10	1.88
Butler et al. 1993	LP3	1.12	2.80	2.51
Butler et al. 1993	LP4	3.20	1.80	0.56

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1993	R2	3.90	6.70	1.72
Butler et al. 1993	R2	3.70	5.90	1.59
Butler et al. 1993	ST2	4.10	6.00	1.46
Butler et al. 1994	GUN2	28.00	49.45	1.77
Butler et al. 1994	GUN2	28.00	5.90	0.21
Butler et al. 1994	HCC1	21.00	21.98	1.05
Butler et al. 1994	HCC1	21.00	42.00	2.00
Butler et al. 1994	NFK3	2.00	5.00	2.50
Butler et al. 1994	SMF	4.80	21.44	4.47
Butler et al. 1994	SMF	4.80	5.26	1.10
Butler et al. 1994	SMF	4.80	8.40	1.75
Butler et al. 1994	SMF	4.80	9.40	1.96
Formation 2012	CC-1A	12.24	10.51	0.86
Formation 2012	CC-1A	12.24	9.33	0.76
Formation 2012	CC-1A	12.57	9.95	0.79
Formation 2012	CC-1A	12.24	16.85	1.38
Formation 2012	CC-1A	13.55	14.03	1.04
Formation 2012	CC-3A	5.45	10.44	1.92
Formation 2012	CC-3A	5.45	9.20	1.69
Formation 2012	CC-3A	5.48	11.25	2.05
Formation 2012	CC-3A	14.50	15.38	1.06
Formation 2012	CC-3A	14.50	19.68	1.36
Formation 2012	CC-150	4.46	5.83	1.31
Formation 2012	CC-150	4.46	8.67	1.94
Formation 2012	CC-150	4.70	5.20	1.11
Formation 2012	CC-150	7.03	10.14	1.44
Formation 2012	CC-150	14.32	7.83	0.55
Formation 2012	CC-350	3.16	6.28	1.99
Formation 2012	CC-350	3.16	8.53	2.70
Formation 2012	CC-350	4.20	5.78	1.38
Formation 2012	CC-350	11.45	11.50	1.00
Formation 2012	CC-350	11.45	7.95	0.69
Formation 2012	CC-75	3.11	4.05	1.30
Formation 2012	CC-75	3.11	5.35	1.72
Formation 2012	CC-75	3.97	3.18	0.80
Formation 2012	CC-75	4.16	10.32	2.48
Formation 2012	CC-75	4.16	6.60	1.59
Formation 2012	DC-600	8.53	8.54	1.00
Formation 2012	DC-600	8.53	6.20	0.73
Formation 2012	DC-600	8.65	5.85	0.68
Formation 2012	DC-600	7.83	12.83	1.64

Brown trout (*Salmo trutta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	7.83	10.54	1.35
Formation 2012	HS	15.70	16.52	1.05
Formation 2012	HS	15.70	25.00	1.59
Formation 2012	HS	18.70	24.90	1.33
Formation 2012	HS	27.80	32.63	1.17
Formation 2012	HS	27.80	22.80	0.82
Formation 2012	HS-3	11.40	20.60	1.81
Formation 2012	HS-3	11.40	18.83	1.65
Formation 2012	HS-3	13.41	17.89	1.33
Formation 2012	HS-3	24.70	23.68	0.96
Formation 2012	HS-3	26.55	28.97	1.09
Formation 2012	LSV-2C	22.62	19.45	0.86
Formation 2012	LSV-2C	22.62	12.78	0.56
Formation 2012	LSV-2C	26.31	22.67	0.86
Formation 2012	LSV-2C	30.00	19.53	0.65
Formation 2012	LSV-2C	26.95	20.96	0.78
Formation 2012	LSV-4	9.54	16.20	1.70
Formation 2012	LSV-4	9.54	15.18	1.59
Formation 2012	SFTC-1	2.42	3.68	1.52
Formation 2012	SFTC-1	3.21	2.25	0.70
Formation 2012	SFTC-1	1.63	6.70	4.11
Formation 2012	SFTC-1	2.49	2.64	1.06
Hamilton and Buhl 2005	CC	6.70	9.70	1.45
McDonald and Strosher 1998	ER 747	4.29	4.80	1.12

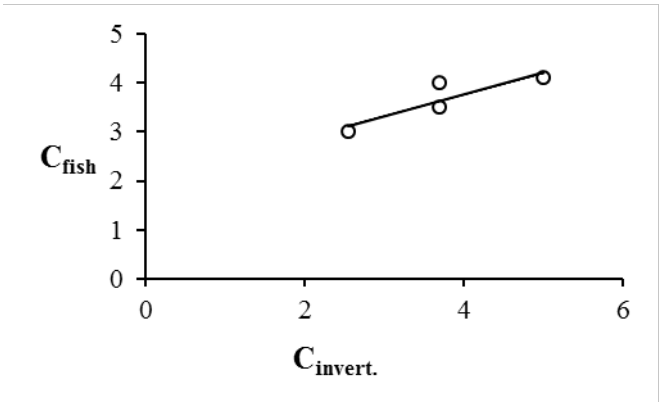


Median ratio: 1.38

R^2: 0.64
F: 151.8
df: 85
P: < 0.001

Bullhead (*Ameiurus sp.*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME3	2.55	3.00	1.18
Butler et al. 1993	R2	3.70	3.50	0.95
Butler et al. 1993	R2	3.70	4.00	1.08
Butler et al. 1994	BSW1	5.00	4.10	0.82



Median ratio: 1.01

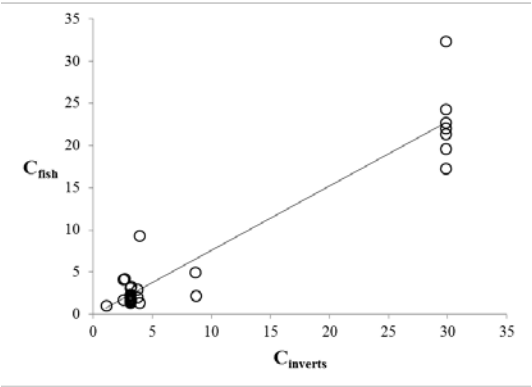
R^2: 0.77
F: 6.58
df: 2
P: 0.13
Not used because P > 0.05

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	7	29.80	21.36	0.72
Butler et al. 1991	7	29.80	22.05	0.74
Butler et al. 1991	7	29.80	17.27	0.58
Butler et al. 1991	7	29.80	19.62	0.66
Butler et al. 1991	7	29.80	22.76	0.76
Butler et al. 1991	7	29.80	24.33	0.82
Butler et al. 1991	7	29.80	32.40	1.09
Butler et al. 1993	LP4	3.20	1.65	0.52
Butler et al. 1993	LP4	3.20	3.30	1.03
Butler et al. 1993	R2	3.90	9.30	2.39
Butler et al. 1993	R2	3.90	1.33	0.34
Butler et al. 1993	R2	3.70	2.04	0.55
Butler et al. 1993	R2	3.70	3.00	0.81
Butler et al. 1995	SJ1	2.50	1.73	0.69
Butler et al. 1995	SJ1	2.50	4.10	1.64
Butler et al. 1995	TT	1.07	1.00	0.94
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	5.00	0.58
Mueller et al. 1991	R1	8.70	2.20	0.25
Roddy et al. 1991	18	3.10	1.40	0.45
Roddy et al. 1991	18	3.10	1.60	0.52
Roddy et al. 1991	18	3.10	1.70	0.55

Channel catfish (*Ictalurus punctatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	1.90	0.61
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	1.50	0.48
Roddy et al. 1991	18	3.10	1.70	0.55
Roddy et al. 1991	18	3.10	1.80	0.58
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	3.10	1.00



Median ratio: 0.74

R^2: 0.91
F: 332.8
df: 32
P: < 0.001

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	10.30	2.15
Butler et al. 1991	7	29.80	25.80	0.87
Butler et al. 1991	7	29.80	31.00	1.04
Butler et al. 1991	7	29.80	40.00	1.34
Butler et al. 1991	7	29.80	50.00	1.68
Butler et al. 1991	9	4.10	3.90	0.95
Butler et al. 1991	3	6.20	2.20	0.35
Butler et al. 1993	D2	1.45	3.70	2.55
Butler et al. 1993	F2	7.50	5.80	0.77
Butler et al. 1993	R2	4.30	5.00	1.16
Butler et al. 1993	R2	3.90	4.80	1.23
Butler et al. 1993	R2	3.70	3.30	0.89
Butler et al. 1994	GUN2	28.00	63.00	2.25
Butler et al. 1994	NFK2	3.10	4.90	1.58

Common carp (*Cyprinus carpio*)

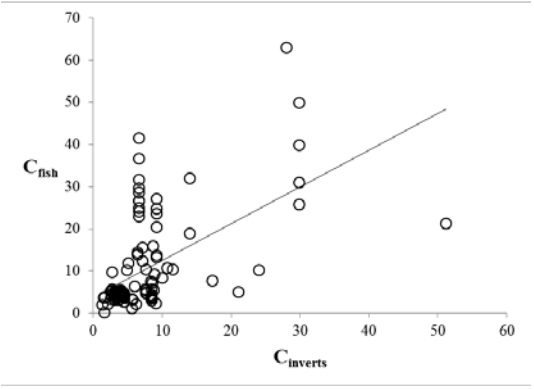
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	BSW1	5.00	12.00	2.40
Butler et al. 1994	RB1	21.00	5.10	0.24
Butler et al. 1995	ME4	1.55	3.90	2.52
Butler et al. 1995	ME4	1.55	3.70	2.39
Butler et al. 1995	ME4	1.55	3.80	2.45
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	ME3	2.55	5.20	2.04
Butler et al. 1995	SJ1	2.50	5.30	2.12
Butler et al. 1995	SJ1	2.50	3.40	1.36
Butler et al. 1995	MN1	2.70	5.80	2.15
Butler et al. 1995	MN1	2.70	9.80	3.63
Butler et al. 1995	MN1	2.70	5.40	2.00
Butler et al. 1997	MN5	8.60	16.00	1.86
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	3.30	1.10
GEI 2013	SWB	7.06	12.50	1.77
GEI 2013	SWB	7.06	15.61	2.21
GEI 2013	SW11	8.41	3.14	0.37
GEI 2013	SW11	8.41	3.52	0.42
GEI 2013	SW11	8.41	3.66	0.44
GEI 2013	SW11	8.41	3.85	0.46
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	3.60	0.43
GEI 2013	SW11	8.41	3.79	0.45
GEI 2013	SW11	8.41	3.95	0.47
GEI 2013	SW11	8.41	4.14	0.49
GEI 2013	SW11	8.41	4.34	0.52
GEI 2013	SW11	8.41	3.56	0.42
GEI 2013	SW2-1	6.60	26.73	4.05
GEI 2013	SW2-1	6.60	26.74	4.05
GEI 2013	SW2-1	6.60	28.74	4.36
GEI 2013	SW2-1	6.60	29.73	4.51
GEI 2013	SW2-1	6.60	41.57	6.30
GEI 2013	SW2-1	6.60	22.96	3.48
GEI 2013	SW2-1	6.60	24.27	3.68
GEI 2013	SW2-1	6.60	25.09	3.80
GEI 2013	SW2-1	6.60	31.74	4.81
GEI 2013	SW2-1	6.60	36.81	5.58
GEI 2013	SW2-1	9.14	13.29	1.45
GEI 2013	SW2-1	9.14	13.77	1.51
GEI 2013	SW2-1	9.14	20.49	2.24
GEI 2013	SW2-1	9.14	24.84	2.72

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW2-1	9.14	23.65	2.59
GEI 2013	SW2-1	9.14	27.27	2.99
GEI 2013	SW4-1	3.33	3.55	1.07
GEI 2013	SW4-1	3.33	4.68	1.41
GEI 2013	SW4-1	3.33	3.91	1.18
GEI 2013	SW4-1	3.33	4.36	1.31
GEI 2013	SW4-1	3.33	4.48	1.35
GEI 2013	SW4-1	3.33	4.60	1.38
GEI 2013	SW4-1	3.33	4.78	1.44
GEI 2013	SW9	4.45	2.73	0.61
GEI 2013	SW9	4.45	2.99	0.67
GEI 2013	SW9	4.45	3.64	0.82
GEI 2013	SW9	4.45	3.80	0.85
GEI 2013	SW9	4.45	3.90	0.88
GEI 2013	SW9	4.45	4.26	0.96
GEI 2013	SW9	4.45	4.53	1.02
GEI 2013	SW9	4.45	3.70	0.83
GEI 2013	SW9	4.45	3.77	0.85
GEI 2013	SW9	4.45	4.14	0.93
GEI 2013	SW9	4.45	4.41	0.99
GEI 2013	SW9	4.45	4.50	1.01
GEI 2013	SW9	4.45	4.69	1.05
GEI 2013	SW88	3.96	3.88	0.98
GEI 2013	SW88	3.96	5.33	1.35
GEI 2013	SW88	3.96	5.49	1.39
GEI 2013	SW88	3.96	5.66	1.43
Grasso et al. 1995	9	7.59	4.70	0.62
Grasso et al. 1995	9	7.59	4.93	0.65
Grasso et al. 1995	9	7.59	5.51	0.73
Lambing et al. 1994	S34	14.00	19.00	1.36
Lambing et al. 1994	S34	14.00	32.00	2.29
Low and Mullins 1990	5	5.60	1.20	0.21
Low and Mullins 1990	7	1.60	0.30	0.19
May et al. 2008	KR	17.20	7.78	0.45
May et al. 2008	NSCL	10.70	10.80	1.01
May et al. 2008	NSK	8.81	9.33	1.06
May et al. 2008	NSP	24.00	10.30	0.43
May et al. 2008	SSAL	11.50	10.50	0.91
May et al. 2008	SSAU	8.35	7.59	0.91
May et al. 2008	SSO	10.00	8.48	0.85
May et al. 2008	SSW	7.60	10.40	1.37
Mueller et al. 1991	R2	6.40	14.40	2.25

Common carp (*Cyprinus carpio*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.00	2.19
Mueller et al. 1991	R1	8.70	5.60	0.64
Mueller et al. 1991	A3	6.00	6.50	1.08
Mueller et al. 1991	A6	5.60	3.40	0.61
Mueller et al. 1991	A2	8.50	7.30	0.86
Peterson et al. 1991	7	3.83	4.24	1.11
Peterson et al. 1991	7	3.83	4.41	1.15
Peterson et al. 1991	7	3.83	4.73	1.23
Peterson et al. 1991	7	3.83	5.16	1.35
Peterson et al. 1991	7	3.83	5.21	1.36
Roddy et al. 1991	18	3.10	3.20	1.03
Roddy et al. 1991	18	3.10	3.90	1.26
Roddy et al. 1991	18	3.10	4.60	1.48
Roddy et al. 1991	18	3.10	4.70	1.52
Roddy et al. 1991	18	3.10	4.80	1.55
Roddy et al. 1991	18	3.10	5.30	1.71
Lemly 1985	Badin Lake	5.70	3.17	0.56
Lemly 1985	Belews Lake	51.15	21.29	0.42
Lemly 1985	High Rock Lake	9.05	2.45	0.27
Rinella and Schuler 1992	Harney Lake	2.05	2.20	1.07
Rinella and Schuler 1992	S. Malheur Lake	1.20	2.00	1.67



Median ratio: 1.20

R^2: 0.32
F: 54.27
df: 116
P: < 0.001

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	HCRT	2.81	0.49	0.18
Mason et al. 2000	HCRT	2.81	1.18	0.42
Mason et al. 2000	HCRT	2.81	1.97	0.70
GEI 2013	SW4-1	3.33	4.65	1.40
GEI 2013	SW4-1	3.33	4.96	1.49

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW4-1	3.33	5.52	1.66
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.31	1.90
GEI 2013	SW4-1	3.33	6.53	1.96
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	LG1	3.37	3.41	1.01
GEI 2013	LG1	3.37	3.58	1.06
GEI 2013	LG1	3.37	3.75	1.11
GEI 2013	LG1	3.37	3.78	1.12
GEI 2013	LG1	3.37	4.10	1.22
GEI 2013	LG1	3.39	3.23	0.95
GEI 2013	LG1	3.39	3.72	1.10
GEI 2013	LG1	3.39	3.74	1.10
GEI 2013	LG1	3.39	3.78	1.12
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.03	1.19
GEI 2013	LG1	3.39	4.12	1.22
GEI 2013	LG1	3.39	5.11	1.51
GEI 2013	LG1	3.39	5.21	1.54
GEI 2013	LG1	3.39	5.34	1.58
GEI 2013	LG1	3.56	3.28	0.92
GEI 2013	LG1	3.56	3.37	0.95
GEI 2013	LG1	3.56	3.82	1.07
GEI 2013	LG1	3.56	3.86	1.09
GEI 2013	LG1	3.56	4.02	1.13
GEI 2013	LG1	3.56	4.16	1.17
GEI 2013	LG1	3.56	4.49	1.26
GEI 2013	LG1	3.56	4.53	1.27
GEI 2013	LG1	3.56	4.63	1.30
GEI 2013	LG1	3.56	4.77	1.34
GEI 2013	CC1	3.76	5.43	1.44
GEI 2013	CC1	3.76	5.57	1.48
GEI 2013	CC1	3.76	6.51	1.73
GEI 2013	CC1	3.76	6.71	1.78
GEI 2013	CC1	3.76	7.12	1.89
GEI 2013	CC1	4.69	3.99	0.85
GEI 2013	CC1	4.69	4.06	0.87
GEI 2013	CC1	4.69	4.08	0.87
GEI 2013	CC1	4.69	4.25	0.91
GEI 2013	CC1	4.69	4.44	0.95
GEI 2013	CC1	4.69	4.48	0.96
GEI 2013	CC1	4.69	4.50	0.96

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	4.72	1.01
GEI 2013	CC1	4.69	5.24	1.12
GEI 2013	CC1	4.69	5.44	1.16
GEI 2013	CC1	5.86	4.98	0.85
GEI 2013	CC1	5.86	5.39	0.92
GEI 2013	CC1	5.86	5.77	0.99
GEI 2013	CC1	5.86	6.39	1.09
GEI 2013	CC1	5.86	6.43	1.10
GEI 2013	CC1	5.86	6.50	1.11
GEI 2013	CC1	5.86	6.57	1.12
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.42	1.27
GEI 2013	CC1	5.86	7.47	1.28
GEI 2014	Bond Creek, BC-2	2.96	2.46	0.83
GEI 2014	Bond Creek, BC-2	2.96	3.22	1.09
GEI 2014	Bond Creek, BC-2	2.96	2.64	0.89
GEI 2014	Bond Creek, BC-2	2.96	2.96	1.00
GEI 2014	Bond Creek, BC-2	2.96	4.47	1.51
GEI 2014	Bond Creek, BC-3	3.02	3.09	1.02
GEI 2014	Bond Creek, BC-3	3.02	2.91	0.96
GEI 2014	Bond Creek, BC-3	3.02	3.45	1.14
GEI 2014	Bond Creek, BC-3	3.02	2.69	0.89
GEI 2014	Bond Creek, BC-3	3.02	3.30	1.09
GEI 2014	Bond Creek, BC-3	5.87	3.44	0.59
GEI 2014	Bond Creek, BC-3	5.87	2.62	0.45
GEI 2014	Bond Creek, BC-3	5.87	3.23	0.55
GEI 2014	Cow Camp Creek, CC-2	5.65	3.05	0.54
GEI 2014	Cow Camp Creek, CC-2	5.65	3.69	0.65
GEI 2014	Cow Camp Creek, CC-2	5.65	3.84	0.68
GEI 2014	Cow Camp Creek, CC-2	5.65	4.44	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	3.98	0.70
GEI 2014	Hazy Creek, C-HC1	8.03	6.84	0.85
GEI 2014	Hazy Creek, C-HC1	8.03	3.78	0.47
GEI 2014	Hazy Creek, C-HC1	8.03	5.81	0.72
GEI 2014	Hazy Creek, C-HC1	8.03	4.29	0.53
GEI 2014	Hazy Creek, C-HC1	8.03	3.59	0.45
GEI 2014	Laurel Fork, C-LF1	12.73	6.52	0.51
GEI 2014	Laurel Fork, C-LF1	12.73	6.81	0.54
GEI 2014	Laurel Fork, C-LF1	12.73	5.11	0.40
GEI 2014	Laurel Fork, C-LF1	12.73	5.16	0.41
GEI 2014	Laurel Fork, C-LF1	12.73	5.46	0.43
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.81	0.63

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.89	0.81
GEI 2014	Little Marsh Fork, C-LMF1	6.02	3.58	0.60
GEI 2014	Little Marsh Fork, C-LMF1	6.02	4.81	0.80
GEI 2014	Little Marsh Fork, C-LMF1	6.02	5.82	0.97
GEI 2014	Dry Creek, DC-1	3.37	4.62	1.37
GEI 2014	Dry Creek, DC-1	3.37	5.15	1.53
GEI 2014	Dry Creek, DC-1	3.37	6.46	1.92
GEI 2014	Dry Creek, DC-1	3.37	5.73	1.70
GEI 2014	Dry Creek, DC-1	3.37	4.59	1.36
GEI 2014	Dry Creek, DC-1	2.29	4.94	2.16
GEI 2014	Dry Creek, DC-1	2.29	4.04	1.76
GEI 2014	Dry Creek, DC-1	2.29	3.62	1.58
GEI 2014	Dry Creek, DC-1	2.29	3.84	1.68
GEI 2014	Dry Creek, DC-1	2.15	4.02	1.87
GEI 2014	Dry Creek, DC-1	2.15	3.60	1.67
GEI 2014	Dry Creek, DC-1	2.15	3.28	1.52
GEI 2014	Dry Creek, DC-1	2.15	3.03	1.41
GEI 2014	Dry Creek, DC-1	2.15	4.01	1.86
GEI 2014	Dry Creek, DC-1	1.97	3.51	1.78
GEI 2014	Dry Creek, DC-2	3.42	5.32	1.56
GEI 2014	Dry Creek, DC-2	3.42	4.62	1.35
GEI 2014	Dry Creek, DC-2	3.42	4.43	1.30
GEI 2014	Dry Creek, DC-2	3.42	4.56	1.34
GEI 2014	Dry Creek, DC-2	3.42	6.38	1.87
GEI 2014	Dry Creek, DC-2	3.42	2.96	0.87
GEI 2014	Dry Creek, DC-2	3.42	3.58	1.05
GEI 2014	Dry Creek, DC-2	3.42	3.22	0.94
GEI 2014	Dry Creek, DC-2	3.42	4.07	1.19
GEI 2014	Dry Creek, DC-2	3.42	3.28	0.96
GEI 2014	Dry Creek, DC-2	3.16	6.52	2.07
GEI 2014	Dry Creek, DC-2	3.16	4.92	1.56
GEI 2014	Dry Creek, DC-2	3.16	3.10	0.98
GEI 2014	Dry Creek, DC-2	3.16	3.14	1.00
GEI 2014	Dry Creek, DC-2	3.16	4.36	1.38
GEI 2014	Dry Creek, DC-2	3.16	3.12	0.99
GEI 2014	Dry Creek, DC-2	3.16	5.40	1.71
GEI 2014	Dry Creek, DC-2	2.93	2.85	0.97
GEI 2014	Dry Creek, DC-2	2.93	4.94	1.69
GEI 2014	Dry Creek, DC-2	2.93	5.17	1.76
GEI 2014	Dry Creek, DC-2	2.93	3.47	1.18
GEI 2014	Dry Creek, DC-2	2.93	2.49	0.85
GEI 2014	Dry Creek, DC-3	7.18	23.79	3.31

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Dry Creek, DC-3	7.18	16.06	2.24
GEI 2014	Dry Creek, DC-3	7.18	24.43	3.40
GEI 2014	Dry Creek, DC-3	7.18	22.20	3.09
GEI 2014	Dry Creek, DC-3	7.18	26.28	3.66
GEI 2014	Dry Creek, DC-3	9.23	21.48	2.33
GEI 2014	Dry Creek, DC-3	9.23	21.24	2.30
GEI 2014	Dry Creek, DC-3	9.23	21.46	2.33
GEI 2014	Dry Creek, DC-3	9.23	22.48	2.44
GEI 2014	Dry Creek, DC-3	9.23	18.80	2.04
GEI 2014	Dry Creek, DC-3	9.23	16.87	1.83
GEI 2014	Dry Creek, DC-4	19.42	15.86	0.82
GEI 2014	Dry Creek, DC-4	19.42	12.76	0.66
GEI 2014	Dry Creek, DC-4	19.42	28.50	1.47
GEI 2014	Dry Creek, DC-4	19.42	18.14	0.93
GEI 2014	Dry Creek, DC-4	19.42	17.55	0.90
GEI 2014	Dry Creek, DC-4	18.10	34.60	1.91
GEI 2014	Dry Creek, DC-4	18.10	23.70	1.31
GEI 2014	Foidel Creek, FOC-1	3.06	8.00	2.61
GEI 2014	Foidel Creek, FOC-1	3.06	9.68	3.16
GEI 2014	Foidel Creek, FOC-1	3.06	8.86	2.89
GEI 2014	Foidel Creek, FOC-1	3.06	2.51	0.82
GEI 2014	Foidel Creek, FOC-1	3.06	2.86	0.93
GEI 2014	Foidel Creek, FOC-1	3.06	4.24	1.39
GEI 2014	Foidel Creek, FOC-1	3.06	3.27	1.07
GEI 2014	Foidel Creek, FOC-1	3.06	5.03	1.64
GEI 2014	Foidel Creek, FOC-2	2.18	2.07	0.95
GEI 2014	Foidel Creek, FOC-2	2.18	3.06	1.41
GEI 2014	Foidel Creek, FOC-2	2.18	3.82	1.76
GEI 2014	Foidel Creek, FOC-2	2.18	2.26	1.04
GEI 2014	Foidel Creek, FOC-2	2.18	2.02	0.93
GEI 2014	Foidel Creek, FOC-2	2.18	2.28	1.05
GEI 2014	Foidel Creek, FOC-2	2.18	2.44	1.12
GEI 2014	Foidel Creek, FOC-2	2.18	2.62	1.21
GEI 2014	Grassy Creek, GC-2	4.20	5.28	1.26
GEI 2014	Grassy Creek, GC-2	4.20	6.13	1.46
GEI 2014	Grassy Creek, GC-2	4.20	6.29	1.50
GEI 2014	Grassy Creek, GC-2	4.20	4.80	1.15
GEI 2014	Grassy Creek, GC-2	4.20	4.59	1.09
GEI 2014	Grassy Creek, GC-2	4.58	3.27	0.71
GEI 2014	Grassy Creek, GC-2	4.58	5.50	1.20
GEI 2014	Grassy Creek, GC-2	4.58	3.64	0.80
GEI 2014	Grassy Creek, GC-2	4.58	4.29	0.94

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-2	4.58	3.01	0.66
GEI 2014	Grassy Creek, GC-2	3.96	7.48	1.89
GEI 2014	Grassy Creek, GC-2	3.96	6.12	1.55
GEI 2014	Grassy Creek, GC-2	3.96	8.61	2.18
GEI 2014	Grassy Creek, GC-2	3.96	7.09	1.79
GEI 2014	Grassy Creek, GC-2	3.96	5.06	1.28
GEI 2014	Grassy Creek, GC-2	3.96	4.95	1.25
GEI 2014	Grassy Creek, GC-2	3.96	3.85	0.97
GEI 2014	Grassy Creek, GC-2	3.96	5.32	1.34
GEI 2014	Grassy Creek, GC-2	3.96	4.04	1.02
GEI 2014	Grassy Creek, GC-2	3.96	4.25	1.08
GEI 2014	Grassy Creek, GC-2	3.97	3.48	0.88
GEI 2014	Grassy Creek, GC-2	3.97	3.86	0.97
GEI 2014	Grassy Creek, GC-2	3.97	4.08	1.03
GEI 2014	Grassy Creek, GC-2	3.97	4.58	1.15
GEI 2014	Grassy Creek, GC-2	3.97	3.53	0.89
GEI 2014	Grassy Creek, GC-3	4.54	6.07	1.34
GEI 2014	Grassy Creek, GC-3	4.54	7.25	1.60
GEI 2014	Grassy Creek, GC-3	4.54	5.25	1.16
GEI 2014	Grassy Creek, GC-3	4.54	5.65	1.25
GEI 2014	Grassy Creek, GC-3	4.54	10.75	2.37
GEI 2014	Grassy Creek, GC-3	4.55	3.30	0.73
GEI 2014	Grassy Creek, GC-3	4.55	3.98	0.88
GEI 2014	Grassy Creek, GC-3	4.55	3.46	0.76
GEI 2014	Grassy Creek, GC-3	4.55	4.14	0.91
GEI 2014	Grassy Creek, GC-3	4.55	3.81	0.84
GEI 2014	Grassy Creek, GC-3	4.34	11.05	2.55
GEI 2014	Grassy Creek, GC-3	4.34	7.22	1.66
GEI 2014	Grassy Creek, GC-3	4.34	9.61	2.22
GEI 2014	Grassy Creek, GC-3	4.34	6.03	1.39
GEI 2014	Grassy Creek, GC-3	4.34	3.95	0.91
GEI 2014	Grassy Creek, GC-3	4.34	4.82	1.11
GEI 2014	Grassy Creek, GC-3	4.34	5.18	1.19
GEI 2014	Grassy Creek, GC-3	4.34	4.46	1.03
GEI 2014	Grassy Creek, GC-3	4.34	4.21	0.97
GEI 2014	Grassy Creek, GC-3	4.35	2.97	0.68
GEI 2014	Grassy Creek, GC-3	4.35	4.16	0.96
GEI 2014	Grassy Creek, GC-3	4.35	4.29	0.99
GEI 2014	Grassy Creek, GC-3	4.35	3.69	0.85
GEI 2014	Grassy Creek, GC-3	4.35	4.73	1.09
GEI 2014	Grassy Creek, GC-4	5.10	4.30	0.84
GEI 2014	Grassy Creek, GC-4	5.10	5.17	1.01

Creek chub (*Semotilus atromaculatus*)

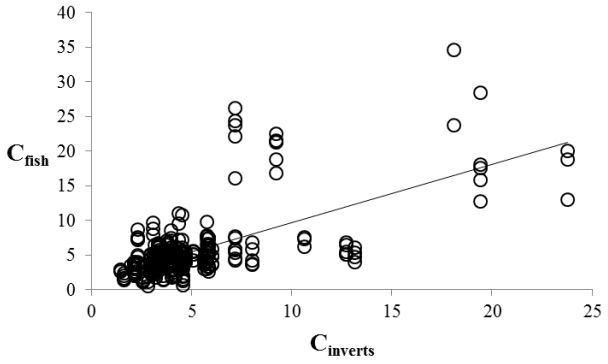
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Grassy Creek, GC-4	5.10	5.46	1.07
GEI 2014	Grassy Creek, GC-4	5.10	5.63	1.10
GEI 2014	Grassy Creek, GC-4	5.10	5.15	1.01
GEI 2014	Grassy Creek, GC-4	5.76	7.72	1.34
GEI 2014	Grassy Creek, GC-4	5.76	6.04	1.05
GEI 2014	Grassy Creek, GC-4	5.76	7.88	1.37
GEI 2014	Grassy Creek, GC-4	5.76	9.77	1.70
GEI 2014	Grassy Creek, GC-4	5.76	7.35	1.28
GEI 2014	Grassy Creek, GC-4	5.76	4.17	0.72
GEI 2014	Grassy Creek, GC-4	5.76	4.86	0.84
GEI 2014	Grassy Creek, GC-4	5.76	5.02	0.87
GEI 2014	Grassy Creek, GC-4	5.76	4.79	0.83
GEI 2014	Grassy Creek, GC-4	5.76	6.56	1.14
GEI 2014	Grassy Creek, GC-4	5.76	5.31	0.92
GEI 2014	Grassy Creek, GC-4 US	13.16	4.05	0.31
GEI 2014	Grassy Creek, GC-4 US	13.16	4.80	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	5.41	0.41
GEI 2014	Grassy Creek, GC-4 US	13.16	6.05	0.46
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.96	0.69
GEI 2014	Big Horse Creek, H-BHC3	5.78	2.97	0.51
GEI 2014	Big Horse Creek, H-BHC3	5.78	3.84	0.66
GEI 2014	Sally Fork, H-BLB2	1.64	2.42	1.48
GEI 2014	Sally Fork, H-BLB2	1.64	1.65	1.01
GEI 2014	Sally Fork, H-BLB2	1.64	1.68	1.03
GEI 2014	Sally Fork, H-BLB2	1.64	2.02	1.23
GEI 2014	Sally Fork, H-BLB2	1.64	1.46	0.89
GEI 2014	Hubberson Gulch, HG-2	3.44	4.41	1.28
GEI 2014	Hubberson Gulch, HG-2	3.44	3.56	1.04
GEI 2014	Hubberson Gulch, HG-2	3.44	4.48	1.30
GEI 2014	Hubberson Gulch, HG-2	1.46	2.95	2.03
GEI 2014	Hubberson Gulch, HG-2	1.46	2.66	1.83
GEI 2014	Hubberson Gulch, HG-2	1.46	2.87	1.97
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	3.04	0.75
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.81	0.45
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.35	0.58
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	1.91	0.47
GEI 2014	Jack Smith (Bear) Branch, H-JSB1	4.03	2.83	0.70
GEI 2014	Laurel Creek, H-LC1	4.57	1.29	0.28

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Laurel Creek, H-LC1	4.57	2.04	0.45
GEI 2014	Laurel Creek, H-LC1	4.57	1.49	0.33
GEI 2014	Laurel Creek, H-LC1	4.57	1.85	0.41
GEI 2014	Laurel Creek, H-LC1	4.57	0.67	0.15
GEI 2014	Lick Creek, H-LKC1	2.59	1.83	0.71
GEI 2014	Lick Creek, H-LKC1	2.59	1.40	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.41	0.54
GEI 2014	Lick Creek, H-LKC1	2.59	1.19	0.46
GEI 2014	Lick Creek, H-LKC1	2.59	1.22	0.47
GEI 2014	Mud River, H-MR3	3.86	4.75	1.23
GEI 2014	Mud River, H-MR3	3.86	4.60	1.19
GEI 2014	Mud River, H-MR3	3.86	5.06	1.31
GEI 2014	Mud River, H-MR3	3.86	3.32	0.86
GEI 2014	Mud River, H-MR3	3.86	4.19	1.08
GEI 2014	Mud River, H-MR5	3.58	1.51	0.42
GEI 2014	Mud River, H-MR5	3.58	1.43	0.40
GEI 2014	Mud River, H-MR5	3.58	1.98	0.55
GEI 2014	Mud River, H-MR5	3.58	3.80	1.06
GEI 2014	Mud River, H-MR5	3.58	3.44	0.96
GEI 2014	Sugartree Branch, H-SB1	10.62	7.29	0.69
GEI 2014	Sugartree Branch, H-SB1	10.62	7.56	0.71
GEI 2014	Sugartree Branch, H-SB1	10.62	6.20	0.58
GEI 2014	Middle Creek, MC-1	3.21	2.75	0.86
GEI 2014	Middle Creek, MC-1	3.21	4.74	1.48
GEI 2014	Middle Creek, MC-1	3.21	4.01	1.25
GEI 2014	Middle Creek, MC-1	3.21	3.94	1.23
GEI 2014	Middle Creek, MC-1	3.21	3.63	1.13
GEI 2014	Middle Creek, MC-1	3.21	1.83	0.57
GEI 2014	Middle Creek, MC-1	3.21	1.85	0.57
GEI 2014	Middle Creek, MC-1	3.21	2.50	0.78
GEI 2014	Middle Creek, MC-1	3.21	2.33	0.73
GEI 2014	Middle Creek, MC-1	3.21	2.56	0.80
GEI 2014	Middle Creek, MC-2	4.19	1.93	0.46
GEI 2014	Middle Creek, MC-2	4.19	1.89	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.51	0.60
GEI 2014	Middle Creek, MC-2	4.19	1.87	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.13	0.51
GEI 2014	Sage Creek, SC-3	2.29	7.33	3.20
GEI 2014	Sage Creek, SC-3	2.29	7.23	3.16
GEI 2014	Sage Creek, SC-3	2.29	7.60	3.32
GEI 2014	Sage Creek, SC-3	2.29	4.77	2.08
GEI 2014	Sage Creek, SC-3	2.29	8.70	3.80

Creek chub (*Semotilus atromaculatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	Sage Creek, SC-4	23.79	18.77	0.79
GEI 2014	Sage Creek, SC-4	23.79	20.08	0.84
GEI 2014	Sage Creek, SC-4	23.79	13.05	0.55
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.41	1.04
GEI 2014	Scotchmans Gulch, SG-1A	7.16	7.65	1.07
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.47	0.62
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.83	0.81
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.37	0.75
GEI 2014	Scotchmans Gulch, SG-1A	7.16	5.69	0.80
GEI 2014	Scotchmans Gulch, SG-1A	7.16	4.31	0.60



Median ratio: 1.06

R^2: 0.41
F: 214.7
df: 303
P: < 0.001

Cutthroat trout (*Oncorhynchus clarkii*)

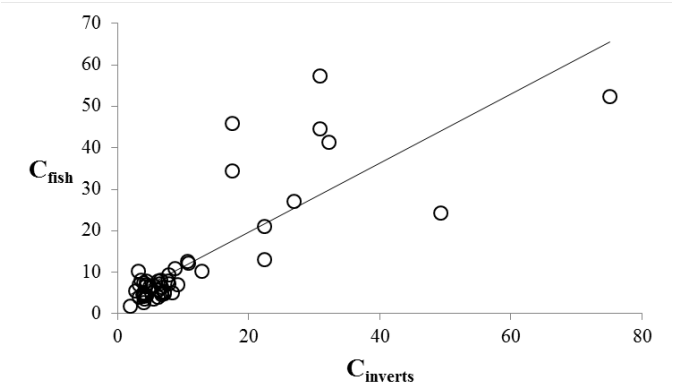
Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ShpC	1.90	1.80	0.95
McDonald and Strosher 1998	ER 745	2.74	5.40	1.97
Hamilton and Buhl 2005	SC	4.10	3.50	0.85
McDonald and Strosher 1998	ER 747	4.29	6.57	1.53
Hamilton and Buhl 2005	UAC	5.00	6.60	1.32
Hamilton and Buhl 2004	ACM	6.70	6.30	0.94
Hamilton and Buhl 2005	DC	8.70	11.00	1.26
McDonald and Strosher 1998	ER 746	10.70	12.71	1.19
Hamilton and Buhl 2005	BGS	10.80	12.20	1.13
Hamilton and Buhl 2004	DVC	12.80	10.20	0.80
Hamilton and Buhl 2004	UEMC	26.90	27.00	1.00
Hamilton and Buhl 2004	LEMC	75.20	52.30	0.70
Minnow 2007	BA6	3.27	6.98	2.13
Minnow 2007	AL4	3.92	4.44	1.13
Minnow 2007	MI5	4.00	5.12	1.28

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	EL12	4.01	7.42	1.85
Minnow 2007	EL14	4.41	4.52	1.02
Minnow 2007	FO9	4.44	7.80	1.76
Minnow 2007	MI3	6.21	5.65	0.91
Minnow 2007	MI2	6.69	5.16	0.77
Minnow 2007	EL1	7.08	4.82	0.68
Minnow 2007	LI8	7.81	9.36	1.20
Minnow 2007	FO10	17.51	45.94	2.62
Minnow 2007	HA7	22.41	21.10	0.94
Minnow 2007	CL11	30.87	57.27	1.86
Orr et al. 2012	Alexander Creek	3.92	2.71	0.69
Orr et al. 2012	Elk River 1	6.23	5.61	0.90
Orr et al. 2012	Elk River 1	6.23	7.94	1.27
Orr et al. 2012	Elk River 1	7.08	5.35	0.76
Orr et al. 2012	Elk River 12	3.81	4.58	1.20
Orr et al. 2012	Elk River 12	4.01	4.89	1.22
Orr et al. 2012	Fording River 22	3.10	10.28	3.32
Orr et al. 2012	Fording River 23	7.72	7.92	1.03
Orr et al. 2012	Fording River 9	4.44	6.92	1.56
Orr et al. 2012	Line Creek 8	6.61	7.99	1.21
Orr et al. 2012	Line Creek 8	7.81	7.13	0.91
Orr et al. 2012	Michel Creek 2	8.38	5.13	0.61
Orr et al. 2012	Michel Creek 2	6.69	4.63	0.69
Orr et al. 2012	Michel Creek 3	5.42	3.51	0.65
Orr et al. 2012	Michel Creek 3	6.21	4.02	0.65
Orr et al. 2012	Michel Creek 5	4.00	4.12	1.03
Orr et al. 2012	Fording River MP1	5.49	6.84	1.25
Orr et al. 2012	Barnes Lake Wetland 6	3.27	3.92	1.20
Orr et al. 2012	Clode Pond 11	32.22	41.27	1.28
Orr et al. 2012	Clode Pond 11	30.87	44.70	1.45
Orr et al. 2012	Elk Lakes 14	6.40	7.14	1.12
Orr et al. 2012	Elk Lakes 14	4.41	4.35	0.99
Orr et al. 2012	Fording River Oxbow 10	49.26	24.34	0.49
Orr et al. 2012	Fording River Oxbow 10	17.51	34.41	1.97
Orr et al. 2012	Henretta Lake 27	9.16	6.90	0.75
Orr et al. 2012	O'Rourke Lake 1	3.63	8.05	2.22
Orr et al. 2012	Harmer Pond 7	22.41	13.08	0.58

Cutthroat trout (*Oncorhynchus clarkii*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.12

R^2: 0.66
F: 95.81
df: 50
P: < 0.001

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	4	1.80	2.10	1.17
Birkner 1978	22	11.30	11.00	0.97
Birkner 1978	27	34.60	79.00	2.28
Birkner 1978	23	15.50	34.50	2.23
Birkner 1978	1	1.75	2.10	1.20
Butler et al. 1991	10	4.80	8.10	1.69
Butler et al. 1991	3	6.20	9.50	1.53
Butler et al. 1993	SP2	3.40	6.00	1.76
Butler et al. 1993	SP2	3.15	8.20	2.60
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.80	3.17
Butler et al. 1993	U1	2.45	6.40	2.61
Butler et al. 1993	R2	3.90	6.60	1.69
Butler et al. 1993	R2	3.70	6.60	1.78
Butler et al. 1993	ST2	4.50	12.80	2.84
Butler et al. 1993	ST2	4.10	7.60	1.85
Butler et al. 1993	ST2	4.10	16.00	3.90
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	R1	4.00	11.00	2.75
Butler et al. 1993	SB2	3.75	5.70	1.52
Butler et al. 1993	SB2	3.75	8.60	2.29
Butler et al. 1993	SB2	3.65	9.90	2.71
Butler et al. 1993	WSB2	4.75	17.10	3.60
Butler et al. 1993	WSB2	3.60	4.20	1.17
Butler et al. 1993	WSB2	3.60	10.00	2.78

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	WSB2	3.00	8.10	2.70
Butler et al. 1994	CRC	7.50	20.40	2.72
Butler et al. 1994	CF1	3.60	7.90	2.19
Butler et al. 1994	GUN2	28.00	7.50	0.27
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	TGC	4.90	11.00	2.24
Butler et al. 1994	AD	2.70	9.60	3.56
Butler et al. 1994	LSW1	3.90	73.00	18.72
Butler et al. 1994	OMD	73.00	13.00	0.18
Butler et al. 1994	PSW1	3.70	22.00	5.95
Butler et al. 1994	MKP	32.00	51.00	1.59
Butler et al. 1995	AK	0.78	2.60	3.35
Butler et al. 1995	AK	0.78	2.90	3.74
Butler et al. 1995	AK	0.78	2.80	3.61
Butler et al. 1995	DD	0.86	3.40	3.95
Butler et al. 1995	DD	0.86	3.90	4.53
Butler et al. 1995	DD	0.86	3.60	4.19
Butler et al. 1995	HD1	0.83	3.90	4.73
Butler et al. 1995	HD1	0.83	2.50	3.03
Butler et al. 1995	HD1	0.83	2.60	3.15
Butler et al. 1995	HD2	0.98	1.50	1.53
Butler et al. 1995	HD2	0.98	1.60	1.63
Butler et al. 1995	ME1	3.40	5.60	1.65
Butler et al. 1995	ME2	1.25	4.80	3.84
Butler et al. 1995	ME4	1.55	1.40	0.90
Butler et al. 1995	ME4	1.55	5.90	3.81
Butler et al. 1995	ME3	2.55	4.30	1.69
Butler et al. 1995	ME3	2.55	5.30	2.08
Butler et al. 1995	ME3	2.55	4.40	1.73
Butler et al. 1995	SD	1.40	4.90	3.50
Butler et al. 1995	SD	1.40	3.00	2.14
Butler et al. 1995	SD	1.40	4.00	2.86
Butler et al. 1995	WC	6.75	18.40	2.73
Butler et al. 1995	WC	6.75	22.90	3.39
Butler et al. 1995	WC	6.75	26.40	3.91
Butler et al. 1995	YJ2	1.65	11.00	6.67
Butler et al. 1995	YJ2	1.65	4.00	2.42
Butler et al. 1997	MNP2	4.40	11.00	2.50
Butler et al. 1997	MUD2	3.45	7.70	2.23
Butler et al. 1997	MUD2	3.45	12.00	3.48
Butler et al. 1997	MUD2	3.45	6.50	1.88
Butler et al. 1997	WCP	9.70	10.00	1.03

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	WCP	9.70	15.00	1.55
Butler et al. 1997	TR25	1.80	4.00	2.22
Butler et al. 1997	TR25	1.80	5.20	2.89
Butler et al. 1997	TR25	1.80	6.00	3.33
Butler et al. 1997	TRH	1.60	4.20	2.63
Butler et al. 1997	TRH	1.60	4.30	2.69
Butler et al. 1997	TRH	1.60	2.20	1.38
Butler et al. 1997	TRH	1.60	3.00	1.88
Butler et al. 1997	MN5	8.60	7.30	0.85
Butler et al. 1997	MNP1	0.70	1.70	2.43
Butler et al. 1997	MNP1	0.70	1.80	2.57
GEI 2013	SWA1	3.64	4.07	1.12
GEI 2013	SWA1	3.64	4.68	1.29
GEI 2013	SWA1	3.64	4.76	1.31
GEI 2013	SWA1	3.64	5.45	1.50
GEI 2013	SWA1	3.64	5.71	1.57
GEI 2013	SWA1	3.64	3.62	1.00
GEI 2013	SWA1	3.64	3.72	1.02
GEI 2013	SWA1	3.64	4.43	1.22
GEI 2013	SWA1	3.64	4.52	1.24
GEI 2013	SWA1	3.64	4.66	1.28
GEI 2013	SWA1	2.81	4.48	1.60
GEI 2013	SWA1	2.81	4.53	1.61
GEI 2013	SWA1	2.81	5.00	1.78
GEI 2013	SWA1	2.81	5.24	1.87
GEI 2013	SWA1	2.81	5.76	2.05
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	3.98	1.42
GEI 2013	SWA1	2.81	4.04	1.44
GEI 2013	SWA1	2.81	4.33	1.54
GEI 2013	SWA1	2.81	4.81	1.71
GEI 2013	SWB	7.06	7.38	1.05
GEI 2013	SWB	7.06	8.49	1.20
GEI 2013	SWB	7.06	8.72	1.24
GEI 2013	SWB	7.06	9.80	1.39
GEI 2013	SWB	7.06	8.61	1.22
GEI 2013	SWB	7.06	9.02	1.28
GEI 2013	SWB	7.06	9.11	1.29
GEI 2013	SWB	7.06	9.30	1.32
GEI 2013	SWB	7.06	9.53	1.35
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	11.22	1.51

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SWB	7.44	12.25	1.65
GEI 2013	SWB	7.44	12.43	1.67
GEI 2013	SWB	7.44	12.46	1.68
GEI 2013	SWB	7.44	9.36	1.26
GEI 2013	SWB	7.44	9.46	1.27
GEI 2013	SWB	7.44	9.78	1.32
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.66	1.43
GEI 2013	SW11	8.41	5.70	0.68
GEI 2013	SW11	8.41	7.05	0.84
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW11	8.41	4.68	0.56
GEI 2013	SW11	8.41	5.29	0.63
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	5.38	0.64
GEI 2013	SW2-1	6.60	12.83	1.95
GEI 2013	SW2-1	6.60	14.80	2.24
GEI 2013	SW2-1	6.60	20.13	3.05
GEI 2013	SW2-1	6.60	26.75	4.06
GEI 2013	SW2-1	6.60	30.48	4.62
GEI 2013	SW2-1	6.60	12.51	1.90
GEI 2013	SW2-1	6.60	16.70	2.53
GEI 2013	SW2-1	6.60	17.21	2.61
GEI 2013	SW2-1	6.60	18.27	2.77
GEI 2013	SW2-1	6.60	20.66	3.13
GEI 2013	SW2-1	9.14	13.31	1.46
GEI 2013	SW2-1	9.14	15.63	1.71
GEI 2013	SW2-1	9.14	15.77	1.73
GEI 2013	SW2-1	9.14	16.79	1.84
GEI 2013	SW2-1	9.14	17.00	1.86
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	19.39	2.12
GEI 2013	SW2-1	9.14	22.50	2.46
GEI 2013	SW1	7.82	9.11	1.16
GEI 2013	SW1	7.82	9.15	1.17
GEI 2013	SW1	7.82	11.15	1.43
GEI 2013	SW1	7.82	11.23	1.44
GEI 2013	SW1	7.82	13.76	1.76
GEI 2013	SW1	7.82	9.82	1.26
GEI 2013	SW1	7.82	8.45	1.08
GEI 2013	SW1	7.82	8.88	1.14
GEI 2013	SW1	7.82	9.41	1.20

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	11.07	1.42
GEI 2013	SW1	6.54	7.01	1.07
GEI 2013	SW1	6.54	7.86	1.20
GEI 2013	SW1	6.54	7.98	1.22
GEI 2013	SW1	6.54	8.23	1.26
GEI 2013	SW1	6.54	8.50	1.30
GEI 2013	SW1	6.54	9.48	1.45
GEI 2013	SW1	6.54	9.95	1.52
GEI 2013	SW1	6.54	10.09	1.54
GEI 2013	SW1	6.54	10.19	1.56
GEI 2013	SW4-1	3.33	5.88	1.77
GEI 2013	SW4-1	3.33	5.89	1.77
GEI 2013	SW4-1	3.33	6.07	1.83
GEI 2013	SW4-1	3.33	6.61	1.99
GEI 2013	SW4-1	3.33	6.87	2.07
GEI 2013	SW4-1	3.33	4.85	1.46
GEI 2013	SW4-1	3.33	5.25	1.58
GEI 2013	SW4-1	3.33	5.39	1.62
GEI 2013	SW4-1	3.33	6.11	1.84
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	SW9	4.45	5.57	1.25
GEI 2013	SW9	4.45	5.93	1.33
GEI 2013	SW9	4.45	6.14	1.38
GEI 2013	SW9	4.45	6.20	1.39
GEI 2013	SW9	4.45	6.56	1.47
GEI 2013	SW9	4.45	7.57	1.70
GEI 2013	SW88	3.96	4.73	1.20
GEI 2013	SW88	3.96	4.96	1.25
GEI 2013	SW88	3.96	5.55	1.40
GEI 2013	SW88	3.96	5.56	1.41
GEI 2013	SW88	3.96	6.32	1.60
GEI 2013	SW88	3.96	5.13	1.30
GEI 2013	SW88	3.96	5.86	1.48
GEI 2013	SW88	3.96	6.07	1.53
GEI 2013	CC1	3.76	3.79	1.01
GEI 2013	CC1	3.76	5.23	1.39
GEI 2013	CC1	3.76	7.36	1.96
GEI 2013	CC1	3.76	8.69	2.31
GEI 2013	CC1	3.76	9.07	2.41
GEI 2013	CC1	4.69	5.92	1.26
GEI 2013	CC1	4.69	7.68	1.64
GEI 2013	CC1	4.69	7.59	1.62

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	CC1	4.69	6.49	1.39
GEI 2013	CC1	4.69	7.14	1.52
GEI 2013	CC1	5.86	6.68	1.14
GEI 2013	CC1	5.86	7.73	1.32
GEI 2013	CC1	5.86	7.88	1.35
GEI 2013	CC1	5.86	8.45	1.44
GEI 2013	CC1	5.86	11.69	2.00
GEI 2013	CC1	5.86	9.21	1.57
GEI 2013	CC1	5.86	9.70	1.66
GEI 2013	LG1	3.56	4.81	1.35
GEI 2013	LG1	3.56	4.86	1.37
GEI 2013	LG1	3.56	5.05	1.42
GEI 2013	LG1	3.56	5.47	1.54
GEI 2013	LG1	3.56	5.56	1.56
GEI 2013	LG1	3.56	3.72	1.05
GEI 2013	LG1	3.56	4.09	1.15
GEI 2013	LG1	3.56	3.26	0.92
GEI 2013	LG1	3.56	3.35	0.94
GEI 2013	LG1	3.56	4.20	1.18
GEI 2013	LG1	3.39	3.60	1.06
GEI 2013	LG1	3.39	3.89	1.15
GEI 2013	LG1	3.39	4.27	1.26
GEI 2013	LG1	3.39	4.45	1.31
GEI 2013	LG1	3.39	5.18	1.53
GEI 2013	LG1	3.39	5.51	1.63
Grasso et al. 1995	17	1.91	6.59	3.45
Grasso et al. 1995	17	1.91	6.60	3.46
Grasso et al. 1995	17	1.91	7.30	3.82
Grasso et al. 1995	10	1.85	2.74	1.48
Grasso et al. 1995	10	1.85	2.79	1.51
Grasso et al. 1995	10	1.85	2.90	1.57
Lambing et al. 1994	S46	6.20	5.10	0.82
Lambing et al. 1994	S48	3.05	2.50	0.82
Lambing et al. 1994	S11	14.50	11.00	0.76
Lambing et al. 1994	S11	14.50	33.00	2.28
Lambing et al. 1994	S34	14.00	25.00	1.79
Lambing et al. 1994	S39	5.85	7.90	1.35
Lambing et al. 1994	S39	5.85	21.00	3.59
Lemly 1985	Badin Lake	5.70	1.50	0.26
Lemly 1985	Belews Lake	51.15	13.60	0.27
Lemly 1985	High Rock Lake	9.05	1.89	0.21
GEI 2014	DC-4	19.42	27.69	1.43

Fathead minnow (*Pimephales promelas*)

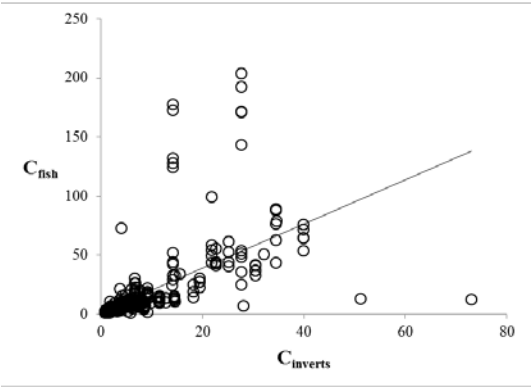
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	DC-4	19.42	27.88	1.44
GEI 2014	DC-4	19.42	23.05	1.19
GEI 2014	DC-4	19.42	30.61	1.58
GEI 2014	DC-4	18.10	19.32	1.07
GEI 2014	DC-4	18.10	14.48	0.80
GEI 2014	DC-4	18.10	25.42	1.40
GEI 2014	FOC-2	2.18	4.13	1.90
GEI 2014	FOC-2	2.18	4.10	1.89
GEI 2014	FOC-2	2.18	5.50	2.53
GEI 2014	FOC-2	2.18	4.85	2.23
GEI 2014	FOC-2	2.18	4.65	2.14
GEI 2014	SC-1	5.85	7.95	1.36
GEI 2014	SC-1	5.85	7.62	1.30
GEI 2014	SC-1	5.85	7.88	1.35
GEI 2014	SC-1	5.85	8.46	1.45
GEI 2014	SC-1	5.85	7.29	1.25
GEI 2014	SC-1	5.85	8.94	1.53
GEI 2014	SC-1	5.85	8.28	1.42
GEI 2014	SC-1	5.85	8.62	1.47
GEI 2014	SC-1	5.85	6.17	1.05
GEI 2014	SC-1	5.85	6.09	1.04
GEI 2014	SC-1	5.85	9.23	1.58
GEI 2014	SC-1	5.85	10.00	1.71
GEI 2014	SC-1	5.85	8.12	1.39
GEI 2014	SC-1	5.85	6.71	1.15
GEI 2014	SC-1	5.85	8.34	1.43
GEI 2014	SC-1	4.94	10.22	2.07
GEI 2014	SC-1	4.94	10.86	2.20
GEI 2014	SC-1	4.94	9.82	1.99
GEI 2014	SC-1	4.94	9.45	1.91
GEI 2014	SC-1	4.94	10.30	2.09
GEI 2014	SC-2	14.33	15.86	1.11
GEI 2014	SC-2	14.33	15.08	1.05
GEI 2014	SC-2	14.33	13.33	0.93
GEI 2014	SC-2	14.33	12.04	0.84
GEI 2014	SC-2	14.33	13.82	0.96
GEI 2014	SC-2	11.44	9.64	0.84
GEI 2014	SC-2	11.44	14.94	1.31
GEI 2014	SC-2	11.44	10.91	0.95
GEI 2014	SC-2	11.44	16.06	1.40
GEI 2014	SC-2	11.44	14.60	1.28
GEI 2014	SC-2	12.75	13.81	1.08

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-2	12.75	14.10	1.11
GEI 2014	SC-2	12.75	10.68	0.84
GEI 2014	SC-3	11.41	11.65	1.02
GEI 2014	SC-3	11.41	10.95	0.96
GEI 2014	SC-3	11.41	10.84	0.95
GEI 2014	SC-3	11.41	13.48	1.18
GEI 2014	SC-3	8.58	7.70	0.90
GEI 2014	SC-3	8.58	6.46	0.75
GEI 2014	SC-3	8.58	6.97	0.81
GEI 2014	SC-3	8.58	10.64	1.24
GEI 2014	SC-3	8.58	7.85	0.91
GEI 2014	SC-3	5.75	13.75	2.39
GEI 2014	SC-3	5.75	11.19	1.95
GEI 2014	SC-3	5.75	12.68	2.20
GEI 2014	SC-4	7.39	6.33	0.86
GEI 2014	SC-4	7.39	14.39	1.95
GEI 2014	SC-4	5.18	2.72	0.53
GEI 2014	SC-4	5.18	11.95	2.31
GEI 2014	SC-4	5.18	7.98	1.54
GEI 2014	SC-4	5.18	6.75	1.30
GEI 2014	SC-6	39.87	72.33	1.81
GEI 2014	SC-6	39.87	76.00	1.91
GEI 2014	SC-6	39.87	64.73	1.62
GEI 2014	SC-6	39.87	54.09	1.36
GEI 2014	SC-6	39.87	64.64	1.62
GEI 2014	SC-6	34.35	76.89	2.24
GEI 2014	SC-6	34.35	89.67	2.61
GEI 2014	SC-6	34.35	63.32	1.84
GEI 2014	SC-6	34.35	88.44	2.57
GEI 2014	SC-6	34.35	44.15	1.29
GEI 2014	SC-6	27.54	51.65	1.88
GEI 2014	SC-6	27.54	35.92	1.30
GEI 2014	SC-6	27.54	25.55	0.93
GEI 2014	SC-6	27.54	54.25	1.97
GEI 2014	SC-6	27.54	48.94	1.78
GEI 2014	SC-6	27.54	204.26	7.42
GEI 2014	SC-6	27.54	143.62	5.22
GEI 2014	SC-6	27.54	192.93	7.01
GEI 2014	SC-6	27.54	171.89	6.24
GEI 2014	SC-6	27.54	171.36	6.22
GEI 2014	SC-8	22.62	43.12	1.91
GEI 2014	SC-8	22.62	43.36	1.92

Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-8	22.62	55.81	2.47
GEI 2014	SC-8	22.62	44.60	1.97
GEI 2014	SC-8	22.62	41.67	1.84
GEI 2014	SC-9	30.36	37.18	1.22
GEI 2014	SC-9	30.36	41.32	1.36
GEI 2014	SC-9	30.36	37.20	1.23
GEI 2014	SC-9	30.36	33.25	1.10
GEI 2014	SC-9	30.36	41.94	1.38
GEI 2014	SC-8	21.77	99.40	4.57
GEI 2014	SC-8	21.77	54.47	2.50
GEI 2014	SC-8	21.77	59.07	2.71
GEI 2014	SC-8	21.77	43.70	2.01
GEI 2014	SC-8	21.77	50.18	2.31
GEI 2014	SC-9	25.06	40.82	1.63
GEI 2014	SC-9	25.06	61.80	2.47
GEI 2014	SC-9	25.06	44.74	1.79
GEI 2014	SC-9	25.06	52.97	2.11
GEI 2014	SC-8	14.15	52.46	3.71
GEI 2014	SC-8	14.15	29.43	2.08
GEI 2014	SC-8	14.15	44.58	3.15
GEI 2014	SC-8	14.15	33.44	2.36
GEI 2014	SC-8	14.15	42.86	3.03
GEI 2014	SC-8	14.15	128.33	9.07
GEI 2014	SC-8	14.15	173.33	12.25
GEI 2014	SC-8	14.15	132.34	9.35
GEI 2014	SC-8	14.15	124.90	8.83
GEI 2014	SC-8	14.15	177.97	12.58



Median ratio: 1.57

R^2: 0.35
F: 185.7
df: 344
P: < 0.001

Flannelmouth sucker (*Catostomus latipinnis*)

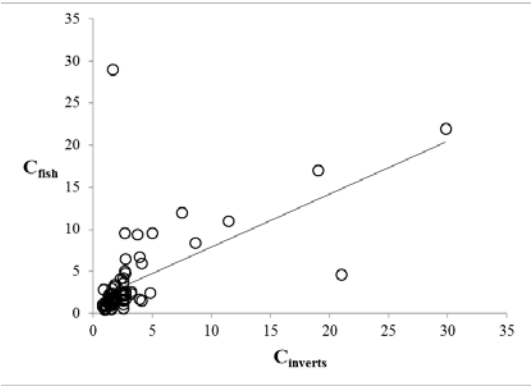
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	2.50	0.52
Butler et al. 1991	7	29.80	22.00	0.74
Butler et al. 1991	4	3.90	1.70	0.44
Butler et al. 1991	9	4.10	1.50	0.37
Butler et al. 1991	9	4.10	6.00	1.46
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	P1	1.50	2.40	1.60
Butler et al. 1993	LP3	1.12	0.92	0.83
Butler et al. 1993	LP3	1.12	1.40	1.26
Butler et al. 1993	LP4	3.20	2.40	0.75
Butler et al. 1993	LP4	3.20	2.60	0.81
Butler et al. 1994	CRC	7.50	12.00	1.60
Butler et al. 1994	LZA1	19.00	17.00	0.89
Butler et al. 1994	BSW1	5.00	9.60	1.92
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	COL1	1.50	0.50	0.33
Butler et al. 1994	COL1	1.50	0.60	0.40
Butler et al. 1994	COL1	1.50	0.63	0.42
Butler et al. 1994	COL1	1.50	0.92	0.61
Butler et al. 1994	COL1	1.50	1.00	0.67
Butler et al. 1994	COL1	1.50	1.60	1.07
Butler et al. 1994	COL1	1.50	1.70	1.13
Butler et al. 1994	COL1	1.50	1.80	1.20
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	RB3	1.60	29.00	18.13
Butler et al. 1994	RB1	21.00	4.60	0.22
Butler et al. 1994	LSW1	3.90	6.70	1.72
Butler et al. 1994	PSW1	3.70	9.40	2.54
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	AK	0.78	0.90	1.16
Butler et al. 1995	AK	0.78	0.82	1.06
Butler et al. 1995	AK	0.78	1.10	1.42
Butler et al. 1995	HD1	0.83	2.90	3.52
Butler et al. 1995	HD2	0.98	0.49	0.50
Butler et al. 1995	HD2	0.98	0.54	0.55
Butler et al. 1995	HD2	0.98	0.62	0.63
Butler et al. 1995	HD2	0.98	0.96	0.98
Butler et al. 1995	ME2	1.25	1.60	1.28
Butler et al. 1995	ME2	1.25	1.40	1.12
Butler et al. 1995	ME2	1.25	2.00	1.60
Butler et al. 1995	ME2	1.25	2.20	1.76
Butler et al. 1995	ME4	1.55	1.50	0.97

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	ME4	1.55	1.30	0.84
Butler et al. 1995	ME4	1.55	1.90	1.23
Butler et al. 1995	ME4	1.55	2.40	1.55
Butler et al. 1995	ME4	1.55	3.00	1.94
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	1.70	0.67
Butler et al. 1995	ME3	2.55	2.10	0.82
Butler et al. 1995	ME3	2.55	2.40	0.94
Butler et al. 1995	ME3	2.55	3.60	1.41
Butler et al. 1995	SJ1	2.50	1.71	0.68
Butler et al. 1995	SJ1	2.50	1.50	0.60
Butler et al. 1995	SJ1	2.50	2.20	0.88
Butler et al. 1995	SJ1	2.50	0.61	0.24
Butler et al. 1995	SJ1	2.50	1.10	0.44
Butler et al. 1995	SJ1	2.50	4.20	1.68
Butler et al. 1995	YJ2	1.65	1.60	0.97
Butler et al. 1995	YJ2	1.65	2.40	1.45
Butler et al. 1995	MN1	2.70	6.50	2.41
Butler et al. 1995	MN1	2.70	1.70	0.63
Butler et al. 1995	MN1	2.70	4.80	1.78
Butler et al. 1995	MP	1.60	1.20	0.75
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1997	MN3	2.70	2.30	0.85
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MUD	2.30	4.10	1.78
Butler et al. 1997	MUD	2.30	2.70	1.17
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	MN4	2.65	5.10	1.92
Butler et al. 1997	MN4	2.65	9.60	3.62
Butler et al. 1997	MN5	8.60	8.40	0.98
Butler et al. 1997	MNQ	1.80	2.10	1.17
Butler et al. 1997	MNQ	1.80	3.20	1.78
Butler et al. 1997	MNQ	1.80	3.50	1.94

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio

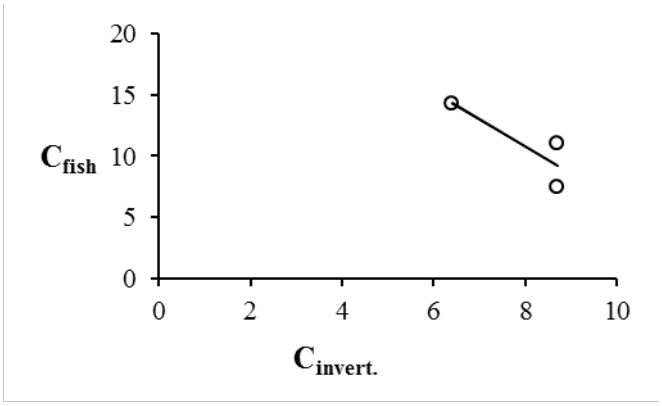


Median ratio: 0.98

R^2: 0.36
F: 41.6
df: 73
P: < 0.001

Gizzard shad (*Dorosoma cepedianum*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mueller et al. 1991	R2	6.40	14.30	2.23
Mueller et al. 1991	R1	8.70	7.50	0.86
Mueller et al. 1991	R1	8.70	11.00	1.26



Median ratio: 1.26

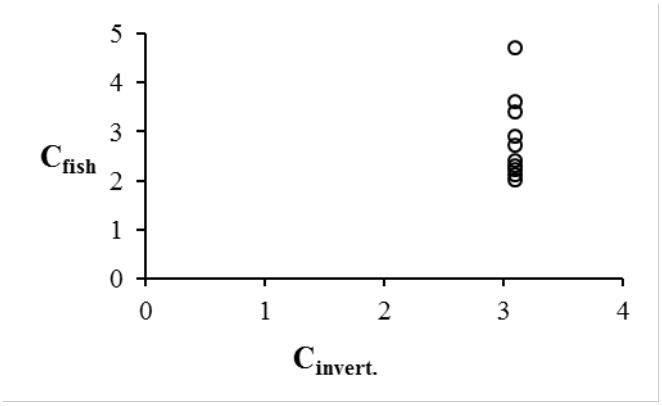
R^2: 0.74
F: 2.78
df: 1
P: 0.39
Not used because P > 0.05 and negative slope.

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.00	0.65
Roddy et al. 1991	18	3.10	2.10	0.68
Roddy et al. 1991	18	3.10	2.20	0.71
Roddy et al. 1991	18	3.10	2.30	0.74
Roddy et al. 1991	18	3.10	2.40	0.77
Roddy et al. 1991	18	3.10	2.70	0.87

Goldeye (*Hiodon alosoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	4.70	1.52



Median ratio: 0.82

R^2: 0.0
F: 0.0
df: 8
P: 1.0

Not used because no slope and P>0.05.

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	10	4.80	7.90	1.65
Butler et al. 1991	7	29.80	15.20	0.51
Butler et al. 1991	7	29.80	25.10	0.84
Butler et al. 1991	3	6.20	6.40	1.03
Butler et al. 1994	LZA1	19.00	37.00	1.95
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	HD1	0.83	1.30	1.58
Butler et al. 1995	ME3	2.55	5.00	1.96
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	CH1	7.50	9.50	1.27
Butler et al. 1997	MUD2	3.45	7.60	2.20
Butler et al. 1997	MUD2	3.45	7.00	2.03
Butler et al. 1997	TR25	1.80	4.40	2.44
Butler et al. 1997	TRH	1.60	3.30	2.06
GEI 2013	SWA1	2.81	2.96	1.06
GEI 2013	SWA1	2.81	3.21	1.14
GEI 2013	SWA1	2.81	3.24	1.16
GEI 2013	SWA1	2.81	3.69	1.32
GEI 2013	SWA1	2.81	3.88	1.38
GEI 2013	SWB	7.44	11.94	1.61
GEI 2013	SW11	8.41	4.54	0.54

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW11	8.41	4.84	0.58
GEI 2013	SW11	8.41	5.34	0.63
GEI 2013	SW11	8.41	7.00	0.83
GEI 2013	SW11	8.41	7.13	0.85
GEI 2013	SW9	4.45	4.38	0.98
GEI 2013	SW9	4.45	5.06	1.14
GEI 2013	SW9	4.45	5.53	1.24
GEI 2013	SW9	4.45	5.80	1.30
GEI 2013	SW9	4.45	7.29	1.64
GEI 2013	SW88	3.96	7.14	1.81
GEI 2013	SW88	3.96	7.41	1.87
GEI 2013	LG1	3.39	4.11	1.21
GEI 2013	LG1	3.39	4.33	1.28
GEI 2013	LG1	3.39	5.71	1.68
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	4.00	1.29
Roddy et al. 1991	18	3.10	5.20	1.68
Roddy et al. 1991	18	3.10	5.70	1.84
Lemly 1985	Badin Lake	5.70	2.18	0.38
Lemly 1985	Belews Lake	51.15	13.99	0.27
Lemly 1985	High Rock Lake	9.05	2.10	0.23
GEI 2014	C-BCR2	6.81	9.47	1.39
GEI 2014	C-BCR2	6.81	9.29	1.37
GEI 2014	C-BCR2	6.81	8.04	1.18
GEI 2014	C-CC1	4.40	7.23	1.64
GEI 2014	C-CC1	4.40	11.76	2.67
GEI 2014	C-CC2	5.56	4.59	0.83
GEI 2014	C-CC2	5.56	3.04	0.55
GEI 2014	C-CC2	5.56	5.34	0.96
GEI 2014	C-CF1	3.39	3.62	1.07
GEI 2014	C-CF1	3.39	2.95	0.87
GEI 2014	C-CF1	3.39	3.23	0.95
GEI 2014	C-CF1	3.39	5.55	1.64
GEI 2014	C-CLF1	9.30	3.99	0.43
GEI 2014	C-CLF1	9.30	5.23	0.56
GEI 2014	C-CLF1	9.30	4.75	0.51
GEI 2014	C-CLF1	9.30	4.87	0.52
GEI 2014	C-CLF1	9.30	3.58	0.39
GEI 2014	C-CLF2	6.85	5.76	0.84
GEI 2014	C-CLF2	6.85	5.89	0.86
GEI 2014	C-CLF2	6.85	4.78	0.70

Green sunfish (*Lepomis cyanellus*)

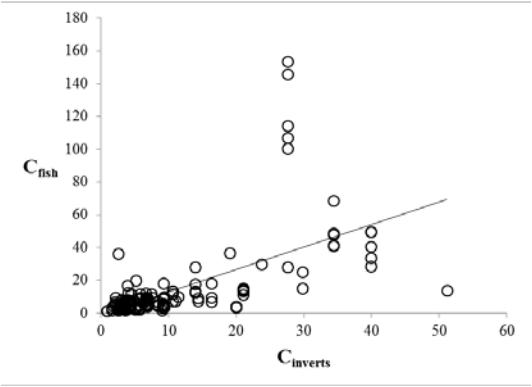
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	C-CLF2	6.85	5.11	0.75
GEI 2014	C-CLF2	6.85	4.10	0.60
GEI 2014	C-LFWOC1	9.32	10.10	1.08
GEI 2014	C-TF1	20.00	4.15	0.21
GEI 2014	C-TF1	20.00	3.47	0.17
GEI 2014	C-TF1	20.00	4.14	0.21
GEI 2014	C-TF1	20.00	4.11	0.21
GEI 2014	C-TF1	20.00	3.41	0.17
GEI 2014	C-WOC1	6.65	12.05	1.81
GEI 2014	C-WOC1	6.65	9.60	1.44
GEI 2014	C-WOC1	6.65	8.66	1.30
GEI 2014	C-WOC1	6.65	5.81	0.87
GEI 2014	C-WOC1	6.65	7.54	1.13
GEI 2014	H-BB1	16.29	9.55	0.59
GEI 2014	H-BB1	16.29	18.27	1.12
GEI 2014	H-BB1	16.29	7.08	0.43
GEI 2014	H-BHC1	5.08	3.69	0.73
GEI 2014	H-BHC1	5.08	2.48	0.49
GEI 2014	H-BHC1	5.08	3.29	0.65
GEI 2014	H-BHC1	5.08	3.49	0.69
GEI 2014	H-BHC1	5.08	3.70	0.73
GEI 2014	H-JSB1	4.03	4.83	1.20
GEI 2014	H-JSB1	4.03	2.57	0.64
GEI 2014	H-JSB1	4.03	3.73	0.93
GEI 2014	H-LF1	9.09	3.12	0.34
GEI 2014	H-LF1	9.09	5.96	0.66
GEI 2014	H-LF1	9.09	4.30	0.47
GEI 2014	H-LF1	9.09	4.02	0.44
GEI 2014	H-LF1	9.09	5.29	0.58
GEI 2014	H-MR2	2.14	9.57	4.47
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	5.80	2.71
GEI 2014	H-MR2	2.14	5.55	2.59
GEI 2014	H-MR2	2.14	6.88	3.22
GEI 2014	H-MR3	3.86	8.09	2.10
GEI 2014	H-MR3	3.86	16.98	4.40
GEI 2014	H-MR3	3.86	6.80	1.76
GEI 2014	H-MR3	3.86	8.52	2.21
GEI 2014	H-MR3	3.86	6.62	1.72
GEI 2014	H-MR4	9.26	9.01	0.97
GEI 2014	H-MR4	9.26	8.78	0.95
GEI 2014	H-MR4	9.26	18.33	1.98

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	H-MR4	9.26	9.84	1.06
GEI 2014	H-MR4	9.26	5.94	0.64
GEI 2014	H-MR5	3.58	5.50	1.54
GEI 2014	H-MR5	3.58	3.52	0.98
GEI 2014	H-MR5	3.58	2.41	0.67
GEI 2014	H-MR5	3.58	3.09	0.86
GEI 2014	H-MR5	3.58	1.94	0.54
GEI 2014	H-MR6	2.49	36.20	14.54
GEI 2014	H-MR6	2.49	2.58	1.04
GEI 2014	H-MR6	2.49	1.94	0.78
GEI 2014	H-MR6	2.49	1.74	0.70
GEI 2014	H-MR6	2.49	2.69	1.08
GEI 2014	H-SB1	10.62	11.90	1.12
GEI 2014	H-SB1	10.62	13.39	1.26
GEI 2014	H-SB1	10.62	7.64	0.72
GEI 2014	H-SB1	10.62	13.45	1.27
GEI 2014	H-SF1	21.05	13.59	0.65
GEI 2014	H-SF1	21.05	14.22	0.68
GEI 2014	H-SF1	21.05	15.27	0.73
GEI 2014	H-SF1	21.05	14.58	0.69
GEI 2014	H-SF1	21.05	11.25	0.53
GEI 2014	H-SF2	13.95	17.74	1.27
GEI 2014	H-SF2	13.95	12.86	0.92
GEI 2014	H-SF2	13.95	12.76	0.91
GEI 2014	H-SF2	13.95	13.41	0.96
GEI 2014	H-SF2	13.95	28.23	2.02
GEI 2014	H-UB1	3.02	4.43	1.47
GEI 2014	H-UB1	3.02	4.91	1.63
GEI 2014	H-UB1	3.02	3.73	1.23
GEI 2014	H-UB1	3.02	8.00	2.65
GEI 2014	H-UB1	3.02	8.36	2.77
GEI 2014	SC-1-25	4.00	12.78	3.19
GEI 2014	SC-2	14.33	9.31	0.65
GEI 2014	SC-2	14.33	7.59	0.53
GEI 2014	SC-2	11.44	10.23	0.89
GEI 2014	SC-2-27	23.76	30.00	1.26
GEI 2014	SC-3	5.75	8.66	1.51
GEI 2014	SC-3	5.75	11.72	2.04
GEI 2014	SC-3	5.75	9.59	1.67
GEI 2014	SC-4	5.18	19.86	3.83
GEI 2014	SC-6	39.87	49.55	1.24
GEI 2014	SC-6	39.87	49.56	1.24

Green sunfish (*Lepomis cyanellus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-6	39.87	33.71	0.85
GEI 2014	SC-6	39.87	28.73	0.72
GEI 2014	SC-6	39.87	40.64	1.02
GEI 2014	SC-6	34.35	41.51	1.21
GEI 2014	SC-6	34.35	48.02	1.40
GEI 2014	SC-6	34.35	68.66	2.00
GEI 2014	SC-6	34.35	41.27	1.20
GEI 2014	SC-6	34.35	48.76	1.42
GEI 2014	SC-6	27.54	28.17	1.02
GEI 2014	SC-6	27.54	106.88	3.88
GEI 2014	SC-6	27.54	114.55	4.16
GEI 2014	SC-6	27.54	100.41	3.65
GEI 2014	SC-6	27.54	153.64	5.58
GEI 2014	SC-6	27.54	145.62	5.29
GEI 2014	S-SC1	11.01	7.47	0.68



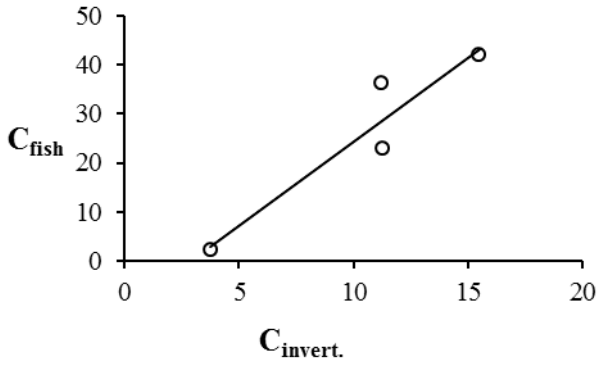
Median ratio: 1.12

R^2: 0.37
F: 93.17
df: 160
P: < 0.001

Iowa darter (*Etheostoma exile*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	7	3.75	2.10	0.56
Birkner 1978	20	11.20	36.30	3.24
Birkner 1978	22	11.30	23.00	2.04
Birkner 1978	23	15.50	41.90	2.70

Iowa darter (*Etheostoma exile*)



Median ratio: 2.37

R^2: 0.90
F: 17.3
df: 2
P: 0.055
Not used because P > 0.05

Largemouth bass (*Micropterus salmoides*)

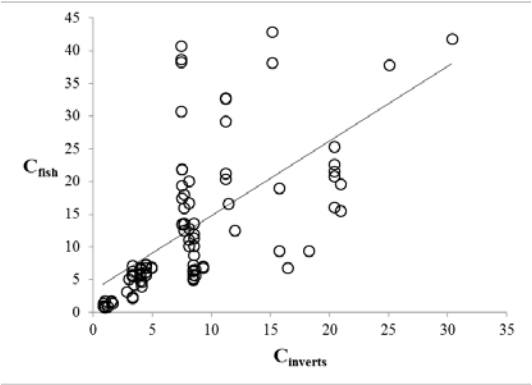
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	MP	1.60	1.40	0.88
Garcia-Hernandez et al. 2000	Cienega de Santa Clara Wetland	3.00	5.10	1.70
GEI 2013	SWA1	2.81	3.17	1.13
GEI 2013	SW11	8.41	5.02	0.60
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	5.19	0.62
GEI 2013	SW11	8.41	6.26	0.74
GEI 2013	SW11	8.41	6.48	0.77
GEI 2013	SW11	8.41	7.22	0.86
GEI 2013	SW4-1	3.33	5.53	1.66
GEI 2013	SW4-1	3.33	5.65	1.70
GEI 2013	SW4-1	3.33	5.72	1.72
GEI 2013	SW4-1	3.33	5.80	1.74
GEI 2013	SW4-1	3.33	6.34	1.91
GEI 2013	SW4-1	3.33	7.14	2.15
GEI 2013	SW9	4.45	5.78	1.30
GEI 2013	SW9	4.45	5.79	1.30
GEI 2013	SW9	4.45	6.19	1.39
GEI 2013	SW9	4.45	6.87	1.54
GEI 2013	SW9	4.45	7.27	1.63
GEI 2013	SW9	4.45	7.36	1.65
GEI 2013	SW88	3.96	4.87	1.23
GEI 2013	SW88	3.96	5.73	1.45
GEI 2013	SW88	3.96	5.77	1.46
GEI 2013	SW88	3.96	5.93	1.50
GEI 2013	SW88	3.96	6.62	1.67
GEI 2013	SW88	3.96	6.84	1.73

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	LG1	3.39	4.29	1.27
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	GT5	4.90	6.80	1.39
Saiki et al. 1993	GT5	4.90	6.90	1.41
Saiki et al. 1993	GT4	4.05	4.70	1.16
Saiki et al. 1993	GT4	4.05	4.00	0.99
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR2	3.30	2.40	0.73
Saiki et al. 1993	SJR3	1.50	1.80	1.20
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR1	0.95	0.80	0.85
Saiki et al. 1993	SJR1	0.95	1.80	1.90
Saiki et al. 1993	ET7	0.86	0.86	1.00
Saiki et al. 1993	ET7	0.86	1.00	1.16
Rinella and Schuler 1992	S. Malheur Lake	1.20	0.92	0.77
Crutchfield 2000	transect 3	11.95	12.52	1.05
Crutchfield 2000	transect 3	11.40	16.67	1.46
Crutchfield 2000	transect 3	9.25	6.83	0.74
Crutchfield 2000	transect 3	9.25	6.99	0.76
Crutchfield 2000	transect 3	8.60	6.59	0.77
Crutchfield 2000	transect 3	8.60	5.69	0.66
Crutchfield 2000	transect 4	20.90	15.53	0.74
Crutchfield 2000	transect 4	20.90	19.68	0.94
Crutchfield 2000	transect 4	15.70	18.95	1.21
Crutchfield 2000	transect 4	15.70	9.43	0.60
Crutchfield 2000	transect 4	16.45	6.83	0.42
Crutchfield 2000	transect 4	18.25	9.43	0.52
GEI 2014	ARB	11.21	20.41	1.82
GEI 2014	ARB	11.21	32.75	2.92
GEI 2014	ARB	11.21	32.73	2.92
GEI 2014	ARB	11.21	29.23	2.61
GEI 2014	ARB	11.21	21.26	1.90
GEI 2014	ARE	20.40	25.35	1.24
GEI 2014	ARE	20.40	20.80	1.02
GEI 2014	ARE	20.40	22.67	1.11
GEI 2014	ARE	20.40	21.57	1.06
GEI 2014	ARE	20.40	16.05	0.79
GEI 2014	ARM	8.51	13.62	1.60
GEI 2014	ARM	8.51	10.13	1.19
GEI 2014	ARM	8.51	12.00	1.41
GEI 2014	ARM	8.51	11.40	1.34

Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARM	8.51	8.71	1.02
GEI 2014	ARM	7.68	12.52	1.63
GEI 2014	ARM	7.68	13.59	1.77
GEI 2014	ARM	7.68	17.99	2.34
GEI 2014	ARM	7.68	13.49	1.76
GEI 2014	ARM	7.68	15.98	2.08
GEI 2014	ARN	8.06	11.16	1.39
GEI 2014	ARN	8.06	12.89	1.60
GEI 2014	ARN	8.06	16.74	2.08
GEI 2014	ARN	8.06	10.12	1.26
GEI 2014	ARN	8.06	20.08	2.49
GEI 2014	ARN	7.49	17.47	2.33
GEI 2014	ARN	7.49	13.48	1.80
GEI 2014	ARN	7.49	19.44	2.60
GEI 2014	ARN	7.49	21.87	2.92
GEI 2014	ARN	7.49	21.91	2.92
GEI 2014	ARN	7.44	30.73	4.13
GEI 2014	ARN	7.44	40.71	5.47
GEI 2014	ARN	7.44	38.75	5.21
GEI 2014	ARN	7.44	38.24	5.14
GEI 2014	SC-5	15.13	38.13	2.52
GEI 2014	SC-5	15.13	42.86	2.83
GEI 2014	SC-9	30.36	41.87	1.38
GEI 2014	SC-9	25.06	37.84	1.51

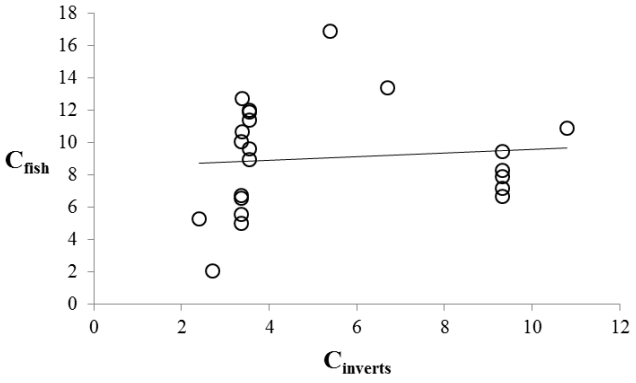


Median ratio: 1.39

R^2: 0.40
F: 61.45
df: 91
P: < 0.001

Longnose dace (*Rhinichthys cataractae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Lambing et al. 1994	S33	2.40	5.30	2.21
Mueller et al. 1991	A1	2.70	2.10	0.78
GEI 2013	LG1	3.37	5.05	1.50
GEI 2013	LG1	3.37	5.57	1.65
GEI 2013	LG1	3.37	6.57	1.95
GEI 2013	LG1	3.37	6.75	2.00
GEI 2013	LG1	3.37	10.08	2.99
GEI 2013	LG1	3.39	10.69	3.15
GEI 2013	LG1	3.39	12.77	3.77
GEI 2013	LG1	3.56	8.95	2.52
GEI 2013	LG1	3.56	9.63	2.71
GEI 2013	LG1	3.56	11.41	3.21
GEI 2013	LG1	3.56	11.94	3.36
GEI 2013	LG1	3.56	12.04	3.39
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	8.32	0.89
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	9.47	1.02
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.94	0.85
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	6.67	0.72
GEI 2014	Left Fork White Oak Creek, C-LFWOC1	9.32	7.19	0.77
Mueller et al. 1991	T1	5.40	16.90	3.13
Hamilton and Buhl 2005	CC	6.70	13.40	2.00
Hamilton and Buhl 2005	BGS	10.80	10.90	1.01

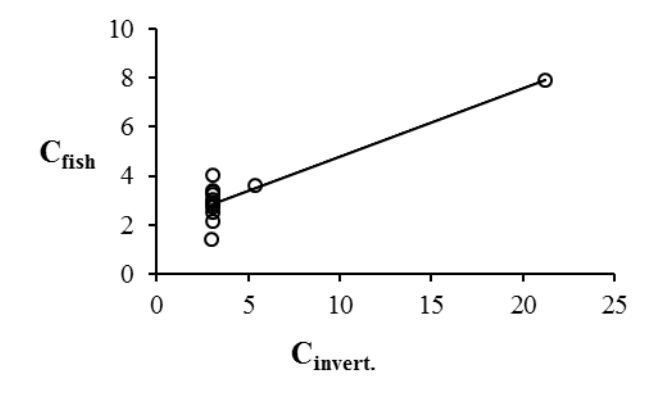


Median ratio: 2.00

R^2: 0.01
F: 0.19
df: 20
P: 0.83
Not used because P > 0.05

Longnose sucker (*Catostomus catostomus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	FL17	3.03	1.40	0.46
Butler et al. 1994	NFK2	3.10	2.10	0.68
Butler et al. 1994	NFK2	3.10	2.50	0.81
Butler et al. 1994	NFK2	3.10	2.70	0.87
Butler et al. 1994	NFK2	3.10	2.80	0.90
Butler et al. 1994	NFK2	3.10	2.90	0.94
Butler et al. 1994	NFK2	3.10	3.00	0.97
Butler et al. 1994	NFK2	3.10	3.20	1.03
Butler et al. 1994	NFK2	3.10	3.30	1.06
Butler et al. 1994	NFK2	3.10	3.40	1.10
Butler et al. 1994	NFK2	3.10	4.00	1.29
Mueller et al. 1991	T1	5.40	3.60	0.67
Minnow 2007	FL17	21.22	7.90	0.37



Median ratio: 0.90

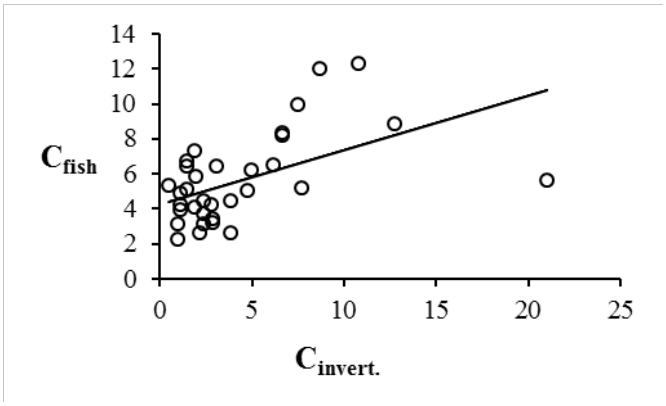
R^2: 0.83
F: 54.66
df: 11
P: <0.001

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	USC	0.50	5.30	10.60
Butler et al. 1993	LP2	1.00	2.20	2.20
Butler et al. 1993	LP2	1.00	3.10	3.10
Butler et al. 1993	LP3	1.12	3.90	3.50
Butler et al. 1993	LP3	1.12	4.20	3.77
Butler et al. 1993	LP3	1.12	4.90	4.39
Butler et al. 1993	P1	1.50	5.10	3.40
Butler et al. 1993	P1	1.50	6.40	4.27
Butler et al. 1993	P1	1.50	6.70	4.47
Hamilton and Buhl 2004	ShpC	1.90	4.10	2.16
Butler et al. 1993	P1	1.95	7.30	3.74
Butler et al. 1994	NFK3	2.00	5.80	2.90

Mottled sculpin (*Cottus bairdii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1997	MN2	2.20	2.60	1.18
Butler et al. 1997	CHK	2.40	3.10	1.29
Butler et al. 1997	CHK	2.40	4.40	1.83
Lambing et al. 1994	S33	2.40	3.70	1.54
Butler et al. 1991	12	2.80	4.20	1.50
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	MN1	2.90	3.40	1.17
Butler et al. 1994	NFK2	3.10	6.40	2.06
Butler et al. 1991	4	3.90	2.60	0.67
Butler et al. 1991	4	3.90	4.40	1.13
Butler et al. 1991	10	4.80	5.00	1.04
Hamilton and Buhl 2005	UAC	5.00	6.20	1.24
Butler et al. 1991	3	6.20	6.50	1.05
Hamilton and Buhl 2004	ACM	6.70	8.30	1.24
Hamilton and Buhl 2005	CC	6.70	8.20	1.22
Butler et al. 1993	F2	7.50	9.90	1.32
Hamilton and Buhl 2004	LBR	7.70	5.20	0.68
Hamilton and Buhl 2005	DC	8.70	12.00	1.38
Hamilton and Buhl 2005	BGS	10.80	12.30	1.14
Hamilton and Buhl 2004	DVC	12.80	8.80	0.69
Butler et al. 1994	HCC1	21.00	5.60	0.27



Median ratio: 1.38

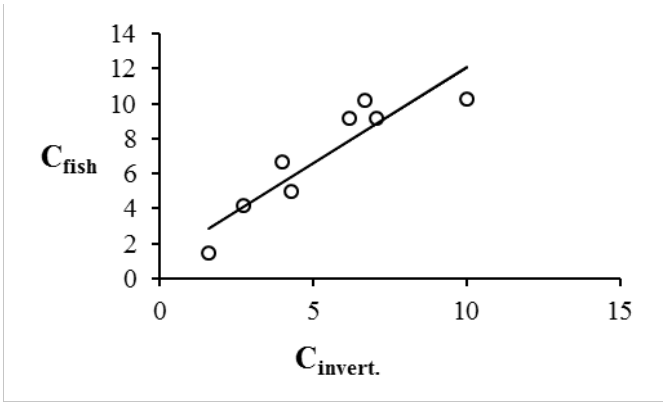
R^2: 0.27
F: 11.62
df: 31
P: < 0.001

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Low and Mullins 1990	7	1.60	1.40	0.88
McDonald and Strosher 1998	ER 745	2.74	4.17	1.52
Minnow 2007	EL12	4.01	6.60	1.65
McDonald and Strosher 1998	ER 747	4.29	4.93	1.15

Mountain whitefish (*Prosopium williamsoni*)

Study	Site	C_{invert}	C_{fish}	Ratio
Minnow 2007	MI3	6.21	9.12	1.47
Minnow 2007	MI2	6.69	10.16	1.52
Minnow 2007	EL1	7.08	9.12	1.29
Minnow 2007	FO23	10.00	10.20	1.02



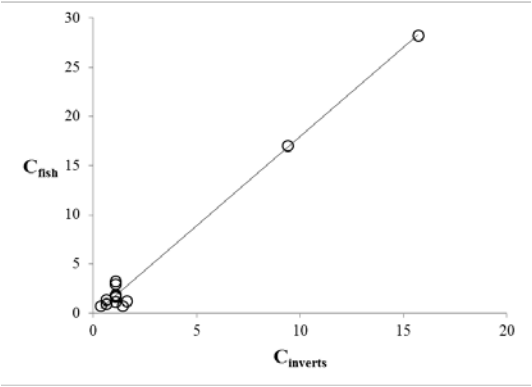
Median ratio: 1.38

R^2: 0.83
F: 30.27
df: 6
P: <0.001

Northern pike (*Esox lucius*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	TT	1.07	2.96	2.78
Butler et al. 1995	TT	1.07	3.24	3.04
Butler et al. 1995	TT	1.07	1.65	1.55
Butler et al. 1995	TT	1.07	1.18	1.11
Butler et al. 1995	TT	1.07	1.90	1.78
Butler et al. 1995	TT	1.07	1.80	1.69
Butler et al. 1995	PU	0.61	0.93	1.52
Butler et al. 1995	PU	0.61	1.40	2.30
Muscatello et al. 2008	David Lake	1.39	0.78	0.56
Muscatello et al. 2008	Delta Lake	9.38	17.02	1.81
Muscatello et al. 2008	Unknown Lake	15.71	28.28	1.80
Muscatello and Janz 2009	Indigo Lake	0.36	0.75	2.08
Muscatello and Janz 2009	Vulture Lake	1.62	1.26	0.78

Northern pike (*Esox lucius*)

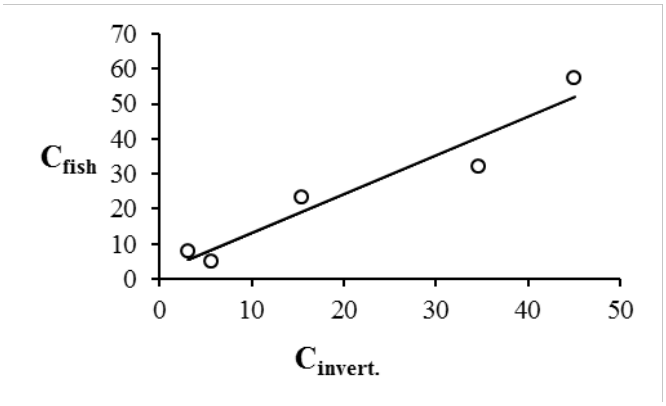


Median ratio: 1.78

R^2: 0.99
F: 982.9
df: 11
P: <0.001

Northern plains killfish (*Fundulus kansae*)

Study	Site	C_{invert}	C_{fish}	Ratio
Birkner 1978	3	3.10	7.70	2.48
Birkner 1978	11	5.65	5.00	0.88
Birkner 1978	23	15.50	23.10	1.49
Birkner 1978	27	34.60	31.90	0.92
Birkner 1978	30	45.05	57.40	1.27



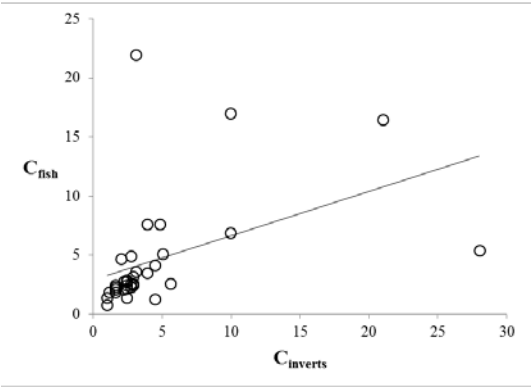
Median ratio: 1.27

R^2: 0.93
F: 37.8
df: 3
P: 0.008

Rainbow trout (*Oncorhynchus mykiss*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	4	3.90	3.50	0.90
Butler et al. 1993	F2	4.80	7.60	1.58
Butler et al. 1993	F2	3.90	7.60	1.95
Butler et al. 1993	LP2	1.00	0.78	0.78
Butler et al. 1993	LP2	1.00	1.40	1.40
Butler et al. 1993	LP3	1.12	1.90	1.70
Butler et al. 1994	GUN2	28.00	5.40	0.19
Butler et al. 1994	HCC1	21.00	16.48	0.78
Butler et al. 1994	NFK3	2.00	4.70	2.35
Butler et al. 1994	NFK2	3.10	21.98	7.09
Butler et al. 1994	NFK2	3.10	3.60	1.16
Butler et al. 1995	MP	1.60	1.88	1.18
Butler et al. 1995	MP	1.60	2.30	1.44
Butler et al. 1995	MP	1.60	2.50	1.56
Butler et al. 1995	MP	1.60	2.10	1.31
Butler et al. 1997	CHK	2.40	1.41	0.59
Butler et al. 1997	CHK	2.40	2.20	0.92
Butler et al. 1997	CHK	2.40	2.50	1.04
Butler et al. 1997	CHK	2.40	2.80	1.17
Butler et al. 1997	CHK	2.40	2.90	1.21
Butler et al. 1997	MN3	2.70	2.28	0.84
Butler et al. 1997	MN3	2.70	2.60	0.96
Butler et al. 1997	MN3	2.70	4.90	1.81
Butler et al. 1997	MN2	2.20	2.10	0.95
Butler et al. 1997	MN2	2.20	2.80	1.27
Butler et al. 1997	MN1	2.90	2.50	0.86
Butler et al. 1997	MN1	2.90	2.60	0.90
Butler et al. 1997	MN1	2.90	3.20	1.10
Butler et al. 1997	WBR	5.05	5.10	1.01
Low and Mullins 1990	5	5.60	2.60	0.46
Casey 2005	Deerlick Creek	4.45	1.29	0.29
Casey 2005	Deerlick Creek	4.45	4.15	0.93
Casey 2005	Luscar Creek	9.95	6.88	0.69
Casey 2005	Luscar Creek	9.95	17.04	1.71

Rainbow trout (*Oncorhynchus mykiss*)



Median ratio: 1.07

R^2: 0.19
F: 7.52
df: 32
P: 0.002

Red shiner (*Cyprinella lutrensis*)

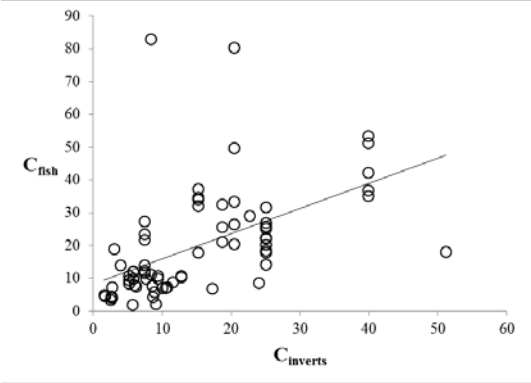
Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	3	6.20	7.70	1.24
Butler et al. 1994	IW	8.35	83.00	9.94
Butler et al. 1994	AD	2.70	7.30	2.70
Butler et al. 1994	LW	3.00	19.00	6.33
Butler et al. 1994	LSW1	3.90	14.00	3.59
Butler et al. 1995	ME4	1.55	5.10	3.29
Butler et al. 1995	ME3	2.55	4.60	1.80
Butler et al. 1995	ME3	2.55	4.20	1.65
Butler et al. 1995	SJ1	2.50	3.50	1.40
Butler et al. 1995	YJ2	1.65	4.50	2.73
Butler et al. 1997	MN4	2.65	4.20	1.58
Butler et al. 1997	MN5	8.60	4.40	0.51
May et al. 2008	KR	17.20	7.03	0.41
May et al. 2008	NSCL	10.70	7.36	0.69
May et al. 2008	NSCU	10.50	7.24	0.69
May et al. 2008	NSK	8.81	5.81	0.66
May et al. 2008	NSP	24.00	8.62	0.36
May et al. 2008	SSAL	11.50	9.00	0.78
May et al. 2008	SSAU	8.35	11.20	1.34
May et al. 2008	SSO	10.00	7.16	0.72
May et al. 2008	SSW	7.60	10.00	1.32
Mueller et al. 1991	A3	6.00	8.10	1.35
Mueller et al. 1991	A2	8.50	7.90	0.93
Lemly 1985	Badin Lake	5.70	2.10	0.37
Lemly 1985	Belews Lake	51.15	18.25	0.36
Lemly 1985	High Rock Lake	9.05	2.18	0.24
GEI 2014	ARE	20.40	49.84	2.44

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	ARE	20.40	80.50	3.95
GEI 2014	ARE	20.40	20.57	1.01
GEI 2014	ARE	20.40	33.37	1.64
GEI 2014	ARE	20.40	26.50	1.30
GEI 2014	ARN	7.44	27.50	3.70
GEI 2014	ARN	7.44	23.58	3.17
GEI 2014	ARN	7.44	21.74	2.92
GEI 2014	FC-4	18.65	21.20	1.14
GEI 2014	FC-4	18.65	32.68	1.75
GEI 2014	FC-4	18.65	25.73	1.38
GEI 2014	GC-1	9.33	10.78	1.16
GEI 2014	GC-1	9.33	9.97	1.07
GEI 2014	SC-2	12.75	10.44	0.82
GEI 2014	SC-2	12.75	10.87	0.85
GEI 2014	SC-3	5.75	12.05	2.10
GEI 2014	SC-3	5.75	12.17	2.12
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-3	5.75	9.93	1.73
GEI 2014	SC-4	7.39	12.26	1.66
GEI 2014	SC-4	7.39	11.68	1.58
GEI 2014	SC-4	7.39	14.15	1.92
GEI 2014	SC-4	5.18	9.58	1.85
GEI 2014	SC-4	5.18	8.43	1.63
GEI 2014	SC-4	5.18	10.83	2.09
GEI 2014	SC-5	15.13	17.96	1.19
GEI 2014	SC-5	15.13	34.71	2.29
GEI 2014	SC-5	15.13	34.05	2.25
GEI 2014	SC-5	15.13	37.28	2.46
GEI 2014	SC-5	15.13	32.18	2.13
GEI 2014	SC-6	39.87	53.60	1.34
GEI 2014	SC-6	39.87	37.00	0.93
GEI 2014	SC-6	39.87	35.11	0.88
GEI 2014	SC-6	39.87	51.39	1.29
GEI 2014	SC-6	39.87	42.31	1.06
GEI 2014	SC-8	22.62	29.20	1.29
GEI 2014	SC-9	25.06	22.55	0.90
GEI 2014	SC-9	25.06	18.02	0.72
GEI 2014	SC-9	25.06	25.94	1.04
GEI 2014	SC-9	25.06	18.68	0.75
GEI 2014	SC-9	25.06	14.28	0.57
GEI 2014	SC-9	25.06	31.67	1.26
GEI 2014	SC-9	25.06	20.43	0.82

Red shiner (*Cyprinella lutrensis*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2014	SC-9	25.06	22.27	0.89
GEI 2014	SC-9	25.06	27.05	1.08
GEI 2014	SC-9	25.06	25.28	1.01



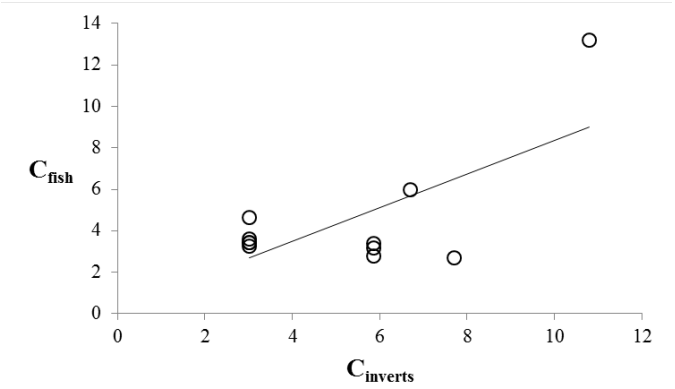
Median ratio: 1.31

R^2: 0.28
F: 26.57
df: 70
P: <0.001

Redside shiner (*Richardsonius balteatus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Hamilton and Buhl 2004	ACM	6.70	6.00	0.90
Hamilton and Buhl 2004	LBR	7.70	2.70	0.35
Hamilton and Buhl 2005	BGS	10.80	13.20	1.22
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	3.44	1.14
GEI 2014	Bond Creek, BC-3	3.02	4.64	1.54
GEI 2014	Bond Creek, BC-3	3.02	3.26	1.08
GEI 2014	Bond Creek, BC-3	5.87	3.18	0.54
GEI 2014	Bond Creek, BC-3	5.87	3.37	0.57
GEI 2014	Bond Creek, BC-3	5.87	2.79	0.47
GEI 2014	Bond Creek, BC-3	3.02	3.58	1.19

Redside shiner (*Richardsonius balteatus*)



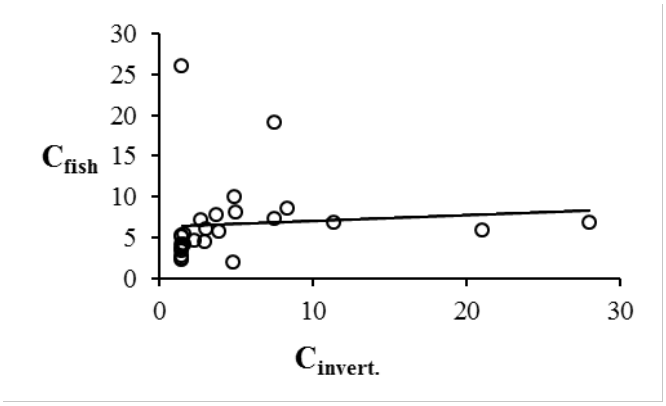
Median ratio: 1.08

R^2: 0.47
F: 7.84
df: 9
P: 0.011

Roundtail chub (*Gila robusta*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1994	COL1	1.50	2.20	1.47
Butler et al. 1994	COL1	1.50	2.50	1.67
Butler et al. 1994	COL1	1.50	2.70	1.80
Butler et al. 1994	COL1	1.50	3.30	2.20
Butler et al. 1994	COL1	1.50	3.70	2.47
Butler et al. 1994	COL1	1.50	4.10	2.73
Butler et al. 1994	COL1	1.50	5.10	3.40
Butler et al. 1994	COL1	1.50	5.30	3.53
Butler et al. 1994	COL1	1.50	26.00	17.33
Butler et al. 1994	RB3	1.60	5.40	3.38
Butler et al. 1995	MP	1.60	4.20	2.63
Butler et al. 1997	MUD	2.30	4.60	2.00
Butler et al. 1994	AD	2.70	7.10	2.63
Butler et al. 1994	LW	3.00	4.50	1.50
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	PSW1	3.70	7.70	2.08
Butler et al. 1994	LSW1	3.90	5.80	1.49
Butler et al. 1991	10	4.80	1.90	0.40
Butler et al. 1994	TGC	4.90	10.00	2.04
Butler et al. 1994	BSW1	5.00	8.10	1.62
Butler et al. 1993	F2	7.50	7.30	0.97
Butler et al. 1994	CRC	7.50	19.00	2.53
Butler et al. 1994	IW	8.35	8.50	1.02
Butler et al. 1997	NW2	11.40	6.90	0.61
Butler et al. 1994	RB1	21.00	5.90	0.28
Butler et al. 1994	GUN2	28.00	6.80	0.24

Roundtail chub (*Gila robusta*)



Median ratio: 1.98

R^2: 0.01
F: 0.18
df: 24
P: 0.834
Not used because P > 0.05

Sand shiner (*Notropis stramineus*)

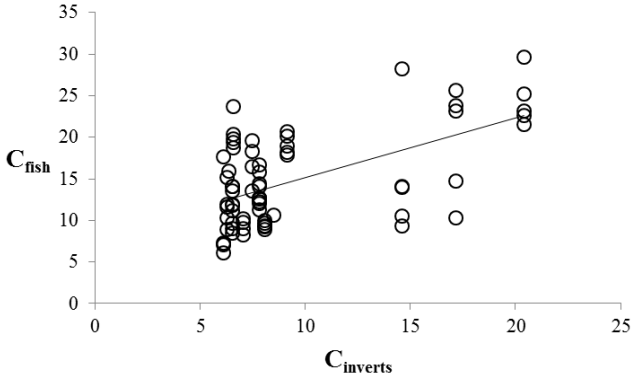
Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	6.54	8.43	1.29
GEI 2013	SW1	6.54	9.02	1.38
GEI 2013	SW1	6.54	9.66	1.48
GEI 2013	SW1	6.54	11.21	1.71
GEI 2013	SW1	6.54	11.85	1.81
GEI 2013	SW1	6.54	11.94	1.83
GEI 2013	SW1	6.54	13.50	2.06
GEI 2013	SW1	6.54	14.05	2.15
GEI 2013	SW1	6.54	14.14	2.16
GEI 2013	SW2-1	6.60	18.70	2.84
GEI 2013	SW2-1	6.60	19.33	2.93
GEI 2013	SW2-1	6.60	19.77	3.00
GEI 2013	SW2-1	6.60	20.39	3.09
GEI 2013	SW2-1	6.60	23.70	3.59
GEI 2013	SWB	7.06	8.27	1.17
GEI 2013	SWB	7.06	9.01	1.28
GEI 2013	SWB	7.06	9.81	1.39
GEI 2013	SWB	7.06	10.22	1.45
GEI 2013	SW1	7.82	11.33	1.45
GEI 2013	SW1	7.82	12.05	1.54
GEI 2013	SW1	7.82	12.22	1.56
GEI 2013	SW1	7.82	12.55	1.60
GEI 2013	SW1	7.82	12.65	1.62
GEI 2013	SW1	7.82	12.68	1.62
GEI 2013	SW1	7.82	14.13	1.81
GEI 2013	SW1	7.82	14.43	1.85
GEI 2013	SW1	7.82	15.87	2.03

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW1	7.82	16.63	2.13
GEI 2013	SW2-1	9.14	17.84	1.95
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	18.98	2.08
GEI 2013	SW2-1	9.14	20.12	2.20
GEI 2013	SW2-1	9.14	20.73	2.27
GEI 2014	Arkansas River, ARE	20.40	21.50	1.05
GEI 2014	Arkansas River, ARE	20.40	23.20	1.14
GEI 2014	Arkansas River, ARE	20.40	22.64	1.11
GEI 2014	Arkansas River, ARE	20.40	25.24	1.24
GEI 2014	Arkansas River, ARE	20.40	29.70	1.46
GEI 2014	Arkansas River, ARM	8.51	10.67	1.25
GEI 2014	Arkansas River, ARN	8.06	9.69	1.20
GEI 2014	Arkansas River, ARN	8.06	9.27	1.15
GEI 2014	Arkansas River, ARN	8.06	9.96	1.24
GEI 2014	Arkansas River, ARN	8.06	9.29	1.15
GEI 2014	Arkansas River, ARN	8.06	8.86	1.10
GEI 2014	Arkansas River, ARN	7.49	13.60	1.82
GEI 2014	Arkansas River, ARN	7.49	18.34	2.45
GEI 2014	Arkansas River, ARN	7.49	16.46	2.20
GEI 2014	Arkansas River, ARN	7.49	19.64	2.62
GEI 2014	Fountain Creek, FC-4	14.59	13.95	0.96
GEI 2014	Fountain Creek, FC-4	14.59	9.34	0.64
GEI 2014	Fountain Creek, FC-4	14.59	14.06	0.96
GEI 2014	Fountain Creek, FC-4	14.59	28.26	1.94
GEI 2014	Fountain Creek, FC-4	14.59	10.53	0.72
GEI 2014	Fountain Creek, FC-4	17.15	10.28	0.60
GEI 2014	Fountain Creek, FC-4	17.15	23.76	1.39
GEI 2014	Fountain Creek, FC-4	17.15	14.77	0.86
GEI 2014	Fountain Creek, FC-4	17.15	23.13	1.35
GEI 2014	Fountain Creek, FC-4	17.15	25.62	1.49
GEI 2014	Fountain Creek, FCP	6.13	17.62	2.88
GEI 2014	Fountain Creek, FCP	6.13	7.32	1.19
GEI 2014	Fountain Creek, FCP	6.13	7.14	1.17
GEI 2014	Fountain Creek, FCP	6.13	6.05	0.99
GEI 2014	Fountain Creek, FCP	6.13	7.11	1.16
GEI 2014	Fountain Creek, FCP	6.35	15.93	2.51
GEI 2014	St. Charles River, SC-4	6.29	11.92	1.90
GEI 2014	St. Charles River, SC-4	6.29	15.14	2.41
GEI 2014	St. Charles River, SC-4	6.29	8.94	1.42
GEI 2014	St. Charles River, SC-4	6.29	10.33	1.64
GEI 2014	St. Charles River, SC-4	6.29	11.58	1.84

Sand shiner (*Notropis stramineus*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.56

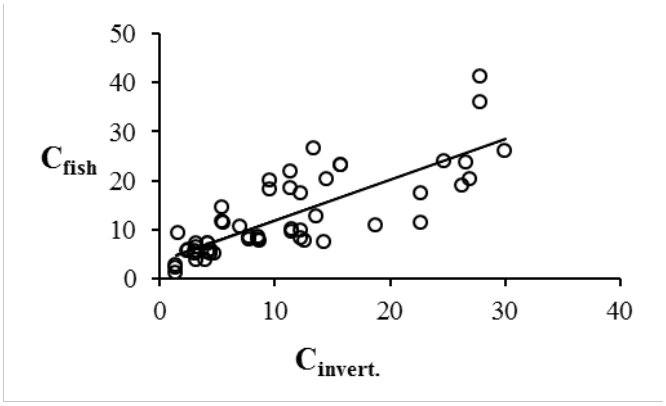
R^2: 0.32
F: 32.15
df: 67
P: <0.001

Sculpin (*Cottoidea*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	BK	1.43	1.16	0.81
Mason et al. 2000	BK	1.43	2.35	1.64
Mason et al. 2000	BK	1.43	2.64	1.84
Formation 2012	SFTC-1	1.63	9.31	5.71
Formation 2012	SFTC-1	2.42	5.68	2.35
Formation 2012	SFTC-1	2.49	5.87	2.36
Formation 2012	CC-75	3.11	5.03	1.62
Formation 2012	CC-75	3.11	5.58	1.79
Formation 2012	CC-350	3.16	6.47	2.05
Formation 2012	CC-350	3.16	7.12	2.26
Formation 2012	SFTC-1	3.21	3.75	1.17
Formation 2012	CC-75	3.97	3.77	0.95
Formation 2012	CC-75	4.16	7.08	1.70
Formation 2012	CC-75	4.16	7.19	1.73
Formation 2012	CC-350	4.20	5.28	1.26
Formation 2012	CC-150	4.46	5.04	1.13
Formation 2012	CC-150	4.46	6.01	1.35
Formation 2012	CC-150	4.70	5.14	1.09
Formation 2012	CC-3A	5.45	11.65	2.14
Formation 2012	CC-3A	5.45	14.45	2.65
Formation 2012	CC-3A	5.48	11.47	2.09
Formation 2012	CC-150	7.03	10.73	1.53
Formation 2012	DC-600	7.83	7.96	1.02
Formation 2012	DC-600	7.83	8.62	1.10

Sculpin (*Cottoidea*)

Study	Site	C_{invert}	C_{fish}	Ratio
Formation 2012	DC-600	8.53	7.87	0.92
Formation 2012	DC-600	8.53	8.50	1.00
Formation 2012	DC-600	8.65	7.63	0.88
Formation 2012	LSV-4	9.54	18.28	1.92
Formation 2012	LSV-4	9.54	20.01	2.10
Formation 2012	HS-3	11.40	18.57	1.63
Formation 2012	HS-3	11.40	21.85	1.92
Formation 2012	CC-350	11.45	9.53	0.83
Formation 2012	CC-350	11.45	10.03	0.88
Formation 2012	CC-1A	12.24	8.34	0.68
Formation 2012	CC-1A	12.24	9.94	0.81
Formation 2012	CC-1A	12.24	17.47	1.43
Formation 2012	CC-1A	12.57	7.78	0.62
Formation 2012	HS-3	13.41	26.63	1.99
Formation 2012	CC-1A	13.55	12.63	0.93
Formation 2012	CC-150	14.32	7.35	0.51
Formation 2012	CC-3A	14.50	20.20	1.39
Formation 2012	HS	15.70	23.23	1.48
Formation 2012	HS	15.70	23.25	1.48
Formation 2012	HS	18.70	10.95	0.59
Formation 2012	LSV-2C	22.62	11.38	0.50
Formation 2012	LSV-2C	22.62	17.47	0.77
Formation 2012	HS-3	24.70	23.93	0.97
Formation 2012	LSV-2C	26.31	18.85	0.72
Formation 2012	HS-3	26.55	23.68	0.89
Formation 2012	LSV-2C	26.95	20.32	0.75
Formation 2012	HS	27.80	35.93	1.29
Formation 2012	HS	27.80	41.30	1.49
Formation 2012	LSV-2C	30.00	25.95	0.87

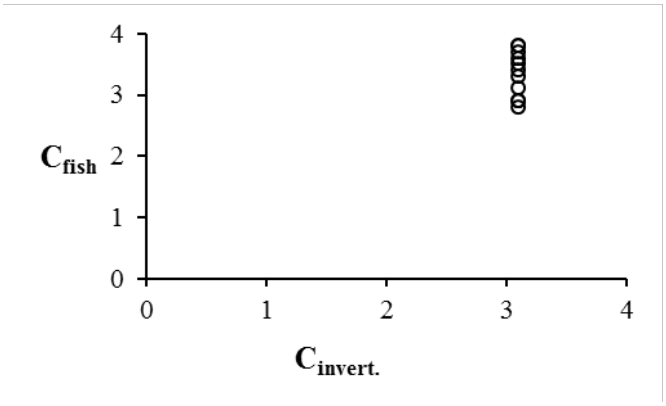


Median ratio: 1.29

R^2: 0.63
F: 87.0
df: 51
P: <0.001

Shorthead redhorse (*Moxostoma macrolepidotum*)

Study	Site	C_{invert}	C_{fish}	Ratio
Roddy et al. 1991	18	3.10	2.80	0.90
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	2.90	0.94
Roddy et al. 1991	18	3.10	3.10	1.00
Roddy et al. 1991	18	3.10	3.30	1.06
Roddy et al. 1991	18	3.10	3.40	1.10
Roddy et al. 1991	18	3.10	3.50	1.13
Roddy et al. 1991	18	3.10	3.60	1.16
Roddy et al. 1991	18	3.10	3.70	1.19
Roddy et al. 1991	18	3.10	3.80	1.23
Roddy et al. 1991	18	3.10	3.80	1.23



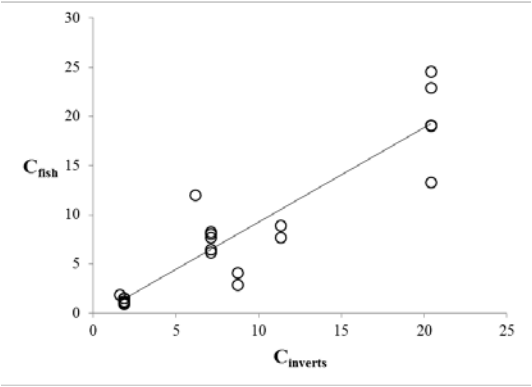
Median ratio: 1.10

R^2: 0.00
F: 0.00
df: 9
P: 1.0
Not used because P > 0.05

Smallmouth bass (*Micropterus dolomieu*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	SU	1.85	1.55	0.84
Butler et al. 1995	SU	1.85	1.22	0.66
Butler et al. 1995	SU	1.85	0.98	0.53
Butler et al. 1995	SU	1.85	1.14	0.62
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1997	MNP3	6.15	12.00	1.95
Mueller et al. 1991	R1	8.70	2.90	0.33
Mueller et al. 1991	R1	8.70	4.10	0.47
GEI 2014	ARE	20.40	24.61	1.21
GEI 2014	ARE	20.40	22.97	1.13
GEI 2014	ARE	20.40	13.28	0.65

Smallmouth bass (*Micropterus dolomieu*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	ARE	20.40	19.06	0.93
GEI 2014	ARE	20.40	19.11	0.94
GEI 2014	ARM	7.10	8.25	1.16
GEI 2014	ARM	7.10	8.04	1.13
GEI 2014	ARM	7.10	7.72	1.09
GEI 2014	ARM	7.10	6.21	0.87
GEI 2014	ARM	7.10	6.51	0.92
GEI 2014	C-SC1	11.30	8.94	0.79
GEI 2014	C-SC1	11.30	7.68	0.68



Median ratio: 0.86

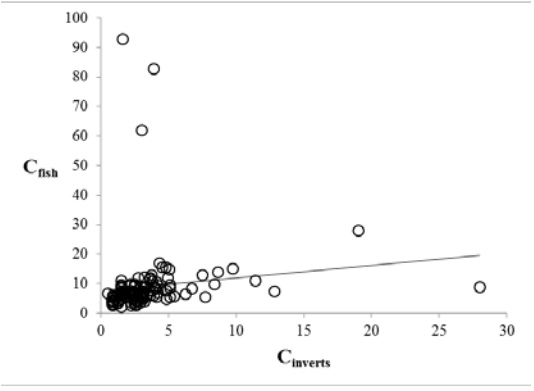
R^2: 0.84
F: 107.1
df: 20
P: <0.001

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1991	10	4.80	4.80	1.00
Butler et al. 1991	9	4.10	5.70	1.39
Butler et al. 1991	3	6.20	6.50	1.05
Butler et al. 1993	SP2	2.75	12.00	4.36
Butler et al. 1993	B2	1.35	5.80	4.30
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	B1	1.25	4.40	3.52
Butler et al. 1993	D1	1.20	3.50	2.92
Butler et al. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.40	2.83
Butler et al. 1993	D2	1.45	4.90	3.38
Butler et al. 1993	D2	1.45	6.80	4.69
Butler et al. 1993	D2	1.45	6.50	4.48
Butler et al. 1993	F2	3.90	8.90	2.28
Butler et al. 1993	P1	1.95	5.50	2.82
Butler et al. 1993	SP1	2.95	7.30	2.47

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	SP1	2.95	8.90	3.02
Butler et al. 1993	SP1	2.95	7.00	2.37
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1993	U1	2.45	6.90	2.82
Butler et al. 1993	U1	2.45	7.30	2.98
Butler et al. 1993	U1	2.45	9.20	3.76
Butler et al. 1993	U1	2.45	9.40	3.84
Butler et al. 1993	U1	2.45	9.80	4.00
Butler et al. 1993	LP3	1.12	6.00	5.38
Butler et al. 1993	LP4	3.20	8.70	2.72
Butler et al. 1993	R2	4.30	17.10	3.98
Butler et al. 1993	R2	3.90	6.00	1.54
Butler et al. 1993	ST2	4.50	15.70	3.49
Butler et al. 1993	ST2	4.10	8.50	2.07
Butler et al. 1993	ST2	4.10	10.70	2.61
Butler et al. 1993	ST2	3.35	9.30	2.78
Butler et al. 1993	R1	4.00	8.50	2.13
Butler et al. 1993	ST1	2.25	6.80	3.02
Butler et al. 1993	SB2	3.60	12.10	3.36
Butler et al. 1993	SB2	3.75	7.80	2.08
Butler et al. 1993	SB2	3.75	10.80	2.88
Butler et al. 1993	SB1	2.15	10.00	4.65
Butler et al. 1993	SB1	2.15	9.50	4.42
Butler et al. 1993	SB1	2.15	7.80	3.63
Butler et al. 1993	WSB2	4.75	15.60	3.28
Butler et al. 1993	WSB2	3.60	11.70	3.25
Butler et al. 1993	WSB2	3.00	6.20	2.07
Butler et al. 1993	WSB2	3.00	7.60	2.53
Butler et al. 1994	CRC	7.50	13.00	1.73
Butler et al. 1994	CF1	3.60	6.10	1.69
Butler et al. 1994	GUN2	28.00	8.90	0.32
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1994	LZA1	19.00	28.00	1.47
Butler et al. 1994	NFK3	2.00	7.10	3.55
Butler et al. 1994	NFK2	3.10	6.90	2.23
Butler et al. 1994	NFK2	3.10	4.80	1.55
Butler et al. 1994	NFK2	3.10	5.40	1.74
Butler et al. 1994	NFK2	3.10	5.70	1.84
Butler et al. 1994	NFK2	3.10	6.10	1.97
Butler et al. 1994	NFK2	3.10	6.20	2.00
Butler et al. 1994	NFK2	3.10	6.30	2.03
Butler et al. 1994	NFK2	3.10	6.40	2.06

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1994	NFK2	3.10	6.70	2.16
Butler et al. 1994	NFK2	3.10	7.40	2.39
Butler et al. 1994	NFK2	3.10	8.70	2.81
Butler et al. 1994	TGC	4.90	12.00	2.45
Butler et al. 1994	BSW1	5.00	15.00	3.00
Butler et al. 1994	COL1	1.50	2.30	1.53
Butler et al. 1994	COL1	1.50	5.00	3.33
Butler et al. 1994	COL1	1.50	7.30	4.87
Butler et al. 1994	COL1	1.50	7.40	4.93
Butler et al. 1994	COL1	1.50	8.40	5.60
Butler et al. 1994	COL1	1.50	8.60	5.73
Butler et al. 1994	COL1	1.50	9.30	6.20
Butler et al. 1994	COL1	1.50	9.60	6.40
Butler et al. 1994	COL1	1.50	11.00	7.33
Butler et al. 1994	RB3	1.60	93.00	58.13
Butler et al. 1994	SMF	4.80	7.80	1.63
Butler et al. 1994	LW	3.00	62.00	20.67
Butler et al. 1994	LSW1	3.90	83.00	21.28
Butler et al. 1994	PSW1	3.70	13.00	3.51
Butler et al. 1995	AK	0.78	4.30	5.55
Butler et al. 1995	AK	0.78	3.10	4.00
Butler et al. 1995	AK	0.78	4.00	5.16
Butler et al. 1995	DD	0.86	5.60	6.51
Butler et al. 1995	DD	0.86	4.40	5.12
Butler et al. 1995	DD	0.86	6.00	6.98
Butler et al. 1995	HD1	0.83	2.80	3.39
Butler et al. 1995	HD1	0.83	3.20	3.88
Butler et al. 1995	HD1	0.83	5.30	6.42
Butler et al. 1995	ME1	3.40	6.40	1.88
Butler et al. 1995	ME2	1.25	6.10	4.88
Butler et al. 1995	ME3	2.55	2.80	1.10
Butler et al. 1995	ME3	2.55	7.00	2.75
Butler et al. 1995	ME3	2.55	5.50	2.16
Butler et al. 1995	NW	5.10	8.70	1.71
Butler et al. 1995	SJ1	2.50	4.30	1.72
Butler et al. 1995	SJ1	2.50	5.10	2.04
Butler et al. 1995	SJ1	2.50	2.90	1.16
Butler et al. 1995	YJ2	1.65	6.50	3.94
Butler et al. 1995	YJ2	1.65	6.30	3.82
Butler et al. 1995	YJ2	1.65	7.10	4.30
Butler et al. 1995	MN1	2.70	5.50	2.04
Butler et al. 1997	CHK	2.40	5.20	2.17

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1997	CHK	2.40	3.80	1.58
Butler et al. 1997	MN3	2.70	6.00	2.22
Butler et al. 1997	MN3	2.70	4.30	1.59
Butler et al. 1997	MN2	2.20	2.70	1.23
Butler et al. 1997	MN2	2.20	3.60	1.64
Butler et al. 1997	MN1	2.90	3.70	1.28
Butler et al. 1997	MUD	2.30	7.20	3.13
Butler et al. 1997	MUD	2.30	6.10	2.65
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1997	WBR	5.05	9.70	1.92
Butler et al. 1997	WBR	5.05	5.50	1.09
Butler et al. 1997	MN4	2.65	7.90	2.98
Butler et al. 1997	MN5	8.60	14.00	1.63
Butler et al. 1997	MNQ	1.80	5.90	3.28
Hamilton and Buhl 2004	DVC	12.80	7.50	0.59
Hamilton and Buhl 2004	USC	0.50	6.90	13.80
Hamilton and Buhl 2004	ACM	6.70	8.50	1.27
Hamilton and Buhl 2004	LBR	7.70	5.60	0.73
Hamilton and Buhl 2005	LiB	5.40	5.80	1.07
Hamilton and Buhl 2005	SLC	9.70	15.20	1.57



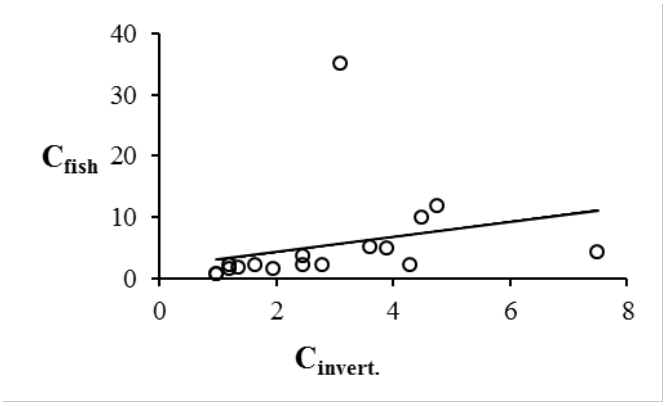
Median ratio: 2.76

R^2: 0.01
F: 1.76
df: 118
P: 0.177
Not used because P > 0.05

Sucker (Catostomidae)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	HD2	0.98	0.68	0.69
Butler et al. 1995	HD2	0.98	0.76	0.78
Butler et al. 1993	D1	1.20	2.30	1.92
Rinella and Schuler 1992	Malheur Lake	1.20	1.60	1.33

Sucker (Catostomidae)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	B2	1.35	1.80	1.33
Butler et al. 1995	YJ2	1.65	2.20	1.33
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1993	U1	2.45	2.30	0.94
Butler et al. 1993	U1	2.45	3.60	1.47
Butler et al. 1991	12	2.80	2.10	0.75
Butler et al. 1994	NFK2	3.10	35.00	11.29
Butler et al. 1993	SB2	3.60	5.10	1.42
Butler et al. 1993	R2	3.90	5.00	1.28
Butler et al. 1993	R2	4.30	2.20	0.51
Butler et al. 1993	ST2	4.50	10.00	2.22
Butler et al. 1993	WSB2	4.75	11.80	2.48
Butler et al. 1993	F2	7.50	4.20	0.56



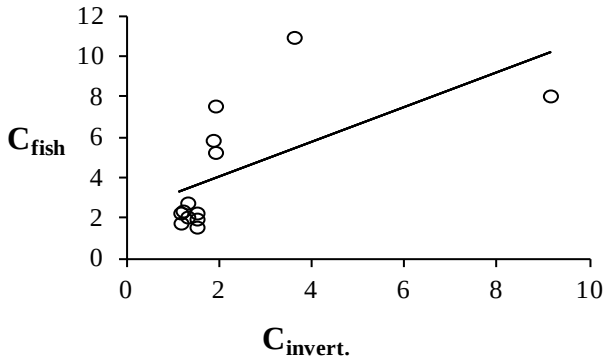
Median ratio: 1.33

R^2: 0.07
F: 1.10
df: 15
P: 0.360
Not used because P > 0.05

Sunfish (Centrarchidae)

Study	Site	C_{invert}	C_{fish}	Ratio
Welsh and Maughan 1994	outfall drain	1.15	2.30	2.00
Welsh and Maughan 1994	Pretty Water	1.16	1.80	1.56
Welsh and Maughan 1994	Hart Mine Marsh	1.20	2.40	2.00
Welsh and Maughan 1994	outfall drain	1.30	2.10	1.62
Welsh and Maughan 1994	outfall drain	1.30	2.80	2.15
Welsh and Maughan 1994	Pretty Water	1.50	1.60	1.07
Welsh and Maughan 1994	Old Channel	1.50	2.00	1.33
Welsh and Maughan 1994	Pretty Water	1.50	2.30	1.53
Welsh and Maughan 1994	Cibola Lake	1.85	5.90	3.19
Welsh and Maughan 1994	Cibola Lake	1.90	5.30	2.79
Welsh and Maughan 1994	Cibola Lake	1.90	7.60	4.00
Welsh and Maughan 1994	Oxbow Lake	3.60	11.00	3.06

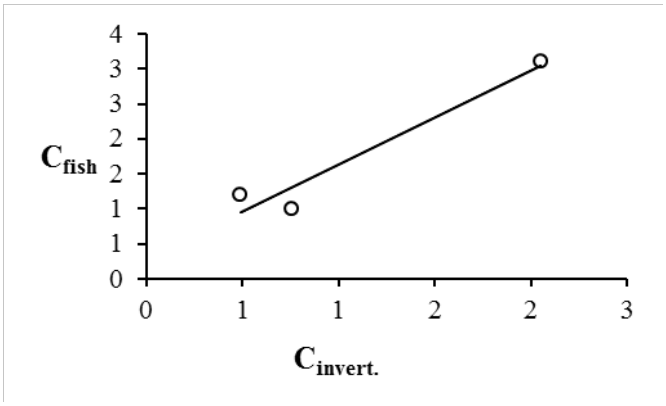
Sunfish (Centrarchidae)				
GEI 2013	SW2-1	9.14	8.10	0.89



Median ratio: 2.00

R^2: 0.38
F: 6.66
df: 11
P: 0.013

Tui chub (*Gila bicolor*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Sorenson & Schwarzbach 1991	5	0.49	1.20	2.45
Sorenson & Schwarzbach 1991	4	0.76	1.00	1.32
Rinella and Schuler 1992	Harney Lake	2.05	3.10	1.51

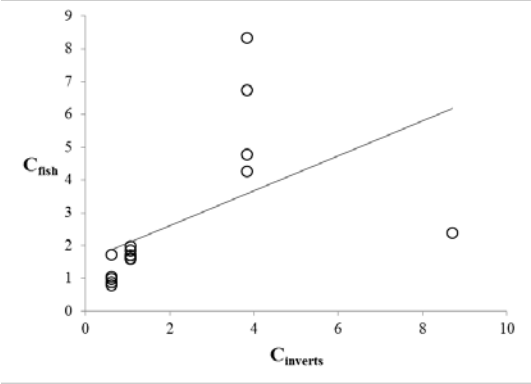


Median ratio: 1.51

R^2: 0.94
F: 15.9
df: 1
P: 0.175
Not used because P > 0.05

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.86	1.75
Butler et al. 1995	TT	1.07	1.62	1.52
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	TT	1.07	2.00	1.88

Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	PU	0.61	1.72	2.82
Butler et al. 1995	PU	0.61	1.05	1.73
Butler et al. 1995	PU	0.61	0.81	1.33
Butler et al. 1995	PU	0.61	1.00	1.64
Butler et al. 1995	PU	0.61	0.89	1.46
Mueller et al. 1991	R1	8.70	2.40	0.28
Peterson et al. 1991	7	3.83	4.27	1.11
Peterson et al. 1991	7	3.83	4.79	1.25
Peterson et al. 1991	7	3.83	6.76	1.77
Peterson et al. 1991	7	3.83	8.35	2.18



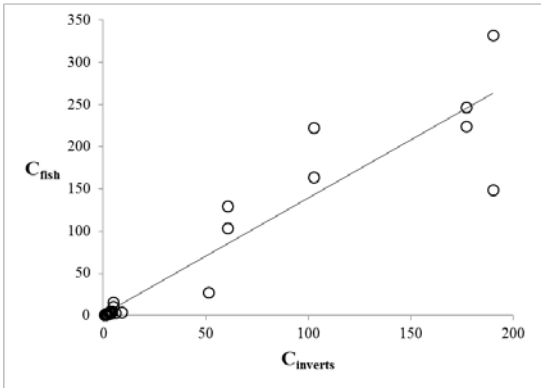
Median ratio: 1.60

R^2: 0.28
F: 5.46
df: 14
P: 0.018

Western mosquitofish (*Gambusia affinis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	SWA1	3.64	2.91	0.80
GEI 2013	SWA1	2.81	3.01	1.07
GEI 2013	SWA1	2.81	3.49	1.24
GEI 2013	SWA1	2.81	3.66	1.30
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.27	1.52
Lemly 1985	Badin Lake	5.70	3.35	0.59
Lemly 1985	Belews Lake	51.15	27.20	0.53
Lemly 1985	High Rock Lake	9.05	3.54	0.39
Saiki and Lowe 1987	Kesterson Pond 11	60.65	130.00	2.14
Saiki and Lowe 1987	Kesterson Pond 11	60.65	104.00	1.71
Saiki and Lowe 1987	Kesterson Pond 2	177.00	224.00	1.27
Saiki and Lowe 1987	Kesterson Pond 2	177.00	247.00	1.40
Saiki and Lowe 1987	Kesterson Pond 8	102.50	164.00	1.60

Western mosquitofish (*Gambusia affinis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Saiki and Lowe 1987	Kesterson Pond 8	102.50	223.00	2.18
Saiki and Lowe 1987	San Luis Drain	190.00	149.00	0.78
Saiki and Lowe 1987	San Luis Drain	190.00	332.00	1.75
Saiki and Lowe 1987	Volta Pond 26	1.42	1.28	0.90
Saiki and Lowe 1987	Volta Pond 26	1.42	1.24	0.87
Saiki and Lowe 1987	Volta Wasteway	2.23	1.35	0.61
Saiki and Lowe 1987	Volta Wasteway	2.23	1.36	0.61
Saiki et al. 1993	ET6	0.85	1.00	1.18
Saiki et al. 1993	ET6	0.85	1.30	1.54
Saiki et al. 1993	GT5	4.90	16.00	3.27
Saiki et al. 1993	GT5	4.90	11.00	2.24
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT4	4.05	4.90	1.21
Saiki et al. 1993	SJR2	3.30	4.50	1.36
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki et al. 1993	SJR1	0.95	0.95	1.01
Saiki et al. 1993	SJR1	0.95	1.30	1.38
Saiki et al. 1993	ET7	0.86	0.90	1.05
Saiki et al. 1993	ET7	0.86	1.00	1.16



Median ratio: 1.21

R^2: 0.89
F: 263.3
df: 33
P: <0.001

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1993	LP3	1.12	2.50	2.24
Butler et al. 1993	B1	1.25	2.60	2.08
Butler et al. 1993	D2	1.45	1.90	1.31
Butler et al. 1993	D2	1.45	2.50	1.72

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	P1	1.50	1.70	1.13
Butler et al. 1993	P1	1.50	1.80	1.20
Butler et al. 1995	MP	1.60	1.40	0.88
Butler et al. 1995	SU	1.85	1.20	0.65
GEI 2014	Arkansas River, ARB	11.21	14.90	1.33
GEI 2014	Arkansas River, ARB	11.21	20.39	1.82
GEI 2014	Arkansas River, ARB	11.21	13.82	1.23
GEI 2014	Arkansas River, ARB	11.21	8.36	0.75
GEI 2014	Arkansas River, ARB	11.21	10.88	0.97
GEI 2014	Arkansas River, ARB	11.21	21.55	1.92
GEI 2014	Arkansas River, ARB	11.21	18.70	1.67
GEI 2014	Arkansas River, ARB	11.21	24.53	2.19
GEI 2014	Arkansas River, ARB	11.21	15.02	1.34
GEI 2014	Arkansas River, ARB	11.21	28.29	2.52
GEI 2014	Arkansas River, ARE	20.4	18.21	0.89
GEI 2014	Arkansas River, ARE	20.4	19.54	0.96
GEI 2014	Arkansas River, ARE	20.4	15.27	0.75
GEI 2014	Arkansas River, ARE	20.4	11.37	0.56
GEI 2014	Arkansas River, ARE	20.4	17.86	0.88
GEI 2014	Arkansas River, ARE	20.4	10.62	0.52
GEI 2014	Arkansas River, ARE	20.4	17.51	0.86
GEI 2014	Arkansas River, ARE	20.4	24.66	1.21
GEI 2014	Arkansas River, ARE	20.4	18.92	0.93
GEI 2014	Arkansas River, ARE	20.4	21.70	1.06
GEI 2014	Arkansas River, ARM	8.51	10.13	1.19
GEI 2014	Arkansas River, ARM	8.51	9.24	1.09
GEI 2014	Arkansas River, ARM	8.51	8.30	0.97
GEI 2014	Arkansas River, ARM	8.51	10.09	1.19
GEI 2014	Arkansas River, ARM	7.68	16.18	2.11
GEI 2014	Arkansas River, ARM	7.68	13.21	1.72
GEI 2014	Arkansas River, ARM	7.68	11.96	1.56
GEI 2014	Arkansas River, ARM	7.68	8.58	1.12
GEI 2014	Arkansas River, ARM	7.68	9.73	1.27
GEI 2014	Arkansas River, ARM	7.1	8.19	1.15
GEI 2014	Arkansas River, ARM	7.1	7.96	1.12
GEI 2014	Arkansas River, ARN	7.49	9.15	1.22
GEI 2014	Arkansas River, ARN	7.49	8.61	1.15
GEI 2014	Arkansas River, ARN	7.49	7.06	0.94
GEI 2014	Arkansas River, ARN	7.49	11.57	1.54
GEI 2014	Arkansas River, ARN	7.49	11.56	1.54
GEI 2014	Arkansas River, ARN	7.44	21.20	2.85
GEI 2014	Arkansas River, ARN	7.44	23.28	3.13

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Arkansas River, ARN	7.44	20.85	2.80
GEI 2014	Arkansas River, ARN	7.44	25.91	3.48
GEI 2014	Bond Creek, BC-2	2.96	3.06	1.03
GEI 2014	Bond Creek, BC-2	2.96	3.54	1.19
GEI 2014	Bond Creek, BC-2	2.96	3.04	1.03
GEI 2014	Bond Creek, BC-3	3.02	2.76	0.91
GEI 2014	Bond Creek, BC-3	3.02	2.85	0.94
GEI 2014	Bond Creek, BC-3	3.02	2.47	0.82
GEI 2014	Bond Creek, BC-3	3.02	2.03	0.67
GEI 2014	Bond Creek, BC-3	3.02	2.23	0.74
GEI 2014	Cow Camp Creek, CC-2	5.65	4.46	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	4.45	0.79
GEI 2014	Cow Camp Creek, CC-2	5.65	6.19	1.09
GEI 2014	Cow Camp Creek, CC-2	5.65	4.71	0.83
GEI 2014	Cow Camp Creek, CC-2	5.65	5.38	0.95
GEI 2014	Seng Creek, C-SC1	11.302	20.32	1.80
GEI 2014	Dry Creek, DC-4	19.42	26.07	1.34
GEI 2014	Dry Creek, DC-4	19.42	22.55	1.16
GEI 2014	Dry Creek, DC-4	19.42	14.29	0.74
GEI 2014	Dry Creek, DC-4	19.42	14.25	0.73
GEI 2014	Dry Creek, DC-4	19.42	14.67	0.76
GEI 2014	Dry Creek, DC-4	18.1	29.83	1.65
GEI 2014	Dry Creek, DC-4	18.1	30.65	1.69
GEI 2014	Dry Creek, DC-4	18.1	20.87	1.15
GEI 2014	Dry Creek, DC-4	18.1	12.06	0.67
GEI 2014	Fountain Creek, FC-4	18.65	24.54	1.32
GEI 2014	Fountain Creek, FCP	6.13	5.33	0.87
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.88	0.96
GEI 2014	Fountain Creek, FCP	6.13	5.75	0.94
GEI 2014	Fountain Creek, FCP	6.13	4.37	0.71
GEI 2014	Fountain Creek, FCP	5.38	8.50	1.58
GEI 2014	Fountain Creek, FCP	5.38	5.94	1.10
GEI 2014	Fountain Creek, FCP	5.38	5.97	1.11
GEI 2014	Fountain Creek, FCP	5.38	5.76	1.07
GEI 2014	Fountain Creek, FCP	5.38	5.61	1.04
GEI 2014	Fountain Creek, FCP	6.35	15.82	2.49
GEI 2014	Fountain Creek, FCP	6.35	5.68	0.90
GEI 2014	Fountain Creek, FCP	6.35	10.17	1.60
GEI 2014	Fountain Creek, FCP	6.35	12.34	1.94
GEI 2014	Fountain Creek, FCP	6.35	10.64	1.68
GEI 2014	Foidel Creek, FOC-2	2.175	1.74	0.80

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	Foidel Creek, FOC-2	2.175	1.25	0.57
GEI 2014	Foidel Creek, FOC-2	2.175	1.76	0.81
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	1.64	0.76
GEI 2014	Foidel Creek, FOC-2	2.175	2.11	0.97
GEI 2014	Foidel Creek, FOC-2	2.175	2.29	1.05
GEI 2014	Grassy Creek, GC-2	4.195	4.45	1.06
GEI 2014	Grassy Creek, GC-2	4.195	4.42	1.05
GEI 2014	Grassy Creek, GC-2	4.195	2.51	0.60
GEI 2014	Grassy Creek, GC-3	4.535	2.78	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.535	2.84	0.63
GEI 2014	Grassy Creek, GC-3	4.535	4.37	0.96
GEI 2014	Grassy Creek, GC-3	4.535	2.89	0.64
GEI 2014	Grassy Creek, GC-3	4.545	4.29	0.94
GEI 2014	Grassy Creek, GC-3	4.545	3.16	0.69
GEI 2014	Grassy Creek, GC-3	4.545	2.76	0.61
GEI 2014	Grassy Creek, GC-3	4.34	4.12	0.95
GEI 2014	Grassy Creek, GC-3	4.35	3.96	0.91
GEI 2014	Grassy Creek, GC-4	5.1	0.93	0.18
GEI 2014	Grassy Creek, GC-4	5.1	1.40	0.27
GEI 2014	Grassy Creek, GC-4	5.1	4.12	0.81
GEI 2014	Grassy Creek, GC-4	5.76	7.04	1.22
GEI 2014	Grassy Creek, GC-4	5.76	7.42	1.29
GEI 2014	Grassy Creek, GC-4	5.76	4.22	0.73
GEI 2014	Grassy Creek, GC-4	5.76	4.75	0.82
GEI 2014	Grassy Creek, GC-4 US	13.16	6.32	0.48
GEI 2014	Grassy Creek, GC-4 US	13.16	4.74	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.98	0.38
GEI 2014	Grassy Creek, GC-4 US	13.16	4.75	0.36
GEI 2014	Grassy Creek, GC-4 US	13.16	4.88	0.37
GEI 2014	Middle Creek, MC-1	3.21	2.03	0.63
GEI 2014	Middle Creek, MC-1	3.21	2.18	0.68
GEI 2014	Middle Creek, MC-1	3.21	1.79	0.56
GEI 2014	Middle Creek, MC-1	3.21	2.28	0.71
GEI 2014	Middle Creek, MC-1	3.21	2.54	0.79
GEI 2014	Middle Creek, MC-2	4.19	2.58	0.62
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	1.86	0.44
GEI 2014	Middle Creek, MC-2	4.19	1.90	0.45
GEI 2014	Middle Creek, MC-2	4.19	2.10	0.50
GEI 2014	St. Charles River, SC-3	5.75	7.74	1.35

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2014	St. Charles River, SC-3	5.75	12.34	2.15
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.76	3.09
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	38.23	2.53
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	46.59	3.08
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	60.66	4.01
GEI 2014	St. Charles River at US Hwy 50, SC-5	15.13	43.70	2.89
GEI 2014	St. Charles River, SC-9	25.06	35.97	1.44
GEI 2014	St. Charles River, SC-9	25.06	38.53	1.54
GEI 2014	St. Charles River at I-25, SC-I	4.335	7.62	1.76
GEI 2014	St. Charles River at I-25, SC-I	4.335	8.20	1.89
GEI 2014	St. Charles River at I-25, SC-I	4.335	6.10	1.41
GEI 2014	St. Charles River at I-25, SC-I	4.335	22.44	5.18
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.29	0.99
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.79	1.11
GEI 2014	St. Charles River at I-25, SC-I	4.335	47.18	10.88
GEI 2014	St. Charles River at I-25, SC-I	4.335	4.88	1.12
GEI 2014	Wildhorse Creek, WHC	56.14	5.43	0.10
GEI 2014	Wildhorse Creek, WHC	34.24	36.23	1.06
GEI 2014	Wildhorse Creek, WHC	34.24	10.22	0.30
GEI 2014	Wildhorse Creek, WHC	34.24	52.16	1.52
GEI 2014	Wildhorse Creek, WHC	34.24	40.81	1.19
GEI 2014	Wildhorse Creek, WHC	34.24	26.45	0.77
GEI 2014	Wildhorse Creek, WHC	62.34	61.90	0.99
GEI 2014	Wildhorse Creek, WHC	62.34	15.88	0.25
GEI 2014	Wildhorse Creek, WHC	62.34	27.40	0.44
GEI 2014	Wildhorse Creek, WHC	62.34	23.10	0.37
Muscatello and Janz 2009	Indigo Lake	0.36	0.99	2.75
Muscatello and Janz 2009	Vulture Lake	1.62	3.37	2.08
Grasso et al. 1995	17	1.91	2.84	1.49
Grasso et al. 1995	17	1.91	3.19	1.67
Grasso et al. 1995	17	1.91	3.44	1.80
Grasso et al. 1995	17	1.91	3.64	1.91

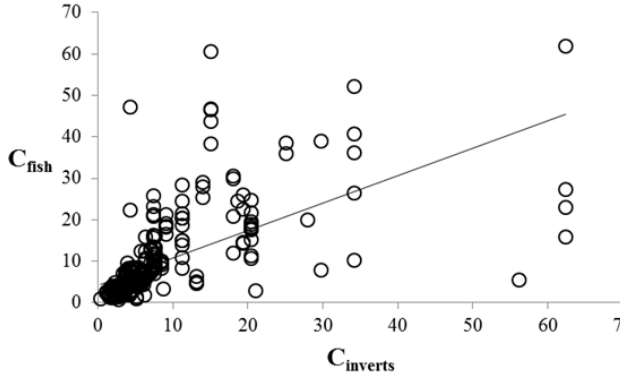
White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Grasso et al. 1995	17	1.91	4.00	2.09
Grasso et al. 1995	17	1.91	4.01	2.10
Butler et al. 1994	NFK3	2.00	3.90	1.95
Butler et al. 1993	ST1	2.25	4.90	2.18
Lambing et al. 1994	S33	2.40	3.50	1.46
Mueller et al. 1991	A1	2.70	4.20	1.56
GEI 2013	SWA1	2.81	2.83	1.01
GEI 2013	SWA1	2.81	3.89	1.39
GEI 2013	SWA1	2.81	4.18	1.49
Mason et al. 2000	HCRT	2.81	0.81	0.29
Mason et al. 2000	HCRT	2.81	1.43	0.51
Mason et al. 2000	HCRT	2.81	1.43	0.51
Butler et al. 1993	WSB2	3.00	3.90	1.30
Butler et al. 1993	SP2	3.15	3.50	1.11
Butler et al. 1993	LP4	3.20	2.80	0.88
GEI 2013	SW4-1	3.33	3.01	0.91
GEI 2013	SW4-1	3.33	3.45	1.04
GEI 2013	SW4-1	3.33	3.50	1.05
GEI 2013	SW4-1	3.33	3.62	1.09
GEI 2013	SW4-1	3.33	4.04	1.22
GEI 2013	SW4-1	3.33	4.08	1.23
GEI 2013	SW4-1	3.33	4.13	1.24
GEI 2013	SW4-1	3.33	4.17	1.25
GEI 2013	SW4-1	3.33	4.34	1.31
GEI 2013	SW4-1	3.33	4.78	1.44
Butler et al. 1993	ST2	3.35	7.00	2.09
GEI 2013	LG1	3.37	3.54	1.05
GEI 2013	LG1	3.37	3.55	1.05
GEI 2013	LG1	3.37	3.90	1.16
GEI 2013	LG1	3.37	3.95	1.17
GEI 2013	LG1	3.37	4.48	1.33
GEI 2013	LG1	3.39	3.00	0.88
GEI 2013	LG1	3.56	2.72	0.77
GEI 2013	LG1	3.56	2.80	0.79
GEI 2013	LG1	3.56	2.89	0.81
GEI 2013	LG1	3.56	2.99	0.84
GEI 2013	LG1	3.56	3.04	0.86
GEI 2013	LG1	3.56	3.08	0.87
GEI 2013	LG1	3.56	3.13	0.88
GEI 2013	LG1	3.56	3.18	0.89
GEI 2013	LG1	3.56	3.25	0.91
GEI 2013	LG1	3.56	3.27	0.92

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	WSB2	3.60	4.30	1.19
Butler et al. 1993	WSB2	3.60	6.30	1.75
GEI 2013	SWA1	3.64	2.83	0.78
GEI 2013	SWA1	3.64	3.39	0.93
GEI 2013	SWA1	3.64	3.47	0.95
GEI 2013	SWA1	3.64	3.55	0.98
GEI 2013	SWA1	3.64	3.63	1.00
GEI 2013	SWA1	3.64	3.75	1.03
Butler et al. 1993	SB2	3.65	4.30	1.18
Butler et al. 1993	R2	3.70	4.20	1.14
Butler et al. 1993	SB2	3.75	4.80	1.28
GEI 2013	CC1	3.76	5.99	1.59
GEI 2013	CC1	3.76	6.56	1.74
GEI 2013	CC1	3.76	7.21	1.92
GEI 2013	CC1	3.76	7.42	1.97
GEI 2013	CC1	3.76	7.62	2.03
Peterson et al. 1991	7	3.83	3.30	0.86
Peterson et al. 1991	7	3.83	4.64	1.21
Butler et al. 1993	R2	3.90	5.40	1.38
Butler et al. 1991	4	3.90	5.30	1.36
GEI 2013	SW88	3.96	4.63	1.17
GEI 2013	SW88	3.96	4.75	1.20
Butler et al. 1993	R1	4.00	9.50	2.38
Butler et al. 1993	ST2	4.10	8.30	2.02
GEI 2013	SW9	4.45	4.07	0.91
GEI 2013	SW9	4.45	4.18	0.94
GEI 2013	SW9	4.45	4.19	0.94
GEI 2013	SW9	4.45	4.20	0.94
GEI 2013	SW9	4.45	4.40	0.99
GEI 2013	SW9	4.45	5.18	1.16
GEI 2013	CC1	4.69	4.51	0.96
GEI 2013	CC1	4.69	4.57	0.98
GEI 2013	CC1	4.69	4.94	1.05
GEI 2013	CC1	4.69	5.02	1.07
GEI 2013	CC1	4.69	5.81	1.24
GEI 2013	CC1	4.69	6.01	1.28
GEI 2013	CC1	4.69	6.43	1.37
GEI 2013	CC1	4.69	7.25	1.55
GEI 2013	CC1	4.69	8.00	1.71
GEI 2013	CC1	4.69	8.52	1.82
Butler et al. 1993	F2	4.80	5.20	1.08
GEI 2013	CC1	5.86	5.00	0.85

White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
GEI 2013	CC1	5.86	5.37	0.92
GEI 2013	CC1	5.86	5.59	0.95
GEI 2013	CC1	5.86	5.71	0.98
GEI 2013	CC1	5.86	5.90	1.01
GEI 2013	CC1	5.86	6.61	1.13
GEI 2013	CC1	5.86	6.79	1.16
GEI 2013	CC1	5.86	6.82	1.16
GEI 2013	CC1	5.86	7.29	1.25
GEI 2013	CC1	5.86	7.48	1.28
Butler et al. 1991	3	6.20	1.80	0.29
GEI 2013	SWB	7.06	7.18	1.02
GEI 2013	SWB	7.06	7.36	1.04
GEI 2013	SWB	7.06	7.98	1.13
GEI 2013	SWB	7.06	8.03	1.14
GEI 2013	SWB	7.06	9.65	1.37
GEI 2013	SWB	7.06	12.76	1.81
GEI 2013	SWB	7.06	12.85	1.82
GEI 2013	SWB	7.06	13.16	1.86
GEI 2013	SWB	7.44	8.21	1.10
GEI 2013	SWB	7.44	8.77	1.18
GEI 2013	SWB	7.44	8.85	1.19
GEI 2013	SWB	7.44	9.87	1.33
GEI 2013	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	13.59	1.83
GEI 2013	SWB	7.44	15.75	2.12
GEI 2013	SWB	7.44	16.40	2.21
Butler et al. 1994	IW	8.35	9.70	1.16
Mueller et al. 1991	R1	8.70	3.40	0.39
GEI 2013	SW2-1	9.14	16.54	1.81
GEI 2013	SW2-1	9.14	18.14	1.99
GEI 2013	SW2-1	9.14	18.54	2.03
GEI 2013	SW2-1	9.14	19.16	2.10
GEI 2013	SW2-1	9.14	21.29	2.33
Lambing et al. 1994	S34	14.00	25.30	1.81
Lambing et al. 1994	S34	14.00	28.00	2.00
Lambing et al. 1994	S34	14.00	29.00	2.07
Butler et al. 1994	HCC1	21.00	3.00	0.14
Butler et al. 1994	GUN2	28.00	20.00	0.71
Butler et al. 1991	7	29.80	7.90	0.27

White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio



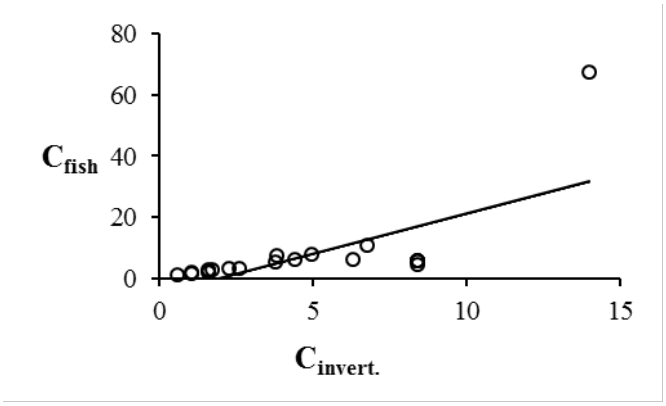
Median ratio: 1.11

R^2: 0.38
F: 174.4
df: 284
P: <0.001

Yellow perch (*Perca flavescens*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	PU	0.61	1.10	1.80
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	TT	1.07	1.70	1.60
Butler et al. 1995	MP	1.60	2.00	1.25
Butler et al. 1995	MP	1.60	2.20	1.38
Butler et al. 1995	MP	1.60	2.70	1.69
Belize et al. 2006	Halfway	1.74	2.72	1.56
Belize et al. 2006	Geneva	2.29	3.30	1.44
Belize et al. 2006	Bethel	2.61	3.09	1.19
Belize et al. 2006	McFarlane	3.79	5.40	1.42
Peterson et al. 1991	7	3.83	7.33	1.91
Belize et al. 2006	Long	4.42	6.28	1.42
Belize et al. 2006	Ramsey	4.97	7.64	1.54
Belize et al. 2006	Windy	6.32	6.06	0.96
Belize et al. 2006	Nelson	6.79	10.68	1.57
GEI 2013	SW11	8.41	4.54	0.54
GEI 2013	SW11	8.41	5.49	0.65
GEI 2013	SW11	8.41	5.50	0.65
GEI 2013	SW11	8.41	5.58	0.66
GEI 2013	SW11	8.41	5.68	0.68
Lambing et al. 1994	S34	14.00	67.00	4.79

Yellow perch (*Perca flavescens*)



Median ratio: 1.42

R^2: 0.46
F: 16.24
df: 19
P: <0.001

Table B-7. Final vertebrate Trophic Transfer Factor (TTF) values, including estimated values using taxonomic classification.

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
alligator gar	*Atractosteus spatula*	Lepistosteiformes	Lepisosteidae	Atractosteus	1.21	All fish
black bullhead	*Ameiurus melas*	Siluriformes	Ictaluridae	Ameiurus	0.85	Exact match
black crappie	*Pomoxis nigromaculatus*	Perciformes	Centrarchidae	Pomoxis	2.67	Exact match
black redhorse	*Moxostoma duquesnei*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
blacknose dace	*Rhinichthys atratulus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Exact match
blue catfish	*Ictalurus furcatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Genus Ictalurus
bluegill	*Lepomis macrochirus*	Perciformes	Centrarchidae	Lepomis	1.03	Exact match
bluehead sucker	*Catostomus discobolus*	Cypriniformes	Catostomidae	Catostomus	1.04	Exact match
brassy minnow	*Hybognathus hankinsoni*	Cypriniformes	Cyprinidae	Hybognathus	1.20	Family Cyprinidae
brook stickleback	*Culaea inconstans*	Gasterosteiformes	Gasterosteidae	Culaea	1.79	Exact match
brook trout	*Salvelinus fontinalis*	Salmoniformes	Salmonidae	Salvelinus	0.88	Exact match
brown bullhead	*Ameiurus nebulosus*	Siluriformes	Ictaluridae	Ameiurus	0.85	Genus Ameiurus
brown trout	*Salmo trutta*	Salmoniformes	Salmonidae	Salmo	1.38	Exact match
bullhead		Siluriformes	Ictaluridae		0.77	Family Ictaluridae
burbot	*Lota lota*	lota	Gadiformes	Lotidae	1.21	All fish
chain pickerel	*Esox niger*	Esociformes	Esocidae	Esox	1.78	Genus Esox
channel catfish	*Ictalurus punctatus*	Siluriformes	Ictaluridae	Ictalurus	0.68	Exact match
common carp	*Cyprinus carpio*	Cypriniformes	Cyprinidae	Cyprinus	1.20	Exact match
common snook	*Centropomus undecimalis*	Perciformes	Centropomidae	Centropomus	1.41	Order Perciformes
crappie	*Pomoxis sp.*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
creek chub	*Semotilus atromaculatus*	Cypriniformes	Cyprinidae	Semotilus	1.06	Exact match
cutthroat trout	*Oncorhynchus clarkii*	Salmoniformes	Salmonidae	Oncorhynchus	1.12	Exact match
desert pupfish	*Cyprinodon macularius*	Cyprinodontiformes	Cyprinodontidae	Cyprinodon	1.24	Order Cyprinodontiformes
dolly varden	*Salvelinus malma*	Salmoniformes	Salmonidae	Salvelinus	0.88	Genus Salvelinus
fathead minnow	*Pimephales promelas*	Cypriniformes	Cyprinidae	Pimephales	1.57	Exact match
flannelmouth sucker	*Catostomus latipinnis*	Cypriniformes	Catostomidae	Catostomus	0.98	Exact match
flathead catfish	*Pylodictis olivaris*	Siluriformes	Ictaluridae	Pylodictus	0.77	Family Ictaluridae
flathead chub	*Platygobio gracilis*	Cypriniformes	Cyprinidae	Platygobio	1.20	Family Cyprinidae
freshwater drum	*Aplodinotus grunniens*	Perciformes	Sciaenidae	Aplodinotus	1.41	Order Perciformes
gizzard shad	*Dorosoma cepedianum*	Clupeiformes	Clupeidae	Dorosoma	1.21	All fish
goldeye	*Hiodon alosoides*	Hiodontiformes	Hiodontidae	Hiodon	1.21	All fish
green sunfish	*Lepomis cyanellus*	Perciformes	Centrarchidae	Lepomis	1.12	Exact match
iowa darter	*Etheostoma exile*	Perciformes	Percidae	Etheostoma	1.51	Family Percidae
kokanee salmon	*Oncorhynchus nerka*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
largemouth bass	*Micropterus salmoides*	Perciformes	Centrarchidae	Micropterus	1.39	Exact match
largescale sucker	*Catostomus macrocheilus*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
longnose dace	*Rhinichthys cataractae*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
longnose sucker	*Catostomus catostomus*	Cypriniformes	Catostomidae	Catostomus	0.90	Exact match
mottled sculpin	*Cottus bairdi*	Scorpaeniformes	Cottidae	Cottus	1.38	Exact match
mountain whitefish	*Prosopium williamsoni*	Salmoniformes	Salmonidae	Prosopium	1.38	Exact match
ninespine stickleback	Pungitius pungitius	pungitius	Gasterosteiformes	Gasterosteidae	1.79	Family Gasterosteidae
northern pike	*Esox lucius*	Esociformes	Esocidae	Esox	1.78	Exact match
northern pikeminnow	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
northern plains killifish	*Fundulus kansae*	Cyprinodontiformes	Fundulidae	Fundulus	1.27	Exact match
northern redbelly dace	*Chrosomus eos*	Cypriniformes	Cyprinidae	Chrosomus	1.20	Family Cyprinidae
northern squawfish	*Ptychocheilus oregonensis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
quillback	*Carpiodes cyprinus*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
rainbow trout	*Oncorhynchus mykiss*	Salmoniformes	Salmonidae	Oncorhynchus	1.07	Exact match
razorback sucker	*Xyrauchen texanus*	Cypriniformes	Catostomidae	Xyrauchen	1.01	Family Catostomidae
red shiner	*Cyprinella lutrensis*	Cypriniformes	Cyprinidae	Cyprinella	1.31	Family Cyprinidae
redbreast sunfish	*Lepomis auritus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redear sunfish	*Lepomis microlophus*	Perciformes	Centrarchidae	Lepomis	1.07	Genus Lepomis
redside shiner	*Richardsonius balteatus*	Cypriniformes	Cyprinidae	Richardsonius	1.08	Exact match
river carpsucker	*Carpiodes carpio*	Cypriniformes	Catostomidae	Carpiodes	1.01	Family Catostomidae
river redhorse	*Moxostoma carinatum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
rock bass	*Ambloplites rupestris*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
roundtail chub	*Gila robusta*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
sacramento perch	*Archoplites interruptus*	Perciformes	Centrarchidae	Archoplites	1.12	Family Centrarchidae
sacramento pikeminnow	*Ptychocheilus grandis*	Cypriniformes	Cyprinidae	Ptychocheilus	1.20	Family Cyprinidae
sailfin molly	*Poecilia latipinna*	Cyprinodontiformes	Poeciliidae	Poecilia	1.21	Family Poeciliidae
sand shiner	*Notropis stramineus*	Cypriniformes	Cyprinidae	Notropis	1.56	Exact match
sauger	*Sander canadensis*	Perciformes	Percidae	Sander	1.60	Genus Sander
sculpin	*Cottus sp.*	Scorpaeniformes	Cottidae	Cottus	1.29	Exact match
shadow bass	*Ambloplites ariommus*	Perciformes	Centrarchidae	Ambloplites	1.12	Family Centrarchidae
shorthead redhorse	*Moxostoma macrolepidotum*	Cypriniformes	Catostomidae	Moxostoma	1.01	Family Catostomidae
silver carp	*Hypophthalmichthys molitrix*	Cypriniformes	Cyprinidae	Hypophthalmichthys	1.20	Family Cyprinidae
smallmouth bass	*Micropterus dolomieu*	Perciformes	Centrarchidae	Micropterus	0.86	Exact match
smallmouth buffalo	*Ictiobus bubalus*	Cypriniformes	Catostomidae	Ictiobus	1.01	Family Catostomidae
speckled dace	*Rhinichthys osculus*	Cypriniformes	Cyprinidae	Rhinichthys	0.71	Genus Rhinichthys
spottail shiner	Notropis hudsonius	hudsonius	Cypriniformes	Cyprinidae	1.56	Genus Notropis
spotted bass	*Micropterus punctulatus*	Perciformes	Centrarchidae	Micropterus	1.12	Genus Micropterus
spotted gar	*Lepisosteus oculatus*	Lepistosteiformes	Lepisosteidae	Lepisosteus	1.21	All fish
stonecat	*Noturus flavus*	Siluriformes	Ictaluridae	Noturus	0.77	Family Ictaluridae
striped bass	*Morone saxatilis*	Perciformes	Moronidae	Morone	1.48	Exact match
striped mullet	*Mugil cephalus*	Mugiliformes	Mugilidae	Mugil	1.21	All fish

Common name	Scientific name	Order	Family	Genus	TTF	TTF source data
sucker		Cypriniformes	Catostomidae		1.01	Family Catostomidae
tilapia		Perciformes	Cichlidae		1.41	Order Perciformes
trout species	*Oncorhynchus sp.*	Salmoniformes	Salmonidae	Oncorhynchus	1.10	Genus Oncorhynchus
tui chub	*Gila bicolor*	Cypriniformes	Cyprinidae	Gila	1.20	Family Cyprinidae
utah sucker	*Catostomus ardens*	Cypriniformes	Catostomidae	Catostomus	1.01	Genus Catostomus
walleye	*Sander vitreus*	Perciformes	Percidae	Sander	1.60	Exact match
western mosquitofish	*Gambusia affinis*	Cyprinodontiformes	Poeciliidae	Gambusia	1.21	Exact match
white bass	*Morone chrysops*	Perciformes	Moronidae	Morone	1.48	Genus Morone
white crappie	*Pomoxis annularis*	Perciformes	Centrarchidae	Pomoxis	2.67	Genus Pomoxis
white sturgeon	*Acipenser transmontanus*	Acipenseriformes	Acipenseridae	Acipenser	1.21	All fish
white sucker	*Catostomus commersonii*	Cypriniformes	Catostomidae	Catostomus	1.11	Exact match
wiper	*Morone chrysops x Moron saxatilis*	Perciformes	Moronidae	Morone	1.48	Genus Morone
yellow perch	*Perca flavescens*	Perciformes	Percidae	Perca	1.42	Exact match

Table B-8. Food web models used to calculate composite TTFs to translate the egg-ovary FCV to a water-column value at aquatic sites where sufficient data was available to calculate an enrichment factor (EF).

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			black bullhead	0.85	Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion		Median of all insects	in	2.14	0.45	Median of all crustaceans	crs	1.41	0.45	Median of all bivalves	bvs	4.29	0.10											2.03	0.85	1.72
Default			black crappie	2.67	Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			blacknose dace	0.71	Eats immature aquatic insects, amphipods, and various other aquatic invertebrates; also eats algae and diatoms, which may be of little nutritional value (Smith 1979, Becker 1983).		Median of all insects	in	2.14	0.50	Median of all invertebrates except bivalves	all	1.48	0.50														1.81	0.71	1.29
Default			blue catfish	0.68	Bottom feeder. Eats mostly crustaceans and aquatic insects when young. Later, fishes and large invertebrates become most important (Moyle 1976). Also scavenges.		Median of all insects	in	2.14	0.36	Median insects and benthic crustaceans	in,bc	1.74	0.20	Median of all bivalves	bvs	4.29	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.36	2.28	0.68	1.56
Default			bluegill	1.03	Feeds opportunistically on aquatic insect larvae, planktonic crustaceans, flying insects, snails, and other small invertebrates; small fishes, fish eggs, crayfish, and algae sometimes are eaten. Larvae and juveniles often eat cladocerans and copepod nauplii. Adults eats mainly aquatic insects, crayfishes, and small fishes, or, in some bodies of water, mostly zooplankton. Feeds at all levels of water column.		Median of all insects	in	2.14	0.68	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.04	1.95	1.03	2.00
Default			bluehead sucker	1.04	Herbivore, Invertivore		TL1	TL1	1.00	0.60	Median of all invertebrates except bivalves	all	1.48	0.40														1.19	1.04	1.24
Default			brassy minnow	1.20	Eats algae, phyto- and zooplankton, benthic invertebrates, surface drift, bottom ooze (Becker 1983).		TL1	TL1	1.00	0.50	Median of planktonic crustacean	pc	1.41	0.40	Median of all insects	in	2.14	0.10										1.28	1.20	1.54

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			brook stickleback	1.79	Eats various aquatic invertebrates (including eggs and larvae), eggs and larvae of fishes, and algae. In a Manitoba lake, was opportunistic but heavily dependent on arthropods (Moodie 1986).		Median of all invertebrates except bivalves	all	1.48	0.80	TL1	TL1	1.00	0.20															1.38	1.79	2.47
Default			brook trout	0.88	Feeds opportunistically on various invertebrate and vertebrate animals, including primarily terrestrial and aquatic insects and planktonic crustaceans.		Median of all insects	in	2.14	0.60	crayfish	cr	1.46	0.10	Median of all bivalves	bvs	4.29	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.22	0.88	1.96
Default			brown bullhead	0.85	Bottom feeder. Young eat chironomid larvae and small crustaceans. Adults eat larger insect larvae and fishes, also fish eggs, mollusks, carrion, and plant material (Becker 1983, Moyle 1976).		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.85	1.79
Default			brown trout	1.38	Eats aquatic and terrestrial insects and their larvae, crustaceans (especially crayfish), molluscs, fishes, and other animals. In streams, young feed mainly on aquatic and terrestrial drift invertebrates; in lakes, they feed on zooplankton and benthic invertebrates (Sublette et al. 1990). Large adults feed on fishes, crayfish, and other benthic invertebrates.		Median of planktonic crustaceans	pc	1.41	0.20	Median of all insects	in	2.14	0.12	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.02	1.38	2.78
Default			bullhead	0.77	Black (not exotic to CO and NM): Omnivorous bottom feeder; often eats aquatic insects, crustaceans, molluscs, occasionally fishes and carrion. Stomach often contain substantial amounts of plant material of unknown nutritional value (Moyle 1976). Juveniles planktivorous; at about 27 mm TL, feed largely on crustaceans and midge larvae		Median of all insects	in	2.14	0.68	Median of all invertebrates except bivalves	all	1.48	0.20	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	2.11	0.77	1.62
Default			burbot	1.21	Young eat mainly immature aquatic insects, crayfish, molluscs, and other deepwater invertebrates. Larger individuals feed mostly on fishes (Becker 1983, Scott and Crossman 1973).		Median of all insects	in	2.14	0.25	Median of all crustaceans	crs	1.41	0.25									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.03	1.21	2.45
Default			channel catfish	0.68	Bottom feeder. Young eat mainly small invertebrates; as they grow, fishes and crayfish become increasingly important, though individuals of all sizes eat abundant aquatic insects. Large fish are mainly piscivorous (Moyle 1976).		Median of all insects	in	2.14	0.48	Median of planktonic crustaceans	pc	1.41	0.20	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	1.97	0.68	1.35

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			common carp	1.20	Omnivorous; adults eat mainly invertebrates, detritus, fish eggs, and plant material (Jester 1974, Becker 1983, Sublette et al. 1990).		Median of all invertebrates except bivalves	all	1.48	0.65	TL1	TL1	1.00	0.35															1.31	1.20	1.58
Default			crappie	2.67	Black: Primarily a midwater feeder; zooplankton and small Diptera larvae predominate in the diet of individuals to 12 cm SL, while fishes and aquatic insects predominate in the diet of larger individuals. White: eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			creek chub	1.06	Feeds opportunistically on various plants and animals, from surface drift to benthos; mostly invertivorous but large individuals often picivorous (Becker 1983). Chironomid larvae and other larval insects important in diet of young.		Median of all invertebrates except bivalves	all	1.48	0.70	TL1	TL1	1.00	0.20									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.46	1.06	1.55
Default			cutthroat trout	1.12	Opportunistic. Inland cutthroats feed primarily on insects (aquatic and terrestrial); often feeds in and especially downstream from riffle areas; some large individuals feed mostly on fishes; also eats zooplankton and crustaceans.		Median of all insects	in	2.14	0.50	Median of all crustaceans	crs	1.41	0.20									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.3	2.04	1.12	2.29
Default			fathead minnow	1.57	Feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990).	expected diet of small invertebrates	Median of all insects	in	2.14	0.60	Median of all crustaceans	crs	1.41	0.20	TL1	TL1	1.00	0.20											1.77	1.57	2.78
Default			flannel mouth sucker	0.98	Herbivore, Invertivore Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25															1.55	0.98	1.52
Default			flathead chub	1.20	Opportunistic; eats aquatic and terrestrial insects and some algae (Olund and Cross 1961)		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20															1.91	1.20	2.30
Default			freshwater drum	1.41	Young feed mainly on minute crustaceans; adults mostly are bottom feeders, eat insect larvae, crustaceans, fishes, and (mostly in rivers) clams and		Median of all crustaceans	crs	1.41	0.44	Median of all insects	in	2.14	0.40	Median of all bivalves	bvs	4.29	0.04					Fish	Median all fish eating median all invertebrat	f+a	2.28	0.12	1.92	1.41	2.71

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					snails (Becker 1983, Scott and Crossman 1973, Lee et al. 1980).																			es						
Default			gizzard shad	1.21	Adults primarily bottom filter-feeding detritivores		TL1	TL1	1.00	1.00																		1.00	1.21	1.21
Default			goldeye	1.21	Young-of-year eat mainly microcrustaceans, also other invertebrates. Older individuals eat mainly aquatic insects obtained at surface but also various other animals, including frogs, fishes, and small mammals.		Median insects and benthic crustaceans	in,bc	1.74	1.00																		1.74	1.21	2.10
Default			green sunfish	1.12	Feeds opportunistically on the larger, more active invertebrates that occur with them, and on small fishes. Young feed mostly on crustaceans (zooplankton) and aquatic insect larvae. Adults eat more large aquatic and terrestrial insects, crayfish, and fishes		Median of all insects	in	2.14	0.58	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.08					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.24	2.05	1.12	2.29
Default			Iowa darter	1.51	Eats mainly various invertebrates; commonly ingested food items of adults are midge larvae, mayfly naiads, and amphipods, and of the young, copepods and cladocerans. Apparently feeds on swimming organisms and those on bottom.	expected diet of small invertebrates	Median of all insects	in	2.14	0.70	amphipods	am	1.22	0.16	crayfish	cr	1.46	0.08	Median of planktonic crustaceans	pc	1.41	0.06						1.90	1.51	2.87
Default			kokanee salmon	1.10	Zooplankton, insects.		Median of planktonic crustaceans	pc	1.41	0.80	Median of all insects	in	2.14	0.20														1.56	1.10	1.71
Default			largemouth bass	1.39	Fry feed mainly on zooplankton. Larger young eat insects, crustaceans, and fish fry. Adults eat mainly fishes, though sometimes prefer crayfish or amphibians (Moyle 1976, Smith 1979).		Median of all insects	in	2.14	0.10	crayfish	cr	1.46	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.18	1.39	3.04
Default			longnose dace	0.71	Eats mainly benthic insects, especially Diptera and mayflies (Becker 1983, Scott and Crossman 1973); also eats algae and plant material (Sublette et al. 1990). Terrestrial insects and fish eggs common in diet of adults from Lake Michigan (see Sublette et al. 1990).		Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20														1.91	0.71	1.36
Default			longnose sucker	0.90	Eats mostly bottom invertebrates (Scott and Crossman 1973).		Median of all invertebrates except bivalves	all	1.48	1.00																		1.48	0.90	1.34

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			mottled sculpin	1.38	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	Median of all crustaceans	crs	1.41	0.10	TL1	TL1	1.00	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.97	1.38	2.72
Default			mountain whitefish	1.38	Feeds actively on aquatic and terrestrial insects. Also feeds on some fish eggs and occasionally on fishes. Bottom-oriented predator (Moyle 1976), occasionally feeds at surface (Sigler and Sigler 1987).		Median of all insects	in	2.14	0.90													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.16	1.38	2.97
Default			ninespine stickleback	1.79	Eats mainly small crustaceans and aquatic insects; sometimes also fish eggs and fry (Becker 1983).		Median of all crustaceans	crs	1.41	0.48	Median of all insects	in	2.14	0.44									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.08	1.80	1.79	3.22
Default			northern pike	1.78	Young initially eat large zooplankton and immature aquatic insects. After 7-10 days fishes begin to enter diet and eventually dominate. Adults feed opportunistically on vertebrates small enough to be engulfed. (Scott and Crossman 1973). Sight feeder.		Median insects and benthic crustaceans	in,bc	1.74	0.05													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.95	2.25	1.78	4.02
Default			northern plains killifish	1.27	Feed effectively at all levels and food habits are generalized. Prefer aquatic insects but also feed on plants.	Montana field guide (http://fieldguide.mt.gov/detail_AFCNB04600.aspx)	Median of all insects	in	2.14	0.80	TL1	TL1	1.00	0.20															1.91	1.27	2.44
Default			northern redbelly dace	1.20	Eats mainly diatoms and filamentous algae, also zooplankton and aquatic insects.		TL1	TL1	1.00	0.70	Median of all insects	in	2.14	0.15	Median insects and benthic crustaceans	in,bc	1.74	0.15											1.28	1.20	1.54
Default			northern squawfish	1.20	Small individuals feed primarily on aquatic and terrestrial insects. Adults feed on fish, insects, insect larvae, crustaceans and some plankton during spring and summer. Fishes are the major component		Median of all insects	in	2.14	0.32	Median of all crustaceans	crs	1.41	0.08									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.17	1.20	2.61

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					of the diet in winter.																									
Default			rainbow trout	1.07	In lakes, feeds mostly on bottom-dwelling invertebrates (e.g., aquatic insects, amphipods, worms, fish eggs, sometimes small fish) and plankton. In streams, feeds primarily on drift organisms. May ingest aquatic vegetation (probably for attached invertebrates).		Median of all insects	in	2.14	0.75													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.25	2.18	1.07	2.33
Default			red shiner	1.31	Eats various small invertebrates (insects, crustaceans), plant material (digestibility may be low), and microorganisms (Becker 1983). In Virgin River, diet dominated by Ceratopongidae, Simuliidae, and Chironomidae (Greger and Deacon 1988).		Median insects and benthic crustaceans	in,bc	1.74	1.00																	1.74	1.31	2.27
Default			redside shiner	1.08	Feeds mainly on aquatic and terrestrial insects; also eats molluscs, plankton, and some small fish and fish eggs. Fry eat zooplankton and algae.		Median of all insects	in	2.14	0.70	Median of planktonic crustaceans	pc	1.41	0.10	Median of all bivalves	bvs	4.29	0.10					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	2.30	1.08	2.48
Default			river carpsucker	1.01	Mostly a bottom feeder, browses on periphyton associated with submerged rocks and debris, ingests various small planktonic plants and animals.		TL1	TL1	1.00	0.75	Median of planktonic crustaceans	pc	1.41	0.25														1.10	1.01	1.11
Default			roundtail chub	1.20	Opportunistic; eats available aquatic and terrestrial insects, gastropods, crustaceans, fishes, and sometimes filamentous algae (Sublette et al. 1990).		Median of all insects	in	2.14	0.55	Median of all crustaceans	crs	1.41	0.15	Median of all bivalves	bvs	4.29	0.15					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.38	1.20	2.86
Default			Sacramento perch	1.12	Opportunistic; diet mainly benthic insect larvae, snails, mid-water insects, zooplankton, and fishes (Moyle et al. 1989). Young feed mainly on small crustaceans, but as they grow Sacramento perch consume more aquatic insect larvae and pupae. Large adults feed mainly on other fishes when available.		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.12	1.44
Default			sailfin molly	1.21	Eats mainly algae, vascular plants, organic detritus, and mosquito larvae (and other small invertebrates).		TL1	TL1	1.00	0.75	Median of all insects	in	2.14	0.25														1.29	1.21	1.56

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			sand shiner	1.56	Eats various aquatic and terrestrial invertebrates (especially chironomids), algae, and (mainly) bottom particulate matter (Becker 1983). Winter diet mostly chironomids larvae and mayfly and stonefly naiads (Ohio, see Sublette et al. 1990)		Median insects and benthic crustaceans	in,bc	1.74	0.75	TL1	TL1	1.00	0.25															1.55	1.56	2.43
Default			sauger	1.60	Larvae eat microcrustaceans. Young eat zooplankton, immature and adult aquatic insects, and fish fry; adults eat small fishes and various invertebrates (Scott and Crossman 1973), or are almost exclusively piscivorous (Burkhead and Jenkins 1991). Sight feeder, adapted to low light.		Median insects and benthic crustaceans	in,bc	1.74	0.36	Median of planktonic crustaceans	pc	1.41	0.10										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.54	2.00	1.60	3.20
Default			sculpin	1.29	Benthic feeder; forages among rocks, mainly on immature aquatic insect larvae, especially mayflies, chironomid midges, and stoneflies; larger individuals also eat caddisflies and crayfish; crustaceans, annelids, fishes (including fish eggs) and plant material also may be eaten; may take swimming prey from water column (Scott and Crossman 1973, Becker 1983).		Median of all insects	in	2.14	0.70	crayfish	cr	1.46	0.15										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.15	2.06	1.29	2.66
Default			shorthead redhorse	1.01	Invertivore		Median of all invertebrates except bivalves	all	1.48	1.00																			1.48	1.01	1.49
Default			smallmouth bass	0.86	Adults almost entirely piscivorous if sufficient prey available		Median of all insects	in	2.14	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.25	0.86	1.93
Default			speckled dace	0.71	An omnivorous benthic feeder, at times feeding on drift in mid-water or rarely at the surface (Schreiber and Minckley 1981). The diet consists mostly of benthic insects, also includes other invertebrates, algae, and detritus (little or no plant material or detritus in some areas) (Sublette et al. 1990, Woodbury 1933, Greger and Deacon 1988). Young feed mainly on zooplankton.		Median of all insects	in	2.14	0.70	Median insects and benthic crustaceans	in,bc	1.74	0.15	TL1	TL1	1.00	0.15											1.91	0.71	1.36

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			stonecat	0.77	Eats mainly bottom invertebrates (insects, crayfish); sometimes also plant material and fishes (Becker 1983, Scott and Crossman 1973).		Median insects and benthic crustaceans	in,bc	1.74	0.70	TL1	TL1	1.00	0.20										Fish	Median all fish eating median all invertebrates	f+a	2.28	0.1	1.65	0.77	1.26
Default			sucker	1.01	White: Larvae feed near surface on protozoans, diatoms, small crustaceans, and bloodworms. Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990). Bluehead: A bottom feeder. Scrapes algae and other organisms from rocks with chisel-like ridges inside each lip; ingests fine organism-laden sediments. May feed in stream riffles, or deeper rocky pools; in lakes it may feed over rocks near shore. May eat aquatic insect larvae. Flannelmouth: Bottom feeder. Reported to feed on diatoms, algae, fragments of higher plants, seeds, and benthic invertebrates (Sigler and Miller 1963; Lee et al. 1980). See Tyus and Minckley 1988 for possible importance of Mormon cricket as food source.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.01	1.25
Default			tilapia	1.41	aureus: Eats mainly phytoplankton. mossambicus: Nonselective omnivore; eats planktonic algae, aquatic plants, invertebrates, and small fishes (Moyle 1976). zilli: Feeds on algae and higher plants, invertebrates, and occasionally eats dead or dying fish.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.41	1.74
Default			tui chub	1.20	Adults opportunistic. They feed on plant material, plankton, insect larvae, crustaceans, fish fry and fish eggs, etc. Young feed on zooplankton. Coarse-rakered form eats more plant material, fine-rakered form more zooplankton.		TL1	TL1	1.00	0.40	Median of planktonic crustaceans	pc	1.41	0.28	Median of all insects	in	2.14	0.28	crayfish	cr	1.46	0.04						1.45	1.20	1.75
Default			Utah sucker	1.01	Bottom feeder. Varied diet; feeds freely on both animal and plant organisms, at all depths throughout the year. Grazes on filamentous algae.		Median of all invertebrates except bivalves	all	1.48	0.50	TL1	TL1	1.00	0.50														1.24	1.01	1.25

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Default			walleye	1.60	Adults feed opportunistically on various fishes and larger invertebrates.		Median insects and benthic crustaceans	in,bc	1.74	0.50													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.5	2.01	1.60	3.21
Default			western mosquitofish	1.21	Opportunistic omnivore; eats mainly small invertebrates, often taken near water surface. Also eats small fishes and, in the absence of abundant animal food, algae and diatoms (Moyle 1976). Mosquitofish are principally carnivorous, and have strong, conical teeth and short guts (Meffe et al. 1983, Turner and Snelson 1984). They are reported to feed on rotifers, snails, spiders, insect larvae, crustaceans, algae, and fish fry, including their own progeny (Barnickol 1941, Minckley 1973, Meffe and Crump 1987). Cannibalism has been documented by several authors (Seale 1917, Krumholz 1948, Walters and Legner 1980, Harrington and Harrington 1982). Plant material is taken occasionally (Barnickol 1941) and may make up a significant portion of the diet during periods of scarcity of animal prey (Harrington and Harrington 1982). Grubb (1972) showed that anuran eggs from temporary ponds were preferentially selected over those breeding in permanent systems.		Median of all insects	in	2.14	0.75	Median of all crustaceans	crs	1.41	0.25																	1.96	1.21	2.37
Default			white bass	1.48	Eats fishes, zooplankton, aquatic insects, oligochaetes, and crayfish; fishes often dominate diet of adults; diet may vary from place to place (Moyle 1976, Sublette et al. 1990).		Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.05	crayfish	cr	1.46	0.05					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.6	2.15	1.48	3.19
Default			white crappie	2.67	Eats fishes, planktonic crustaceans, and aquatic insects; small individuals eat mostly zooplankton, fish tend to predominate in the diet of larger individuals, though zooplankton also consumed (Moyle 1976)		Median of all insects	in	2.14	0.50	Median of planktonic crustaceans	pc	1.41	0.10									Fish	Median all fish eating median all invertebrates	f+a	2.28	0.4	2.12	2.67	5.66
Default			white sturgeon	1.21	A bottom feeder. Young feed mostly on the larvae of aquatic insects, crustaceans, and molluscs. A significant portion of the		Median insects and benthic	in,bc	1.74	0.31	Median of all bivalves	bvs	4.29	0.09									Fish	Median all fish eating median all	f+a	2.28	0.6	2.29	1.21	2.77

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
					diet of larger sturgeon consists of fish.		crustaceans																	invertebrates						
Default			white sucker	1.11	Adults feed opportunistically on bottom organisms, both plant and animal (e.g., chironomid larvae, zooplankton, small crayfishes) (Becker 1983, Sublette et al. 1990).	expected common spp in benthos	TL1	TL1	1.00	0.50	Median of all insects	in	2.14	0.30	Median of planktonic crustaceans	pc	1.41	0.10	crayfish	cr	1.46	0.10						1.43	1.11	1.58
Default			wiper	1.48	Adults are predatory on fishes and larger crustaceans (Hassler 1988).		crayfish	cr	1.46	0.20													Fish	Median all fish eating median all invertebrates	f+a	2.28	0.8	2.11	1.48	3.13
Default			yellow perch	1.42	Larvae and young primarily zooplankton feeders; older young eat mostly invertebrates associated with bottom and with aquatic plants; adults feed among plants and along bottom on larger invertebrates and small fishes (Moyle 1976).		Median insects and benthic crustaceans	in,bc	1.74	0.64	Median of planktonic crustaceans	pc	1.41	0.13	TL1	TL1	1.00	0.07					Fish	Median all fish eating median all invertebrates	f+a	2.28	0.16	1.73	1.42	2.47
Saiki et al. 1993			bluegill	1.03	site-specific: 0.23 chironomid; 0.3 microcrustacea; 0.47 amphipod	stomach analysis	amphipods	am	1.22	0.47	Median of planktonic crustaceans	pc	1.41	0.30	midges	mg	1.90	0.23										1.43	1.03	1.47
Saiki et al. 1993			largemouth bass	1.39	site-specific: 0.73 fish; 0.27 crayfish	stomach analysis	crayfish	cr	1.46	0.27	Saiki bluegill TTFcomp.	BG	1.47	0.73														1.47	1.39	2.04
Saiki et al. 1993			western mosquitofish	1.21	site-specific: 0.89 molluscs, and insects; 0.065 chironomid; 0.045 microcrustacea	stomach contents show a large terrestrial component	Median insects and benthic crustaceans	in,bc	1.74	0.89	midges	mg	1.90	0.07	Median of planktonic crustaceans	pc	1.41	0.05										1.74	1.21	2.10
Formation 2012	Crow Creek - CC150	CC-150	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.38	2.91
Formation 2012	Crow Creek - CC150	CC-150	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.27	mayflies	mf	2.38	0.16										2.12	1.29	2.74

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Crow Creek - 1A	CC-1A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.38	2.96
Formation 2012	Crow Creek - 1A	CC-1A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.79	midges	mg	1.90	0.09	mayflies	mf	2.38	0.12										2.15	1.29	2.78
Formation 2012	Crow Creek - CC350	CC-350	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.38	2.97
Formation 2012	Crow Creek - CC350	CC-350	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.80	midges	mg	1.90	0.07	mayflies	mf	2.38	0.13										2.16	1.29	2.79
Formation 2012	Crow Creek - 3A	CC-3A	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.38	2.97
Formation 2012	Crow Creek - 3A	CC-3A	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.85	midges	mg	1.90	0.05	mayflies	mf	2.38	0.10										2.15	1.29	2.78
Formation 2012	Crow Creek - CC75	CC-75	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.38	2.87
Formation 2012	Crow Creek - CC75	CC-75	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.49	midges	mg	1.90	0.38	mayflies	mf	2.38	0.13										2.08	1.29	2.69
Formation 2012	Deer Creek	DC-600	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.38	3.00
Formation 2012	Deer Creek	DC-600	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.44	midges	mg	1.90	0.21	mayflies	mf	2.38	0.35										2.18	1.29	2.81
Formation 2012	Hoopes Spring - HS	HS	brown trout	1.38	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.38	3.86
Formation 2012	Hoopes Spring - HS	HS	sculpin	1.29	Proportions as described in table B-10		Median of all bivalves	bvs	4.29	0.44	midges	mg	1.90	0.32	blackworms	bw	1.29	0.24										2.81	1.29	3.63
Formation 2012	Hoopes Spring - HS3	HS-3	brown trout	1.38	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.38	2.63
Formation 2012	Hoopes Spring - HS3	HS-3	sculpin	1.29	Proportions as described in table B-10		Median of all crustaceans	crs	1.41	0.40	Median of all insects	in	2.14	0.33	mayflies	mf	2.38	0.27										1.91	1.29	2.47

Reference	Site description	Site ID	Target fish species common name	Fish TTF	Fish prey as described in NatureServe	Fish prey spp comment	1° TL2 TTF species used	1° TL2 spp abbrev.	1° TL2 TTF	1° TL2 proportion	2° TL2 spp used	2° TL2 spp abrev.	2° TL2 TTF	2° TL2 proportion	3° TL2 spp used	3° TL2 spp abrev.	3° TL2 TTF	3° TL2 proportion	4° TL2 spp used	4° TL2 spp abrev.	4° TL2 TTF	4° TL2 proportion	1° TL3 spp	1° TL3 spp used	1° TL3 spp abbrev.	1° TL3 TTF	1° TL3 proportion	Effective TTF	Target fish TTF	TTFcomposite
Formation 2012	Sage Creek - LSV2C	LSV-2C	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.38	3.01
Formation 2012	Sage Creek - LSV2C	LSV-2C	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.57	midges	mg	1.90	0.12	mayflies	mf	2.38	0.31										2.19	1.29	2.83
Formation 2012	Sage Creek - LSV4	LSV-4	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.38	2.88
Formation 2012	Sage Creek - LSV4	LSV-4	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.53	midges	mg	1.90	0.34	mayflies	mf	2.38	0.13										2.09	1.29	2.70
Formation 2012	South Fork Tincup Cr.	SFTC-1	brown trout	1.38	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.38	3.05
Formation 2012	South Fork Tincup Cr.	SFTC-1	sculpin	1.29	Proportions as described in table B-10		Median of all insects	in	2.14	0.93	Median of all bivalves	bvs	4.29	0.03	mayflies	mf	2.38	0.04										2.22	1.29	2.86

Table B-9. Calculation of site-specific invertebrate proportions using invertebrate counts in Formation 2012

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek		Crow Creek									Deer Creek			Hoopes Spring						Sage Creek					Crow Creek					
					Location	SFTC1		CC75			CC150			CC350			DC600			HS			HS3			LSV2C			LSV4		CC1A		CC3A			Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
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	Tolerance	Stream	SF Tincup Creek		Crow Creek									Deer Creek			Hoopes Spring						Sage Creek				Crow Creek						
					Location	SFTC1		CC75			CC150			CC350			DC600			HS			HS3			LSV2C			LSV4	CC1A			CC3A			Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
	sp.																																			
Trichoptera	Glossoma sp.	CN	SC	0														4																		4
Trichoptera	Helicopsyche sp.	CN	SC	3				3			5	4		5	93									2			2						19	81		214
Trichoptera	Hesperophylax sp.	CN	SH	5																			3	1	48		14	4								70
Trichoptera	Hydropsyche sp.	CN	F	4			5	47	50	23	29	17	11	74	97	41	1	14		2				2		8	9	11	53	63	91	29	105	79	151	1012
Trichoptera	Hydroptila sp.	CN	SC	6				8	9	1	1												1			16				1			2			39
Trichoptera	Lepidostoma spp.	SP/CB	SH	1		1	3		7		6	8			67	6		16				2									13	4		2	6	141
Trichoptera	Micrasema sp.	CN	SH	1				8		9	65	1	18	14			3			28			5			4			76	3	3		36			273
Trichoptera	Neothremma sp.	CN	SC	0														4																		4
Trichoptera	Oecetis disjuncta	CN	P	8		2								2	3																	3		7		17
Trichoptera	Onocosmoecus sp.	CB	SH	1		1																														1
Trichoptera	Oligophlebodes sp.	CN	SC	1				11																									2			13
Trichoptera	Parapsyche sp.	CN	P	1				16			5						7						1			2			1							32
Trichoptera	Psychoglypha sp.	SP/CB	CG	1					3																											3
Trichoptera	Rhyacophila spp.	CN	P	0		7	3	4	5	5	9	17	16	3	5	9	16	23	83					11	13		7									236
Trichoptera	Wormaldia spp.	CN	F	3			3				1	8	15	2	5	6	7							3	9				1			3	1	2	11	77
Coleoptera	Ametor sp.	SW	P	5																		1														1
Coleoptera	Brychius sp.	CB	SC	7		2			1	3																10										16
Coleoptera	Cleptelmis sp.	CN	CG/SC	4		3	26									4												5		1			1		6	46
Coleoptera	Dubiraphia sp.	CN	CG/SC	4		3																														3
Coleoptera	Heterlimnius corpulentus	CN/BU	CG/SC	4														30	32			5														67
Coleoptera	Optioservus quadrimaculatus	CN	CG/SC	4		97	267	43	109	68	40	205	153	78	162	167	7	2	5	12			5	21	33	18	132	151	27	153	74	246	69	83	129	2556
Coleoptera	Oreodytes sp.	SW/DV	P	5		6		1																												7
Coleoptera	Paracymus sp.	CN	P/OM	5								1																								1
Coleoptera	Zaitzevia 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						Total
					Location	SFTC1		CC75			CC150			CC350			DC600			HS			HS3			LSV2C			LSV4	CC1A			CC3A			
					Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Odonata	Ophiogomphus sp.	BU	P	1																												2		7		9
Hemiptera	Sigara sp.	SW	P	10		5																														5
Diptera	Anopheles sp.	SW	F	8									1																							1
Diptera	Antocha sp.	BU	CG	3			5	1			4			6															18		2		1			37
Diptera	Atherix sp.	BU	P	2																											26	22	24	3	44	119
Diptera	Chelifera sp.	SP/BU	CG	6				2			1						7			4									1				5			20
Diptera	Dixa sp.	BU	CG	1																	13															13
Diptera	Empididae	SP/BU	P	6									1						5			2														8
Diptera	Ephydridae	BU	CG	6							1								1			1														3
Diptera	Glutops sp.	BU	P	3				1	2									1																		4
Diptera	Hexatoma	BU	P	2		19				9		1			5	4		1						9			4				5			16	1	74
Diptera	Limnophila sp.	BU	P	4							1			3			3						5						5	3			9			29
Diptera	Muscidae	BU	P	6																						1		3								4
Diptera	Pericoma sp.							2			1																									3
Diptera	Probezzia sp.	BU	P	6						3					2		1	1								2							2			11
Diptera	Ptychoptera sp.		CG	7																												1				1
Diptera	Simulium sp.	CN	F	6			18	78	5	30	26	49	17	17	5	102	9	15	8		5	4	13	21	38	24	25	24	12	114	35	8	1	26	31	760
Diptera	Tipula sp.	BU	SH	4					7	3				1					3					3			2			2		3	3			27
Chironomidae (family)	Chironomidae	BU/SP	CG/SH/P	6				188	195	173	143	99	143	68	10	30	33	88	151	92	124	25	23	83		20	43	91	149	36	56	35	41	21	8	2168
Hirudinea (class)	Helobdella sp.		PA/P	6								1																								1
Collembola	Collembola										2																									2
Oligochaeta (class)	Oligochaeta		CG	5			5	15	7	2	6	4	7	8	3	5	3	5	19	72	101	5	3			34		9	8	9			19	56		405
Bivalvia (class)	Pisidium sp.	BU	F	8		2		2	4		2						2	6		2	5	2	2			12					3	1	1	23		69
Gastropoda (class)	Fossaria sp.	CN	SC	8			2			1					2					52	57	27	4	4			8				15		1	1		174
Gastropoda (class)	Amnicola sp.	CN	SC	5										2	2	1	1													3			1			10
Gastropoda (class)	Gyraulus sp.	CN	SC																											1						1
Gastropoda (class)	Mentus sp.	CN	SC																	6																6
Gastropoda (class)	Physella sp.	CN	SC	8		19		3	2	1					3	1				114	55	7	2	6		14	32		1	2			3	23		288

Order	Genus	Habitat / Behavior	Functional Feeding	Tolerance	Stream	SF Tincup Creek				Crow Creek						Deer Creek						Hoopes Spring				Sage Creek						Crow Creek				
					Location	SFTC1		CC75		CC150			CC350			DC600			HS			HS3			LSV2C		LSV4		CC1A			CC3A			Total
			Groups		Date	8/29/2007	9/9/2008	9/2/2006	8/23/2007	9/3/2008	9/1/2006	8/24/2007	9/3/2008	9/1/2006	8/23/2007	9/4/2008	9/7/2006	8/27/2007	9/8/2008	9/8/2006	8/24/2007	9/4/2008	9/6/2006	8/28/2007	9/5/2008	9/8/2006	8/28/2007	9/5/2008	9/5/2006	9/1/2006	8/25/2007	9/6/2008	9/4/2006	8/26/2007	9/7/2008	
Gastropoda (class)	Valvata sp.	CN	SC																		1														1
Amphipoda	Gammarus sp.	SW/BU	OM	6															2			2	4	13	8	1	12				2				44
Ostracoda	Ostracoda	SW	CG	8						1												460	2	9	30	13	8	1	1						525
Tricladida	Polycelis coronata		OM	1									4																						4
Acari (subclass)	Acari		P	8				2		2	3	4		2	6	7														2	2	5			35
					% Subsampled	50	50	12.5	12.5	66.6	12.5	25	50	25	12.5	50	100	33.3	75	50	33.3	100	12.5	33.3	100	25	25	75	12.5	25	25	50	25	25	50	13872
					Total abundance	394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
					Total taxa	24	19	27	25	22	26	24	16	23	24	21	23	23	16	15	13	14	21	22	14	23	21	17	21	20	18	22	30	20	15	
					Total Counts	788	972	4128	4048	741.7417	3720	1928	1068	1908	4288	984	420	1435.435	545.3333	996	1246.246	91	4768	1411.411	280	2164	2128	593.3333	3560	1948	1808	926	1860	2012	1000	
					Density (#/1m2)	2835	3496	14849	14561	2668	13381	6935	3842	6863	15424	3540	1511	5163	1962	3583	4483	327	17151	5077	1007	7784	7655	2134	12806	7007	6504	3331	6691	7237	3597	
						394	486	516	506	494	465	482	534	477	536	492	420	478	409	498	415	91	596	470	280	541	532	445	445	487	452	463	465	503	500	
							880			1516			1481			1505			1307			1004			1346			1518	445			1402			1468	13872

Functional Feeding Groups (FFG): CG = Collector-Gatherer, SC = Scraper, F = Filterer, P = Predator, SH = Shredder, OM = Omnivore, Habitat/Behavior (Hab/Beh): BU = Burrower, SW = Swimmer, CN = Clinger, CB = Climber, SP = Sprawler, DV = Diver

Table B-10. Summary of Formation 2012 invertebrate data.

Phylum	Subphylum	Class	Subclass	Infraclass	Superorder	Order	Lookup ID	Common name	SFTC1		CC75		CC150		CC350		DC600		HS		HS3		LSV2C		LSV4		CC1A		CC3A	
									Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion	Count	Proportion
Arthropoda		Insecta	Pterygota		Ephemeropteroidea	Ephemeroptera	Ephemeroptera	Mayflies	36	0.04	185	0.12	231	0.16	192	0.13	444	0.34	115	0.11	325	0.24	421	0.28	56	0.13	168	0.12	136	0.09
Arthropoda		Insecta	Pterygota		Exopterygota	Plecoptera	Plecoptera	Stoneflies	107	0.12	85	0.06	40	0.03	18	0.01	195	0.15	59	0.06	20	0.01	26	0.02	30	0.07	11	0.01	22	0.01
Arthropoda		Insecta			Amphiesmenoptera	Trichoptera	Trichoptera	Caddisflies	30	0.03	268	0.18	283	0.19	539	0.36	229	0.18	34	0.03	230	0.17	324	0.21	135	0.30	325	0.23	623	0.42
Arthropoda		Insecta	Pterygota	Neoptera	Endopterygota	Coleoptera	Coleoptera	Beetles	631	0.72	234	0.15	408	0.28	457	0.30	76	0.06	18	0.02	65	0.05	327	0.22	29	0.07	507	0.36	311	0.21
Arthropoda		Insecta		Neoptera		Megaloptera	Megaloptera	Alderflies, dobsonflies and fishflies	1	0.00	4	0.00	1	0.00																
Arthropoda		Insecta	Pterygota		Odonatoptera	Odonata	Odonata	Dragonflies and damselflies																			2	0.00	7	0.00
Arthropoda		Insecta		Neoptera	Paraneoptera	Hemiptera	Hemiptera	True bugs (cicadas, aphids, planthoppers, leafhoppers, shield bugs)	5	0.01																				
Arthropoda		Insecta			Panorpida	Diptera	Diptera	True flies	42	0.05	143	0.09	103	0.07	145	0.10	55	0.04	29	0.03	89	0.07	85	0.06	36	0.08	221	0.16	166	0.11
Arthropoda		Insecta				Chironomidae (family)	Chironomidae (family)	Midges			556	0.37	385	0.26	108	0.07	272	0.21	241	0.24	106	0.08	154	0.10	149	0.33	127	0.09	70	0.05
Annelida		Clitellata	Hirudinea				Hirudinea (class)	Leeches					1	0.00																
Arthropoda		Entognatha				Collembola	Collembola	Springtails (not insects!)					2	0.00																
Annelida		Clitellata	Oligochaeta				Oligochaeta (class)	Worms	5	0.01	24	0.02	17	0.01	16	0.01	27	0.02	178	0.18	3	0.00	43	0.03	8	0.02	9	0.01	75	0.05
Mollusca		Bivalvia					Bivalvia (class)	Clams	2	0.00	6	0.00	2	0.00			8	0.01	9	0.01	2	0.00	12	0.01			4	0.00	24	0.02
Mollusca		Gastropoda					Gastropoda (class)	Snails and slugs	21	0.02	7	0.00			11	0.01	1	0.00	319	0.32	16	0.01	54	0.04	1	0.00	21	0.01	29	0.02
Arthropoda	Crustacea	Malacostraca				Amphipoda	Amphipoda	Crustaceans											2	0.00	19	0.01	21	0.01			2	0.00		
Arthropoda	Crustacea	Ostracoda					Ostracoda	Sea shrimp					1	0.00							471	0.35	51	0.03	1	0.00	1	0.00		
Platyhelminthes		Turbellaria				Tricladida	Tricladida	Flatworms							4	0.00														
Arthropoda	Chelicerata	Arachnida	Acari				Acari (subclass)	Mites and ticks			4	0.00	7	0.00	15	0.01											4	0.00	5	0.00
							Total		880		1516		1481		1505		1307		1004		1346		1518		445		1402		1468	
								Midge		0.00		0.37		0.26		0.07		0.21		0.24		0.08		0.10		0.33		0.09		0.05
								Mayfly		0.04		0.12		0.16		0.13		0.34		0.11		0.24		0.28		0.13		0.12		0.09
								Other insects		0.93		0.48		0.56		0.77		0.42		0.14		0.30		0.50		0.52		0.76		0.77
								Molluscs		0.03		0.01		0.00		0.01		0.01		0.33		0.01		0.04		0.00		0.02		0.04
								Crustaceans		0.00		0.00		0.00		0.00		0.00		0.00		0.36		0.05		0.00		0.00		0.00
								Annelids		0.01		0.02		0.01		0.01		0.02		0.18		0.00		0.03		0.02		0.01		0.05
								Other		0.00		0.00		0.01		0.01		0.00		0.00		0.00		0.00		0.00		0.00		0.00

	Total		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00
Take the top 3 that are above 1%		Insects	0.93	Insects	0.50	Insects	0.58	Insects	0.79	Insects	0.44	Midge	0.32	Insects	0.33	Insects	0.57	Insects	0.53	Insects	0.78	Insects	0.85
		Molluscs	0.03	Midge	0.38	Midge	0.27	Midge	0.07	Midge	0.21	Molluscs	0.44	Crustaceans	0.40	Midge	0.12	Midge	0.34	Midge	0.09	Midge	0.05
		Mayfly	0.04	Mayfly	0.13	Mayfly	0.16	Mayfly	0.13	Mayfly	0.35	Worms and leeches	0.24	Mayfly	0.27	Mayfly	0.31	Mayfly	0.13	Mayfly	0.12	Mayfly	0.10
			1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00

APPENDIX C: SUMMARIES OF CHRONIC STUDIES CONSIDERED FOR CRITERIA DERIVATION

See Appendix E for descriptions of other, less conclusive studies with:
Rainbow trout
Fathead minnow
Sacramento splittail
White sucker

See Appendix E for descriptions of invertebrate studies.

Tashjian, D.H., S.J. The, A. Sogomoyan and S.S.O. Hung. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon *(Acipenser transmontanus*). Aquatic Toxicol.79:401-409.

Test Organism: White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Seleno-L-methionine was added to an artificial diet consisting of vitamin-free casein, wheat gluten, egg albumin, dextrin, vitamin mix, BTM-mineral mix, cellulose, corn oil, cod liver oil, choline chloride and santoquin; the measured dietary concentrations were 0.4, 9.6, 20.5, 41.7, 89.8, 191.1 mg Se/kg dw.

Test Duration: 8 weeks

Study Design: 25 juvenile white sturgeon were placed in each of 24 90-L tanks. Treatments were randomly assigned to the 24 tanks resulting in 4 replicates per dietary treatment. Four fish from each tank were sampled after 0, 4 and 8 weeks for weight, length, liver weight, condition factors, hepatosomatic indices, hemocrit, histopathology, and selenium measurement in liver, kidney, muscle and gill tissues. 8 fish after 0 and 8 weeks were sampled for whole body selenium measurement.

Effects Data: Sturgeon survival did not differ significantly among treatment groups after the 8-week exposure with a mean survival rate of 99 across all groups. Fish fed 41.7 to 191.1 mg Se/kg dw exhibited significant declines in body weight (see table). All other endpoints measured were as sensitive or less sensitive to selenium in the diet as body weight.

Mean (SE) white sturgeon moisture, lipid and whole body Se after 8-week exposure

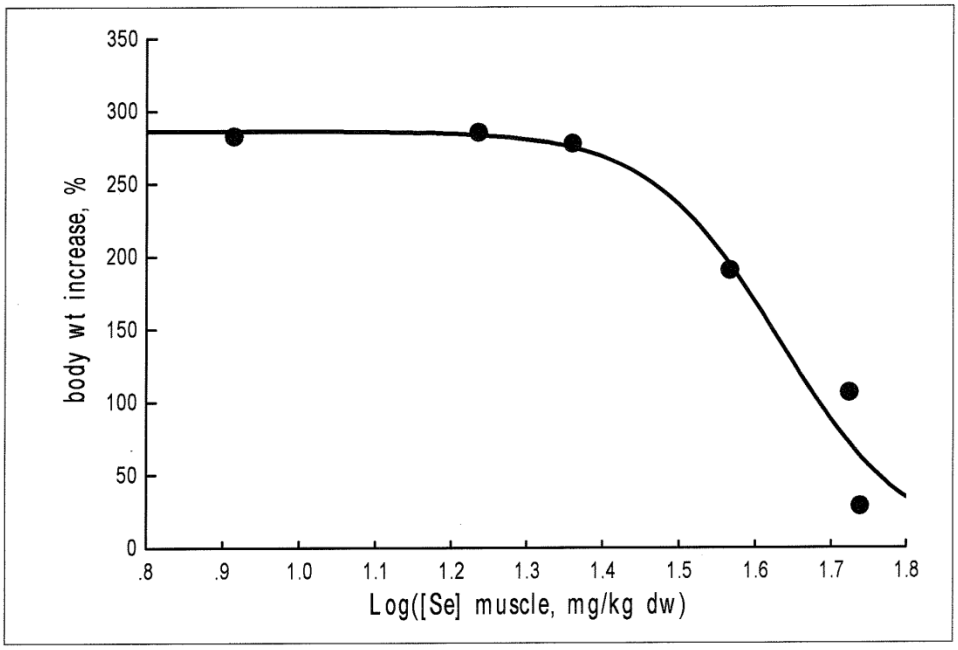
Treatment group	Moisture, % ww	Lipid, % ww	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	76.8 (0.5) b	9.5 (4) abc	8.2 (0.6) e	5.2 (0.4) c
9.6	77.0 (0.7) b	9.5 (0.9) abc	17.2 (0.7) d	11.8 (0.9) b
20.5	76.8 (0.3) b	10.1 (0.4) ab	22.9 (1.5) c	14.7 (0.8) b
41.7	77.3 (0.5) b	9.6 (0.7) abc	36.8 (1.8) b	22.5 (1.4) a
89.8	78.5 (0.3) ab	7.6 (0.4) bcd	52.9 (3.2) a	34.4 (2.3) a
191.1	80.0 (0.4) a	6.1 (0.4) cd	54.8 (2.8) a	27.5 (4.4) a

Mean (SE) white sturgeon body weight increase after 8-week exposure			
Treatment group	Body weight increase (%)	muscle Se, mg/kg dw	whole body Se, mg/kg dw
0.4	282.9 (4.6) a	8.2 (0.6) e	5.2 (0.4) c
9.6	285.5 (9.9) a	17.2 (0.7) d	11.8 (0.9) b
20.5	277.7 (6.1) a	22.9 (1.5) c	14.7 (0.8) b
41.7	191.0 (12.6) b	36.8 (1.8) b	22.5 (1.4) a
89.8	106.5 (5.8) c	52.9 (3.2) a	34.4 (2.3) a
191.1	28.6 (3.6) d	54.8 (2.8) a	27.5 (4.4) a

Letters denote statistical groupings among treatments within each exposure period ($p<0.05$).

Chronic Value: Using the logistic equation with a log transformation of the exposure concentrations (TRAP program), the EC_{10} and EC_{20} values for reduction in body weight are 15.08 and 17.82 mg Se/kg dw whole body and 27.76 and 32.53 mg Se/kg dw muscle tissue.

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

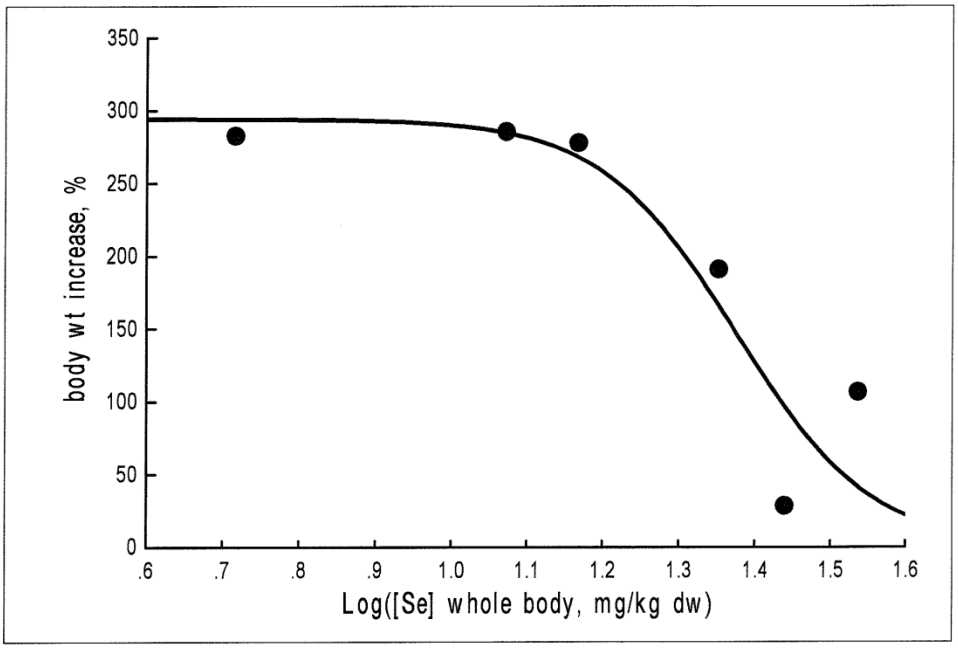
Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.6006	1.6303	0.0314	1.5304	1.7301
S	1.6574	2.938	0.925	-0.005	5.882
Y0	284.2	286.3	18.9	226.1	346.5

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	42.69	33.92	53.72
20.0	32.53	21.17	49.99
10.0	27.76	15.63	49.30
5.0	23.98	11.75	48.93

06/19/2009 10:32 MED Toxic Response Analysis Model, Version 1.03

White sturgeon (Tashjian et al 2006)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3403	1.3750	0.0643	1.1702	1.5797
S	2.283	2.794	1.908	-3.277	8.865
Y0	284.2	294.2	45.0	151.0	437.3

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.71	14.80	37.99
20.0	17.820	6.890	46.090
10.0	15.078	4.160	54.655
5.0	12.926	2.587	64.584

06/19/2009 10:42 MED Toxic Response Analysis Model, Version 1.03

Linville, R.G. 2006. Effects of Excess Selenium on the Health and Reproduction of White Sturgeon (*Acipenser transmontanus*): Implications for San Francisco Bay-Delta. Dissertation. University of California at Davis.

Test Organism: White Sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Selenium was added to the treatment in the form of selenized yeast. Selenized yeast (2.2%; Selenomax®, Ambi Inc.) was added to a commercial salmonid diet and pelleted with fish oil. For the control diet, the selenized yeast mixture contained 1.3% selenized yeast and 98.7 tortula yeast. Only selenized yeast was added to the treatment diet. After pelleting, the diet was allowed to air dry on drying racks.

Test Duration: Females were fed 0.3% body weight/day the experimental diet for 6 months.

Study Design: 16 adult female white sturgeon (approximately 5 years old, mean weight and fork length: 22.71 kg and 134.59 cm) were exposed in a freshwater flow through system to either the control diet (8 females in one tank fed 1.4 mg/kg Se) or treatment (8 females in a separate tank fed 34 mg/kg Se, Se from selenized yeast) for 6 months. After the 6 month dietary exposure, females were induced to spawn and fertilized with non-exposed male milt. Eggs were hatched in jars keeping eggs from each female separate. For each progeny cohort, 3000 larvae were randomly distributed into 3 reps for stage 40 (intestinal portion is void of yolk material, but stomach is not differentiated and is filled with yolk) sampling and 3 reps for stage 45 (yolk sac absorbed, start exogenous feeding) sampling. Se and biological measurements were made in each replicate.

Effects Data: No Se effects were observed for length or weight of larvae. Effects were determined for edema (Table 1), skeletal deformities (Table 2) and larval survival (Table 4). Because the mortalities for each cohort were recorded up to the time the sample was collected for abnormalities, a combined effects variable can be the total proportion of hatched larvae which were both alive and without any abnormalities at stage 45 (Table 4). This was calculated as PS·(1-PA), where PS is the proportion survival in the test chambers prior to sampling and PA is the proportion of the sample of surviving larvae with abnormalities. Binomial confidence limits are included in Table 4 for percent survival and percent abnormalities for each cohort to visualize significant differences among data points and between data points and fitted curves. Such confidence limits cannot be directly calculated for the combined effects variable, for which confidence limits were estimated by combining the lower and upper confidence limits of the individual effects variables using the same equation as above (this slightly overestimates the confidence limit range).

In Table 4, only cohort T2 is significantly different from the controls, based both on larval survival and abnormalities. That this selenium effect is also supported by the microinjection studies of Linville, which showed large abnormality frequencies for egg Se injected with >15 mg/kg, but little or no effect at lower concentrations (this is only supporting information because direct injection of a

specific form of Se is not a complete surrogate for setting effect concentrations for maternally transferred Se). For cohort T3, the data for abnormalities indicate some effects, but cannot be considered a definite effect concentration due to a combination of considerations – overlapping confidence limits with controls, no increase in mortality, limited information on within-cohort variability, and, based on egg concentrations, no effects for cohort T1 at a higher concentration.

EC_{10} Calculations: The combined effects variable is plotted versus Se concentration in the eggs in Figure 1. With only one definite partial effect, TRAP cannot be used to estimate a curve. Instead, the interpolation protocol is applied between the last two points based on specifying the highest no-effect concentration (HNOEC), 11.0 mg/kg, to be the EC_0 in the interpolation equation and specifying the upper control plateau (Y_0 in TRAP) to be average survival of the lower four points. The resultant TRAP slope is 3.0 and the interpolated EC_{10} is 15.6 mg/kg.

The egg EC_{10} of 15.6 mg/kg is slightly lower than the value of 16.3 mg/kg in the previous draft (Figure 3). The lower value was due to the inclusion of larval survival with abnormalities in the endpoint and using interpolation between the last two points rather than a TRAP model of the dataset.

Linville (2006) similarly calculated a 10% effective dose (ED_{10}) of the combined skeletal and edema data of 15.3 mg Se egg/kg dw using a logit regression. Linville (2006) also noted statistically significant differences using a Tukey Honest Significant Difference (HSD) test between Se and control treatments with respect to both the incidence of Stage 45 skeletal and total deformities, respectively, for the maternal transfer study. These author-reported results support the evidence of an effect of selenium in white sturgeon similar to the EC_{10} of 15.6 mg Se/kg egg dw interpolated by TRAP.

The combined effects variable is plotted versus Se concentration in muscle in Figure 2. Unlike for the egg concentration, the muscle concentration for cohort T3, with a small but not significant effect, is greater than that for cohort T1, with no effect, so that TRAP can be used to estimate a curve, although only barely so. This analysis was by tolerance distribution analysis with the log-triangular model. The resultant TRAP estimates are 100% for the control value and 8.8 for the EC_0 (about 11% below the T1 concentration); the standard deviation is 0.14 log units, equivalent to a slope of 3.7. The EC_{10} estimate is 11.9 mg/kg.

Chronic Value: The chronic value for combined deformities and larval survival using egg Se is an EC_{10} of 15.6 mg egg/kg dw. The chronic value for this same endpoint in muscle tissue is an EC_{10} of 11.9 mg muscle/kg dw.

Table 1. Edema deformities.

	Control			Treatment		
	Cohort	**Edema (%)**	**Larval Se (mg/kg dw)**	**Cohort**	**Edema (%)**	**Larval Se (mg/kg dw)**
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	6.67 (1)	7.75
Stage 40	C4	0.00 (3)	1.8	T1	0.00 (3)	11.6
	C5	0.00 (3)	2.88	T2	4.44 ± 2.22 (3)	20.4
				T3	1.67 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	15.56 ± 1.11 (3)	19.4
				T3	0.00 (2)	7.61

Table 2. Skeletal deformities.

	Control			Treatment		
	Cohort	**Skeletal (%)**	**Larval Se (mg/kg dw)**	**Cohort**	**Skeletal (%)**	**Larval Se (mg/kg dw)**
	C3	0.00 (1)	2.43	T1	0.00 (1)	11.6
Stage 36	C4	0.00 (1)	1.69	T2	0.00 (1)	18.4
	C5	0.00 (1)	2.67	T3	10.00 (1)	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.8	T1	0.00 (3)	11.6
	C5	1.11 ± 1.11 (3)	2.88	T2	14.44 ± 1.11 (3)	20.4
				T3	8.33 ± 1.67 (2)	7.22
Stage 45	C4	0.00 (3)	1.96	T1	0.00 (3)	12
	C5	0.00 (3)	2.59	T2	21.11 ± 1.11 (3)	19.4
				T3	13.33 ± 3.33 (2)	7.61

Table 3. Combined edema and skeletal deformities.

Control					Treatment			
	Cohort	Affected (%)	Egg Se (mg/kg)	Larval Se (mg/kg)	Cohort	Abnormal (%)	Egg Se (mg/kg)	Larval Se (mg/kg)
Stage 36	C3	0.00 (1)	2.46	2.43	T1	0.00 (1)	11	11.6
	C4	0.00 (1)	1.61	1.69	T2	0.00 (1)	20.5	18.4
	C5	0.00 (1)	2.68	2.67	T3	16.67 (1)	7.61	7.75
Stage 40	C4	1.11 ± 1.11 (3)	1.61	1.8	T1	0.00 (3)	11	11.6
	C5	1.11 ± 1.11 (3)	2.68	2.88	T2	18.89 ± 1.11 (3)	20.5	20.4
					T3	10.00 ± 0 (2)	7.61	7.22
Stage 45	C4	0.00 (3)	1.61	1.96	T1	0.00 (3)	11	12
	C5	0.00 (3)	2.68	2.59	T2	27.78 ± 2.94 (3)	20.5	19.4
					T3	13.33 ± 3.33 (2)	7.61	7.61

Table 4. Stage 45 data combined abnormalities and percent larval survival.

Cohort	Egg Se (mg/kg)	Muscle Se (mg/kg)	% Survival (95% Binomial CL)	% Abnormal (95% Binomial CL) [# Abnormal][1]	% Alive & w/o Abnormalities (95% Binomial CL)
C4	1.61	1.22	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
C5	2.68	1.48	99.7 (98.9-99.9)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T3	7.61	11.1	>99.6 (98.7-99.8)	13.3 (3.7-24.6) [3,5]	86.4 (74.4-96.3)
T1	11	9.93	>99.6 (98.7-99.8)	0.0 (0.0-4.2) [0,0,0]	99.7 (95.7-99.9)
T2	20.5	15.3	91.6 (90.1-92.8)	27.8 (18.8-38.3) [7,8,10]	66.2 (55.6-75.4)

[1] Bracketed numbers denote abnormal larvae in each of the 2-3 replicates of n=30.

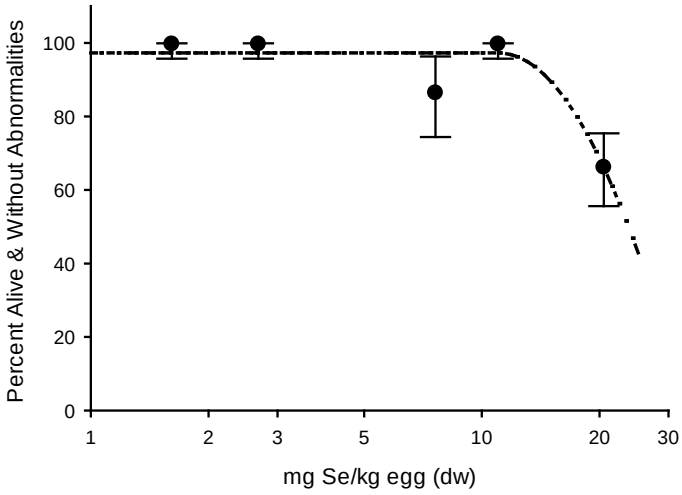


Figure 1. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in eggs. TRAP is used to interpolate between the last two points; EC10 = 15.6 mg Se/kg egg dw.

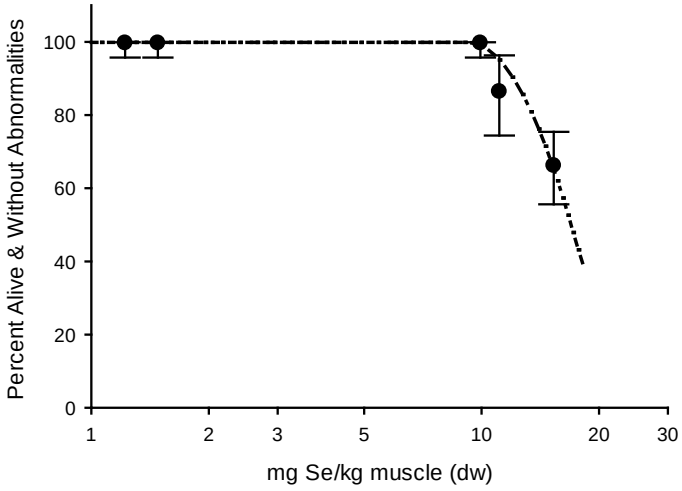
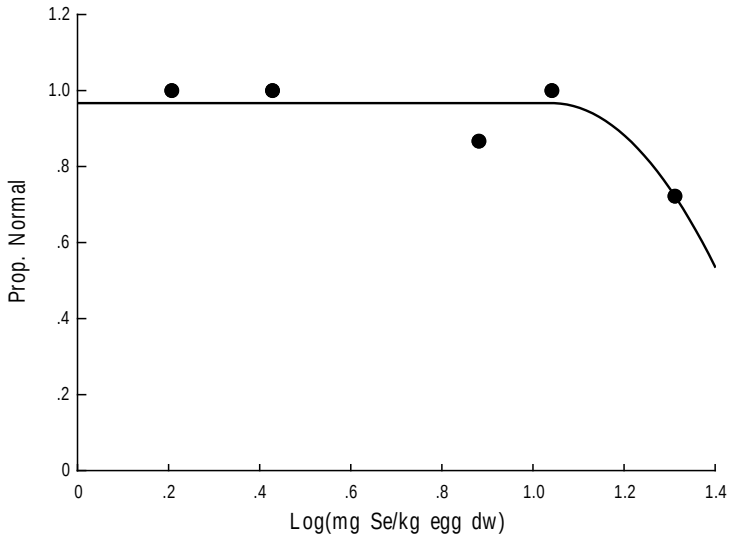


Figure 2. White sturgeon percent alive and without abnormalities as a function of the logarithm of selenium concentrations in female muscle. TRAP tolerance distribution analysis with the log-triangular model; EC10 = 11.9 mg Se/kg muscle dw.



Parameter	Initial	Final
LogX50	1.416	1.4215
S	**2**	**2.6306**
Y0	0.96667	0.96667

Figure 3. TRAP analysis from previous draft. Initial estimate for slope set equal to or less than 2.645 (set to 2 for this figure). EC_{10} = 16.3 mg/kg.

Teh, S.J., X. Deng, D-F Deng, F-C Teh, S.S.O. Hung, T.W. Fan, J. Liu, R.M. Higasi. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (*Pogonichthys macrolepidotus*). Environ. Sci. Technol. 38: 6085-6593.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*); juveniles 7-mos.old

Exposure Route: Dietary only

Dietary Treatments: 8 graded levels of dietary Se; dietary levels obtained by combining selenized yeast with Torula (non-active) yeast. Selenized yeast contained approximately 21% of Se as selenomethionine and proteinaceous Se forms. Diet was formulated as pellets by mixing dry ingredients with water and oil, fan-dried, crumbled and sieved. Analyzed levels: 0.4 (no selenized yeast), 0.7, 1.4, 2.7, 6.6, 12.6, and 57.6 mg/kg.

Fish were fed twice daily with a daily feeding rate of 3% BW in first 5 months and then adjusted to 2% BW thereafter.

Test Duration: 9 months

Study Design: A flow-through system with 40 fish/tank (24 total tanks) was used; each tank held 90 L. Flow rate was 4 L/min. Water temperature was maintained at 23°C for 6 months and then 18°C for last 3 months due to failure of water heating system. 5 fish were sampled from each tank at 5 and 9 months and measured for gross deformities, length, weight, Se in liver and muscle. Sections of the liver were kept for histopathology. Condition factor (100 x BW/length), heptatosomatic index (100 x liver weight/BW), BCF (total organ Se/dietary Se) were determined.

Effects Data: Mortality was observed in the two highest dietary treatments: 10 and 34.3%, respectively. No mortalities were observed in fish fed diets # 12.6 mg/kg. No significant difference in growth of fish fed 12.6 mg/kg Se in diet, but there was in the fish fed 26.6 mg/kg Se. See table below for levels of Se in fish at 9 months and associated effects.

Authors determined prevalence of deformities was higher in fish fed 6.6 and 12.6 mg/kg Se in their diet, however a dose-response relationship did not occur (e.g., no deformities in high concentration). Gross pathology was a more sensitive endpoint than growth.

Summary of effects and assoc. dietary and tissue concentrations in Sacramento splittail after 9 month exp.								
Dietary conc'n mg/kg	0.4	0.7	1.4	2.7	6.6	12.6	26.0	57.6
Se in liver, mg/kg dw	20.1	18.6	20.0	23.0	26.8	31.3	40.4	73.7
Se in muscle, mg/kg dw	6.6	6.9	9.2	10.1	15.1	18.9	29.4	38.7
Liver histopathology (mean lesions scores, N=15)								
Macrophage aggregate	0.13	0.07	0.2	0.27	0.40	0.20	0.20	0.85
Glycogen depletion	0	0	0.2	0	0.4	0.2	0	1.38
Single cell necrosis	0	0	0	0.07	0.13	0	0.07	0.46
Fatty vacuolar degeneration	0	0	0	0.2	0.53	0.07	0.2	0.08
Eosinophilic protein droplets	0	0	0	0	0	0	0.07	0.85
Sum of mean lesion scores	0.13	0.07	0.4	0.54	1.46	0.47	0.54	3.62
Gross Pathology (No. of deformities, N=15)								
Facial deformities (eye, jaw, and mouth)	0	1	0	1	5	3	0	0
Body deformities (kyphosis, lordosis, scoliosis)	0	0	4	2	3	1	1	0
Prevalence of deformity (%)	0	6.7	26.7	20	53.3	26.7	6.7	0

Chronic Value: Using gross pathology as the endpoint (prevalence of deformities, %), the NOAEC is 10.1 mg Se/kg dw and the LOAEC is 15.1 mg/kg Se dw in muscle tissue; MATC or CV = 12.34 mg/kg Se in muscle dw.

The above concentrations in juvenile muscle tissue cannot be exactly translated into an equivalent egg-ovary or whole-body concentration in adult splittail. But using the median egg-ovary to muscle ratio of 1.59 for the family Cyprinidae, the NOEC and MATC would represent 16.1 and 19.6 mg Se/kg egg-ovary. Using the median muscle to whole-body ratio of 1.26 for the family Cyprinidae, the NOEC and MATC would represent 8.04 and 9.83 mg Se/kg whole body. However, appropriateness of these conversion estimates rests upon uncertain assumptions that the muscle concentrations in juvenile splittails equal those of adult splittails under the same exposure conditions, and that splittail tissue ratios are those typical of the family Cyprinidae.

Comments: The authors observed deformities including spinal deformities using fish that were 7-months-old at test initiation. This is the only study in which deformities were observed in fish that were not exposed maternally.

Deng et al. (2008) exposed Sacramento splittail juveniles (21-day post hatch) to dietary selenium and dietary methylmercury in a two factorial design for four weeks. No adverse effects (growth, condition factor, lethargy or abnormalities) were observed in the selenium only exposures. The splittail accumulated approximately 3.5 mg Se/kg ww muscle in the highest dietary exposure (35 mg

Se/kg. Using the average percent moisture in fish muscle of 78.4% (May et al. 2000), the dw Se concentration is 16.2 mg Se/kg muscle indicating the recommended CV does not over-estimate an effect concentration.

Rigby et al. (2010) re-analyzed the juvenile Sacramento splittail data generated in the Teh et al. (2004) study. The authors used logistic regression to estimate EC values for deformities on a culled data set which eliminated the three highest dietary treatments due to their departure from a standard concentration-response relationship. The EC_{10} value for the culled data set was 7.9 mg Se/kg dw muscle which is lower than the recommended CV of 12.3 mg Se/kg dw muscle. Due to the lack of a concentration-response relationship across the entire dietary range and the lack of effects in the Deng et al. (2008) study, an EC_{10} of 7.9 mg Se/kg dw muscle is too uncertain for a recommended CV. Although the recommended CV of 12.3 mg Se/kg dw muscle is based on deformities (an uncertain response), it is considered representative of an effect level for this species because of the significant reductions in growth at the two highest test concentrations.

Bennett, William N., Arthur S. Brooks, and Martin E. Boraas. 1986. Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. Arch. Environ. Contam. Toxicol. 15:513-517.

Test Organism: Fathead minnow (*Pimephales promelas*; 2 to 8 day-old larvae).

Exposure Route: Dietary only
Green alga, *Chlorella pyrenoidosa* were exposed to Se ($H_2{}^{75}SeO_4$) in culture water for 3 days. Rotifers, *Brachionus calyciflorus*, were cultured in chambers with selenium containing green algae at the ratio of 25 μg algae/ml to 50 μg rotifer/ml for 5 hr. The rotifers were filtered to separate them from the algae and immediately heat-killed. The Se concentration in the rotifers was measured for ^{75}Se activity.

Test Duration: 9 to 30 days

Study Design: Selenium uptake by larval fathead minnows was measured in three experiments. Se-contaminated and control rotifers for feeding to larval fish were prepared in advance using the low algae:rotifer ratio. Daily equal volumes of rotifers were divided among five 800 mL polypropylene larval chambers. Three chambers received Se-contaminated rotifers and two received control rotifers. The rotifers were dead at the time of feeding (heat killed).

Larval fish were hatched from eggs spawned in the laboratory. After hatching, active larvae were divided equally among the larval test chambers (daily renewal exposures using dechlorinated Lake Michigan water). Larvae were initially fed rotifers raised on control algae (no selenium). The age of the larvae when first fed Se-contaminated rotifers was 4, 9, and 3 days post-hatch for experiments 1, 2, and 3, respectively. Larval fish were fed Se-contaminated rotifers for 7, 9, and 7 days in the 3 experiments. A post-exposure observation period of 19 and 2 days was used for experiments 1 and 2, respectively. During this time the larvae were fed control rotifers. Daily, larvae from a replicate were removed from the test chamber, washed, placed in a 20 ml vial, and counted for ^{75}Se activity for 20 min. All larvae were then placed in test chambers with fresh food rations. At the end of the study all fish were individually dried and weighed.

	Experiment 1	Experiment 2	Experiment 3
Initial feeding of control diet (days)	3	8	2
Day Se diet first fed	4	9	3
Day Se diet last fed	11	17	9
Observation days on control diet	19	2	0
Age at study termination (days)	30	19	9

Effects Data:

	Experiment 1	Experiment 2	Experiment 3
Mean food Se concentration (mg/kg)	>70	68	55
Food intake (μg rotifers/larva)	50	1330	1190
Initial larvae mean dry wt. at start of Se-laden food (μg)	90	400	100
Final larvae mean dry wt. (μg) at end of test	1470 (Control) 800 (Treatment)[a]	1888 (Control) 1354 (Treatment)[a]	475 (Control) 416 (Treatment)
Final mean larval Se content (μg Se/larva)[b]	0.0062	0.0700	0.0248
Final mean larval Se concentrations (mg Se/kg dw)	43.0	51.7	61.1

[a] Significantly different from the control.
[b] Values when Se-laden feeding was ended.

Selenium was measured in the test water during the feeding exposures, but the concentrations were insignificant (0.84 μg/L). Survival was not affected by the selenium exposures. Preliminary tests showed that fathead minnow larvae would reach plateau concentrations of selenium within the 7- to 9-day exposure periods. The food supply was sufficient to sustain growth of the larvae during the study, according to the authors. The authors state that selenium uptake and higher selenium content in experiment 2 larvae was due to their larger size and ability to consume more rotifers/unit time. Se-exposed larvae were significantly smaller ($p<0.05$) in mass than controls for experiments 1 and 2.

Chronic Value: GM of mean larval Se concentrations measured in the three experiments, i.e., 43.0, 51.7, and 61.1 mg/kg dw WB, respectively, is 51.40 mg Se/kg dw.

Dobbs, M.G., D.S. Cherry, and J. Cairns, Jr. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. Environ. Toxicol. Chem. 15:340-347.

Test Organism: Rotifer (*Brachionus calyciflorus*), and fathead minnow (*Pimephales promelas*) 12 to 24 hr-old at start.

Exposure Route: Dietary and waterborne

Water
Filtered and sterilized natural creek water supplemented with nutrients (Modified Guillard's Woods Hole Marine Biological Laboratory algal culture medium) for algal growth. Sodium selenate (Na_2SeO_4) was added to test water to obtain nominal concentrations of 100, 200, or 400 µg Se/L. Concentrations remained stable and equal in each trophic level.

Control Diet
No selenium was added to the water medium for the alga; green alga was free of selenium for the rotifer; and rotifers were free of selenium for the fathead minnow.

Selenium Diet
Sodium selenate was added to the culture medium for the alga; green alga thereby contained a body burden for the rotifer; and rotifers thereby contained a body burden for the fathead minnow.

Dietary Treatments: Each trophic level had a different treatment. The green alga was exposed directly from the water (1, 108.1, 204.9, 397.6 µg total Se/L); rotifers were exposed from the water (1, 108.1, 204.9, 393.0 µg total Se/L) and the green alga as food (2.5, 33, 40, 50 mg Se/kg dry wt.); and the fathead minnow were exposed from water (1, 108.1, 204.9, 393.0 µg total Se/L) and the rotifer as food (2.5, 47, 53, 60 mg Se/kg dry wt.).

Test Duration: 25 days

Study Design: A flow-through system utilizing a stock solution of filtered and sterilized creek water controlled at 25°C was used to expose three trophic levels of organisms. Approximately one liter of media was pumped from the algal chamber into the rotifer chamber each day. A cell density between 3 and 6×10^6 cells/ml was delivered to the rotifer chambers. Rotifers were started at a density of 151.4 ± 7.7 females/ml and one liter/day of rotifers containing culture water was intermittently pumped into the minnow chamber. (*B. calyciflorus* has a life span of about 7 days at 25°C.) The pump was necessary to overcome the swimming ability of rotifers to avoid an overflow tube. Larval fathead minnows (35/chamber) were prevented from escaping by a screened overflow. Chambers were cleaned daily and aeration was provided. All chambers were duplicated for test replication and water was measured for selenium on days 0, 2, 6, 7, 11, 14, 17, 20, and 24. All algal and rotifer biomass and selenium samples were made on these days. Fathead minnow chambers were measured for biomass, dissolved selenium, and tissue selenium concentrations of days 0, 7, 11, 14, 20, and 24.

Additional measurements were made in the 200 µg Se/L test chambers on the fathead minnow on day 16. Selenium concentrations were maintained near the nominal concentrations and the standard deviation of mean concentrations was less than 4 percent.

Effects Data:

Rotifers. Rotifers did not grow well and demonstrated reduced survival at all selenium exposure concentrations during the 25 day test. By test day 7 only the lowest test concentration (108.1 µg/L) had surviving rotifers which showed a decrease in selenium content from test days 18 through 25. A reduction in rotifer biomass was discernable by test day 4 in the selenium treatments and since all test concentrations had viable rotifer populations present, the effect level was calculated using these data.

Effect of Dietary and Waterborne Selenium on Rotifers after 4 Days Exposure			
Se in water, µg/L	**Se in diet, mg/kg dw**	**Se in rotifer tissue, mg/kg dw**	**rotifer biomass, mg/ml dw**
1	2.5	2.5	0.028
108.1	33	40	0.025
202.4	40	54	0.011
393	50	75	0.003

Fathead minnows. Due to the reduction of rotifer biomass in the higher test concentrations, fish mortality and reduction in fish growth observed in the latter days of the test was difficult to discern between effects from starvation and selenium toxicity. The data from test day 8 was selected for determining the effect of selenium on fathead minnows because starvation could be excluded as a variable.

Effect of Dietary and Waterborne Selenium on Larval Fathead Minnows after 8 Days Exposure			
Se in water, µg/L	**Se in diet, mg/kg dw**	**Se in fathead minnow tissue, mg/kg dw**	**Average fish weight, mg dw**
1	2.5	2.5	0.8
108.1	47	45	0.7
202.4	53	75	0.4
393	60	73	0.2

Chronic Value:

Rotifers	42.36 mg Se/kg dw (EC_{20})
Fish	< 73 mg Se/kg dw (LOAEC) - not amenable to statistical treatment; the LOAEC was based on the observation that a >50 percent reduction in mean fish weight occurred at this tissue concentration.

Schultz, R. and R. Hermanutz. 1990. Transfer of toxic concentrations of selenium from parent to progeny in the fathead minnow (*Pimephales promelas*). Bull. Environ. Contam. Toxicol. 45:568-573.

Test Organism: Fathead minnow (*Pimephales promelas*; Adults)

Exposure Route: Dietary and waterborne
Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Study Design: Four Monticello artificial streams were used for the study which lasted from September 1987 to September 1988. For each study, two streams (treated) were dosed continuously to achieve 10 µg/L and two streams served as controls. Mean selenium concentrations at the head of the treated streams were 9.8 ± 1.2 and 10.3 ± 1.7 µg/L, respectively. The concentrations of selenium measured in the water from controls streams were all less than the detection limit, i.e., 2 µg/L. Spawning platforms were submerged into each stream. One subset of six embryo samples (n = 2000 embryos per sample) were collected from the streams for selenium analysis. Another subset of ten embryo samples were reared in incubation cups receiving the same stream water dosed with sodium selenite via a proportional diluter. The treated embryos in egg cups received an average 9.7 ± 2.6 µg Se/L. Samples of hatched larvae were analyzed for selenium content while others were inspected for occurrence of edema and lordosis. Prior to test termination, female parents were seined. The mean selenium content in the ovaries of seven to eight females from the treated and control streams was reported.

Effects Data: Edema and lordosis occurred in approximately 25 percent of the fish spawned and reared in 10 µg Se/L. Corresponding occurrence in control fish incubated in the egg cups was only 1 and 6 percent, respectively. Table 1 provides the abnormality observations and the selenium residues in the embryos and ovaries from the control and treated streams. Although a case can be made that the Se treatment had a higher rate of edema and lordosis, there are some problems that add uncertainty to the estimation of an effect concentration (R. Erickson, pers. comm.). Heavy mortality/loss of embryo/larvae during monitoring and the erratic occurrence of the abnormalities (e.g., there is a significant incidence of edema in only 3 of 10 replicates for the Se treatment) led to the conclusion that results should not be used for criterion derivation. However, the data from this study support the range of reproductive effect levels determined in other studies. The Se concentration in embryos from the 10 µg/L treatment stream of 3.91 mg/kg ww converts to 25.6 mg/kg dw using 15.3% dw (N=3 range 14.7 – 15.6%) for fathead minnow eggs (R. Erickson, pers. comm). The previous draft used the Se concentrations in the ovaries collected at the end of the study for the effect concentration estimate. However, it was determined that the embryos are a more direct representation of Se exposure and toxicity to the larvae.

Chronic Value: The LOEC for embryos is <25.6 mg Se/kg dw.

Table 1. Percent Abnormalities in Fathead Minnow Larvae and the Associated Selenium Concentrations in Embryos and Ovaries.

Treatment	[Se] embryos, mg/kg ww (SD)	[Se] ovaries, mg/kg ww (SD)	Edema, % (SD)	Lordosis, % (SD)
Control	0.31 (0.01)	0.77 (0.14)	0.9 (2.2)	5.6 (8.8)
10 µg/L	3.91 (1.87)	5.89 (2.21)	24.6 (36.1)	23.4 (20.8)

SD = standard deviation

Beyers, D.W. and Sodergren, C. 2001a. Evaluation of interspecific sensitivity to selenium exposure: Larval razorback sucker versus flannelmouth sucker. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval flannelmouth sucker (*Catostomus latipinnis*) and larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage) Continuous flow diluter supplied a range of aqueous test concentrations <1, 25.4, 50.6, 98.9, and 190.6 µg/L selenate. Well water was used as the dilution water. Across the range of aqueous exposure concentrations, each test chamber was fed the same daily ration of living rotifers containing selenium at <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively. Rotifers accumulated selenium from algae (*Chlorella vulgaris*) exposed to 0, 25, 50, 100, and 200 :g/L selenate.

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1^{st} factor - selenium; 2^{nd} factor - species). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data : No survival effects were observed and there were no decreases in fish weight or length. Fish mass was found to increase as a function of selenium concentration.

Chronic Value: The chronic values for the flannelmouth sucker and razorback sucker were >10.2 and >12.9 mg Se/kg dw, respectively, based on the concentrations of selenium measured in whole-body tissue of larval fish at the highest water and dietary selenium concentrations.

Beyers, D.W. and Sodergren, C. 2001b. Assessment of exposure of larval razorback sucker to selenium in natural waters and evaluation of laboratory-based predictions. Larval Fish Laboratory. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

Test Organism: Larval razorback sucker *(Xyrauchen texanus)*

Exposure Route: Dietary and waterborne - laboratory exposure (28-d early life stage)
Larvae were exposed in a daily static-renewal system to control water (reconstituted very hard) and site waters: De Beque, Orchard Mesa, North Pond diluted 50%, and North Pond. Each water type received either a control diet (rotifers) or a diet previously exposed to the site water (site food: rotifers fed algae exposed to respective site water).

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1^{st} factor - test water type; 2^{nd} factor - rotifers cultured in control water or in site water). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data: No survival effects were observed. There were no significant decreases in growth of fish exposed to both site water and site food compared to fish exposed to control water and control food. There was a significant increase in growth of fish exposed to site water and control food relative to fish exposed to control water and control food ($p<0.0001$). There were reductions in the growth of fish (14%) exposed to site water and site food compared to site water and control food ($p<0.0001$). Due to the lack of a dose-response relationship in both the concentration of selenium in the food (rotifers) and growth, and the concentration of selenium in the fish larvae and growth, the authors did not attribute the effect of site food on the growth of fish to selenium.

Chronic Value: The NOAEC for the razorback sucker larvae in the four site water types based on selenium in whole-body tissue were: De Beque >5.45 mg Se/kg dw; Orchard Mesa >11 mg Se/kg dw; North Pond 50% dilution >41.1 mg Se/kg dw; North Pond >42 mg Se/kg dw. Because no significant effects were observed in larvae exposed to North Pond water at >42 mg Se/kg dw whole-body tissue, this value was selected as the chronic value for the study.

Muscatello, J.R., P.M. Bennett, K.T. Himbeault, A.M. Belknap and D.M. Janz. 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environ. Sci. Technol. 40:6506-6512.

Test Organism: Northern pike (*Esox lucius*)

Exposure Route: Dietary and waterborne - field exposure

Test Duration: Eggs were collected in the field and incubated in the laboratory. The test was terminated when the majority of the fry exhibited swim-up and had absorbed the yolk.

Study Design: The study area was Key Lake uranium milling operation in north-central Saskatoon. Spawning northern pike were collected from four sites, one reference (Davies Creek) and three exposure sites, David Creek near-field (high exposure), Delta Lake (medium exposure), and David Creek far-field (low exposure). The exposure sites were located approximately 2, 10 and 15 km downstream of the effluent discharge. Milt and ova were stripped from ripe fish and eggs were fertilized in the field. Females were saved for metal analysis and age determination. Subsamples of ova (prior to fertilization) were collected for metal analysis.

Although the study sites represent open systems where fish can potentially migrate among sites, radiotelemetry data from tagged adult pike (Muscatello and Janz, unpublished data) indicate high site fidelity at the “high” and “medium” exposure sites (lakes). In contrast, the “low” exposure site likely represents pike that migrated from further downstream sites that were likely of similar Se exposures as the reference site.

Eggs were incubated using a two-way ANOVA experimental design using water collected from reference or exposure sites. So, embryos originating from reference or exposure site females were incubated in either reference or appropriate exposure water. In addition, embryos from reference site females were incubated in water from all four study sites. 50 viable embryos from each individual female were transferred to each of four replicate incubation chambers. Cumulative time to 50% eyed, 50% hatch and 50% swim-up were determined. When the majority of the fry exhibited swim-up and had absorbed the yolk, the remaining fry were preserved and examined for deformities.

Effects Data: Mean egg diameter and fertilization success did not differ among sites. Cumulative embryo mortality throughout incubations was not significantly different among the sites ranging from 45 to 60%. There were no significant differences in the cumulative time to reach 50% eyed embryos, 50% hatch or 50% swim-up among treatments. Differences in the percent total deformities between test waters used during embryo incubation exposures were not significant, so the data were combined for each site (see Table below).

Selenium concentrations in eggs and muscle from female northern pike collected from reference and exposed sites and associated total deformities in embryos					
Site	**Site ID**	**Female**	**[Se] mg/kg dw**		**Total deformities %**
			Egg	**Muscle**	
Davies Creek	Reference	1	3.45	0.86	17
Davies Creek	Reference	2	2.72	1.89	2.5
Davies Creek	Reference	3	3.39	2.56	15.51
Davies Creek	Reference	4	3.72	1.34	7.13
Davies Creek	Reference	5	2.69	1.04	10.41
David Creek (far field)	Low	1	3.39	1.95	20.32
David Creek (far field)	Low	2	4.07	2.04	13.19
David Creek (far field)	Low	3	4.07	1.26	15.33
David Creek (far field)	Low	4	4.07	2.48	18.83
David Creek (far field)	Low	5	3.4	1.26	11.8
Delta Lake	Medium	1	43.19	17	37.8
Delta Lake	Medium	2	24.53	16.52	31.71
Delta Lake	Medium	3	26.14	16.52	26.29
David Creek (near field)	High	1	48.23	47.82	39.5
David Creek (near field)	High	2	N/A*	28.72	N/A*

*female had no eggs

Significant increases in total deformities (edema, skeletal deformities, craniofacial deformities and fin deformities) were observed in fry originating from pike collected at the medium exposure site. Determination of an effect level for the percent total deformities relative to the concentration of selenium in eggs or in female muscle tissue was not amenable to analysis by TRAP. One requirement of TRAP is to have a response greater than 50%, which was not satisfied with the available data.

When data are not amenable to determining an effect level using a software program, such as TRAP, one way to estimate the effect level is to make a direct measurement of effect at an exposure or tissue concentration. For example, if only a control and one exposure concentration, 10 μg/L, were tested in an acute toxicity test and there was 100% survival in the control and 35% in the 10 μg/L, the effect level would be an EC_{35} of 10 **μg**/L. Such an approach was used to estimate effect in the Muscatello et al. data. Because no significant differences were observed in either selenium concentrations in eggs or percent total deformities between the reference and low exposure site, the data from these 10 sites were combined. Similarly, the egg **and muscle** selenium and total deformity data were combined for the 4 medium and high exposure sites. These means, geometric for the selenium concentrations and arithmetic for the percent total deformities, are given in the following table.

	Mean selenium in northern pike egg and muscle and effect values for reference and exposure sites			
Sites	**[Se] in eggs, mg/kg dw (geometric mean)**	**[Se] in muscle, mg/kg dw (geometric mean)**	**Total deformities, % (arithmetic mean)**	**Total deformities, % (accounting for reference deformities and transformed to new scale)[a]**
Reference sites (includes low exposure)	3.462	1.570	13.20	0
exposure sites	34.00	21.70	33.82	23.76

[a] The % total deformities in the reference and exposed sites were normalized to the reference effect (13.2%) and then transformed to a new scale (100%). i.e, Abbott's formula.

The percent affected becomes 24% or an EC_{24} and the effect level is 34.00 mg Se/kg dw in eggs and 21.70 mg Se/kg in muscle**.**

Chronic Value: EC_{24} = 34.00 mg Se/kg dw in eggs. Note: an EC_{10} cannot be estimated with the data.

Hamilton, S.J., K.J. Buhl, N.L. Faerber, R.H. Wiedermeyer and F.A. Bullard. 1990. Toxicity of organic selenium in the diet of chinook salmon. Environ. Toxicol. Chem. 9:347-358.

Test Organism: Chinook salmon (*Oncorhynchus tshawytscha* Walbaum; swim-up larvae)

Exposure Route: Dietary only

Control Diet

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish (1.0 mg Se/kg dw) collected from a reference site.

Selenium Diet #1

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from high-selenium mosquitofish (35.4 mg Se/kg dw) collected from the San Luis Drain, CA, termed SLD diet.

Selenium Diet #2

Oregon moist pellet diet where over half of the salmon meal was replaced with meal from low-selenium mosquitofish same as in the control diet, but fortified with seleno-DL-methionine (35.5 mg Se/kg dw), termed SeMet diet.

Dietary Treatments: Each selenium diet was formulated to contain about 36 mg Se/kg dw as the high exposure treatment. The remaining treatments were achieved by thoroughly mixing appropriate amounts of high-exposure treatment diet with control diet to yield the following nominal concentrations (3, 5, 10, and 18 mg Se/kg dw).

Test Duration: 90 days

Study Design: Each dietary treatment was fed twice each day to swim-up larvae (n=100) in each of two replicate aquaria that received 1 L of replacement water (a reconstituted experimental water that simulated in quality a 1:37 dilution of water from the San Luis Drain, CA minus the trace elements) every 15 minutes (flow-through design). Mortality was recorded daily. Growth was evaluated at 30-day intervals by measuring the total lengths and wet weights of two subsets of individual fish (n=10x2) held in separate 11.5 L growth chambers within each replicate aquarium. Tissue samples were collected for whole-body selenium determinations (dw basis) at 30-day intervals throughout the study; 10, 5, and 2 fish were sampled from each duplicate treatment after 30, 60, and 90 days of exposure, respectively. Concentrations of selenium measured in water were below the limit of detection (1.5-3.1 μg/L) in all dietary selenium exposure concentrations.

Effects Data: The magnitude of reduced growth was most evident in the weight of the fish, although total length was significantly reduced in fish fed high Se-laden diets as well. The effect of increasing dietary selenium on mean larval weight was similar in both the SLD and seleno-methionine diets.

Effect of San Luis Drain Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	3.3	2.68	97.3
5.3	4.5	2.76	93
9.6	8.4	2.8	95
18.2	13.3	2.62	92.4
35.4	29.4	1.4	89

Effect of Seleno-methionine Diet on Growth and Survival of Chinook Salmon Larvae after 60 Days			
Se in diet, mg/kg dw	**Se in chinook salmon, mg/kg dw**	**Mean larval weight, g**	**Survival, %**
1	0.9	3.35	99
3.2	2	3.08	100
5.3	3.1	3.22	95
9.6	5.3	3.07	94.1
18.2	10.4	2.61	92.4
35.4	23.4	1.25	62.5

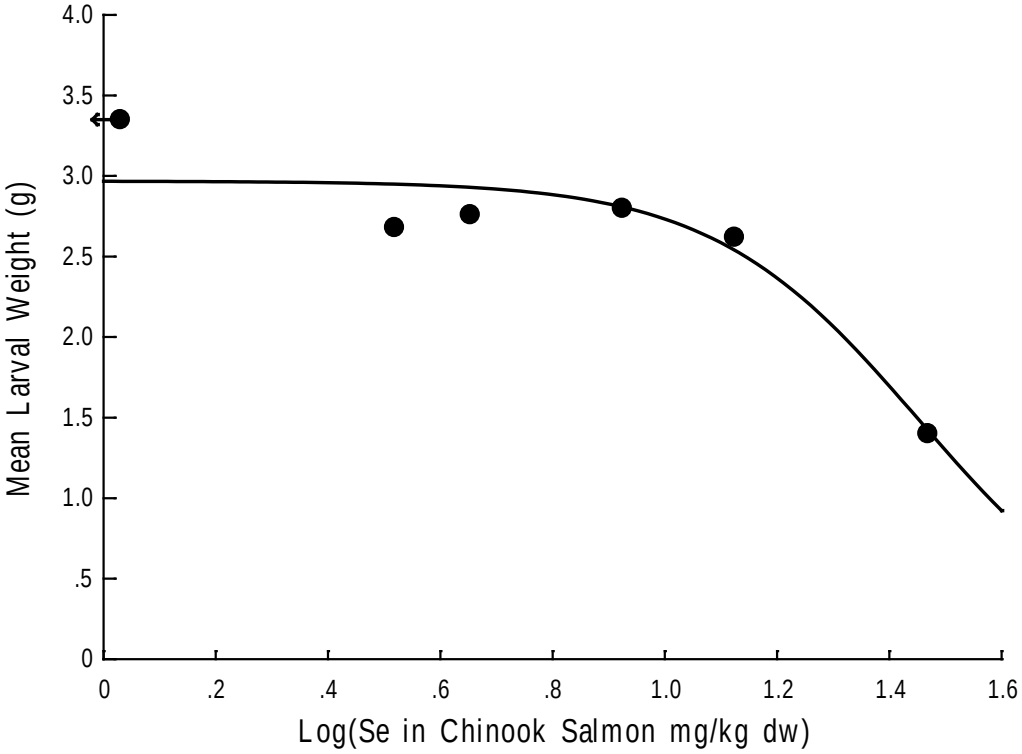
Chronic Value: Due to unacceptable control mortality of swim-up larvae in control treatments after 90 days (33.3 percent - SLD diet; 27.5 percent - SeMet diet), chronic values had to be determined from respective values reported after 60 days (tables above).

Analysis of the elemental composition of the SLD diet indicated that B, Cr, Fe, Mg, Ni and Sr were slightly elevated compared to the control and SeMet diets. No additional analyses were performed to determine the presence of other possible contaminants, i.e., pesticides.

	EC_{20} values		**EC_{10} values**
	Survival (after 60 d of exposure)	**Growth (after 60 d of exposure)**	**Growth (after 60 d of exposure)**
Diet type	**Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**	**Whole body Tissue Se (mg/kg dw)**
SLD	NA[a]	15.73	11.14
SeMet	NA[a]	10.47	7.355

[a] The EC_{20} and EC_{10} values for survival of swim-up larvae versus levels of selenium for the SLD and SeMet dietary exposure could not be estimated using non-linear regression.

Hamilton et al (1990) Chinook Salmon fed SLD Diet
Logistic Equation, Three Parameter Model, Se concentrations log_{10} transformed

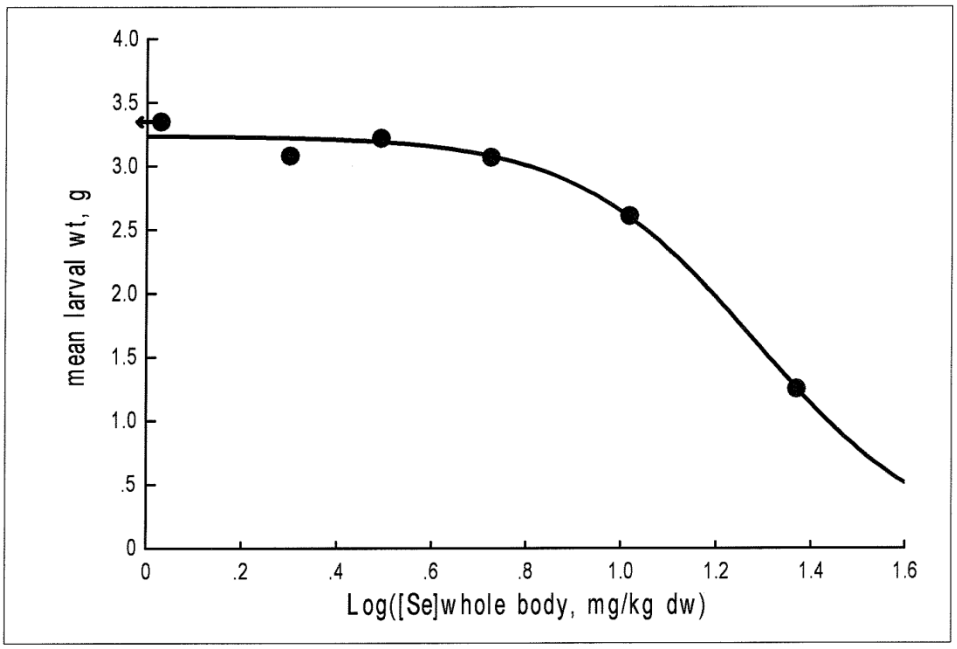


	Guess	FinalEst	SE	95%LCL	95%UCL
LogX50	1.453	1.453	7.30E-02	1.2206	1.6854
StDev	1.353	1.353	6.67E-01	-7.71E-01	3.4769
Y0	2.968	2.968	1.89E-01	2.3651	3.5709

%Effect	Xp Est	95% LCL	95% UCL
50	28.379	16.62	48.458
20	15.734	5.7003	43.431
10	11.143	2.4771	50.127
5	8.1085	1.1213	58.637

	DF	SS	MS	F	P
Total	5	2.0749	0.41498		
Model	2	1.8202	0.91009	10.719	0.95699
Error	3	0.2547	8.49E-02		

Chinook salmon SeMet diet (Hamilton et al. 1990)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3148	1.2823	0.0242	1.2053	1.3593
S	0.6971	1.3214	0.1826	0.7404	1.9025
Y0	3.217	3.239	0.067	3.027	3.452

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	19.156	16.045	22.870
20.0	10.472	7.516	14.591
10.0	7.355	4.595	11.775
5.0	5.312	2.899	9.733

06/19/2009 13:52 MED Toxic Response Analysis Model, Version 1.03

Hilton, J.W. and P.V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). J. Nutr. 113:1241-1248.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only

Low carbohydrate diet (LCD)
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler.

High carbohydrate diet (HCD)
This diet contained cerelose at 25 percent of the diet with cellulose as the filler.

For both diets, the selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The two diets were supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the six different dietary selenium treatments (n = 3 low carbohydrate diet; n= 3 high carbohydrate diet). The six diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw, and the measured concentrations of selenium in the high carbohydrate diet were: 0.7 (control), 6.6, and 11.8 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 weeks

Study Design: Body weights, feed: gain ratios, and total mortalities were determined after each 28-day interval. After 16 weeks, approximately 20 fish were randomly removed from each tank, weighed, and blood was collected for hemoglobin, hematocrit, and plasma glucose, protein, and calcium determination. The livers and kidneys were then dissected. The livers were assayed for glycogen content, and samples of both liver and kidney were assayed for selenium content. Additional subsamples of fish were sacrificed and assayed for selenium content and for ash, crude protein, and moisture content (n=6 per treatment). Finally, 30 fish were killed, their livers and kidneys dissected, and analyzed for Ca, Cu, Fe, Mg, P, and Zn content.

Effects Data: The only overt sign of selenium toxicity was food avoidance observed in trout fed the highest selenium content in both low and high carbohydrate diets, which led to significantly reduced body weight after 16 weeks. There were no significant differences detected between treatment groups in hematological parameters. Kidney, liver, and carcass selenium levels increased with increasing selenium content of the diet, however, only the liver selenium concentrations were significantly affected by dietary selenium level, dietary carbohydrate level, and the interaction between the two treatments. Mineral analysis of the kidney showed significantly higher levels of calcium and phosphorous in trout reared on the two highest levels of dietary selenium. Concentrations of copper in the liver increased significantly with increasing dietary selenium levels and decreasing dietary carbohydrate levels.

Effect of Selenium in Low carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.6	0.8	3.3
6.6	38.3	3.3
11.4	49.3	1.8

Effect of Selenium in High carbohydrate Diet to Rainbow Trout		
Se in diet, mg/kg dw	**Se in trout liver, mg/kg dw**	**Trout weight, kg/100 fish**
0.7	0.6	2.7
6.6	21.0	2.3
11.8	71.7	1.4

Chronic Value: The following table lists the NOAEC, LOAEC and MATC for both diets in liver tissue. EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Diet	**NOAEC, mg Se/kg dw liver**	**LOAEC, mg Se/kg dw liver**	**MATC, mg Se/kg dw liver**
Low carb	38.3	49.3	43.5
high carb	21.0	71.7	38.8

Hicks, B.D., J.W. Hilton, and H.W. Ferguson. 1984. Influence of dietary selenium on the occurrence of nephrocalcinosis in the rainbow trout, *Salmo gairdneri* Richardson. J. Fish Diseases. 7:379-389.

(Note: These data are the exact same as reported for the low carbohydrate diet in **Hilton and Hodson 1983**, with the addition of prevalence of nephrocalcinosis occurring in trout after 16 to 20 weeks of consuming the contaminated test diets).

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 0.6 g each)

Exposure Route: Dietary only
This diet contained capelin oil at 11 percent of the diet with cellulose as the filler. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Treatments: The test diet was supplemented with selenium (as sodium selenite) at the rate of 0, 5, or 10 mg/kg dw to make up the three different dietary selenium treatments. The three diets were fed to duplicate groups of 100 fish. The trout were fed to satiation 3-6 times per day. Measured concentrations of selenium in the low carbohydrate diet were: 0.6 (control), 6.6, and 11.4 mg/kg dw. The tanks received a continuous flow of water with a flow rate of 3-4 liters per minute.

Test Duration: 16 to 20 weeks

Study Design: See Hilton and Hodson (1983). After 20 weeks on the test diets, ten fish were randomly removed from each treatment. Tissues for histopathological examination included the stomach, intestine and pyloric ceca (including pancreas), spleen, liver, heart, kidney, skin, muscle, and gills.

Effects Data: Only effects of selenium on kidney tissue are included in the article. The kidneys of the 10 trout fed the highest selenium content in the diet exhibited normal appearance. Five of these trout exhibited precipitation of calcium in the tubules with some epithelial necrosis, but no loss of epithelial continuity. Extensive mineralized deposition of Ca within the tubules, tubular dilation and necrosis of tubular epithelium, ulceration of tubules, and intestinal Ca mineralization was observed in four of the ten fish.

Chronic Value: Same as for growth of rainbow trout reported by Hilton and Hodson (1983). The MATC estimated for growth of rainbow trout relative to final concentration of selenium in liver tissue of trout reared on the low carbohydrate diet is the GM of 38.3 (NOAEC) and 49.3 (LOAEC) mg/kg dw, or 43.45 mg/kg dw.

EC values could not be determined for this study. Data did not meet minimum requirements for analysis.

Hilton, J.W., P.V. Hodson, and S.J. Slinger. 1980. The requirements and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J. Nutr. 110:2527-2535.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; juvenile; approx. 1.28 g each)

Exposure Route: Dietary only
A casien-torula yeast diet was formulated to contain geometrically increasing levels of selenium from 0 to 15 mg/kg dw. The selenium was supplemented as sodium selenite which was mixed with cellulose and then added to the diet as a selenium premix.

Test Duration: 20 weeks

Study Design: Six test diets were fed to triplicate groups of 75 fish. The trout were fed to satiation 3-4 times per day, 6 days per week, with one feeding on the seventh day. Measured concentrations of selenium in the diet were: 0.07 (control), 0.15, 0.38, 1.25, 3.67, and 13.06 mg/kg dw. The tanks received a continuous flow of dechlorinated tap water from the City of Burlington, Ontario municipal water supply. The waterborne selenium content of this water was 0.4µ g/L. During the experiment, the fish were weighed every 2 weeks with the feeding level adjusted accordingly. Mortalities were noted daily and the feed consumption for each treatment was recorded weekly. After 4 and 16 weeks, three to six fish were randomly removed from each tank, sacrificed, and their livers and kidneys removed and weighed. An additional three to six fish were then obtained from each treatment, killed, and prepared for tissue analysis. Organs and carcasses were freeze-dried for determination of selenium concentration. After 16 weeks, three more fish were removed. Kidney, liver, spleen and dorsal muscle tissue was dissected for examination of histopathology. At the end of 8 and 16 weeks, four to five fish were removed, sacrificed, and a blood sample was taken for hematological measurements (hematocrit, red blood cell count, and blood iron concentration). After 20 weeks, three to four more fish were removed, sacrificed, and a blood sample was taken for measurement of glutathione peroxidase activity.

Effects Data: There were no significant differences detected between treatment groups in histopathology, hematology, or plasma glutathione peroxidase activity. Trout raised on the highest dietary level of selenium (13.06 mg/kg dw) had a significantly lower body weight and a higher number of mortalities (10.7; expressed as number per 10,000 fish days) than trout from the other treatments levels after 20 weeks of exposure.

Effects on Juvenile Rainbow Trout			
Se in diet, mg/kg dw	**Se in Liver, mg/kg dw**	**Weight, g/fish**	**Mortality***
0.07	0.6	3.2	0
0.15	0.95	3.5	0
0.38	2.4	3.7	0.6
1.25	11	4.1	0.6
3.67	40[a]	4.1	0
13.06	100[b]	1.4	10.7

* expressed as number per 10,000 fish-days
[a] NOAEC
[b] LOAEC

Chronic Value: NOAEC = 40 mg Se/kg dw
LOAEC = 100 mg Se/kg dw
MATC = 63.25 mg Se/kg dw

Holm, J. 2002. Sublethal effects of selenium on rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Masters Thesis. Department of Zoology, University of Manitoba, Winnipeg, MB.

Holm, J., V.P. Palace, K. Wautier, R.E. Evans, C.L. Baron, C. Podemski, P. Siwik and G. Sterling. 2003. An assessment of the development and survival of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) exposed to elevated selenium in an area of active coal mining. Proceedings of the 26th Annual Larval Fish Conference 2003, Bergen, Norway. ISBN 82-7461-059-B.

Holm, J., V.P. Palace, P. Siwik, G. Sterling, R. Evans, C. Baron, J. Werner, and K. Wautier. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. Environ. Toxicol. Chem. 24: 2373-2381.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*; spawning adults) and brook trout (*Salvelinus fontinalis*; spawning adults)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the high selenium site ranged from 6 to 32 μg/L. Selenium was not measured at the reference streams; selenium concentrations at reference locations in the area ranged from <0.5 to 2.2 μg/L.

Study Design: Spawning fish were collected at low selenium or reference streams (Deerlick Creek, Wampus Creek and Cold Creek), a slightly elevated selenium stream (Gregg Creek), and an elevated selenium stream (Luscar Creek) in the Northeastern slopes region of Alberta, Canada. An active coal mine is the source of selenium in the elevated streams. Eggs and milt from the spawning trout were expressed by light pressure from abdomen. Individual clutches of eggs were fertilized from a composite volume of milt derived from 3-5 males. Fertilized eggs from individual females were reared to swim-up stage and examined for a number of parameters including percent fertilization, mortality, edema, and deformities (craniofacial, finfold, and spinal malformations). Similar studies were conducted in 2000, 2001 and 2002. One notable difference is that the embryos were incubated at 8°C in 2000 and at 5°C in 2001. The authors noted that 5°C is a better representation of the actual stream temperature during embryo development.

Effects Data : Other than selenium, there were no significant differences in the concentrations of other elements (Al, As, Sb, Ba, Be, Ni, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ag, Sr, Tl, Th, Sn, Ti, U, V, Zn) in trout eggs between the low level and elevated selenium streams. There are two ways to approach determination of effects due to selenium in this study and both are presented here. The first approach determines effects based on a comparison of average conditions between streams (*between streams approach*). For example, if there is a significant difference between the average frequency of deformities in a contaminated stream and reference stream, the effect level for the *between streams approach* would be the average concentration of selenium in the tissue from the contaminated stream. The second approach evaluates individual response variables (e.g., edema, deformities) against the individual selenium tissue concentrations for the combined contaminated and reference stream data set with each year (*within streams approach*). This approach, which results in an

EC estimate (e.g., EC_{10}) if the data meet the model assumptions, is explained below.

Between streams approach: For each sampling location (stream), data for the three years (Tables 1 and 2) were combined in the between streams analysis of variance (ANOVA). For rainbow trout embryos, there were no significant differences in fertilization, time to hatch and mortality between the streams with elevated selenium and the reference streams. ANOVA indicated significant differences in the frequency of embryonic effects between streams (Table 3). The analysis did not prove useful; however, due to a higher occurrence of effects in some of the reference streams relative to the exposed streams (Tables 3 and 4). The between streams analysis, therefore, was not used to determine effect concentrations for rainbow trout.

ANOVA of brook trout data indicated the only significant difference in embryonic abnormalities among sites was craniofacial deformities (Tables 5 and 6). Significant differences were also found for fertilization and larval weight. The highest average percent fertilization was observed at the site with the greatest concentration of selenium in eggs, which indicates that the differences in fertilization among sites were not caused by variation in selenium concentrations. Because the percent of embryos with craniofacial deformities in Luscar Creek was 7.9% (2.1% in Cold Creek), it was not considered biologically meaningful. Likewise the significantly lower larval weights at the exposed sites was not large (16% lower than Cold Creek larvae) and again coupled with the low occurrence of abnormalities by the brook trout, a signature of selenium effects, the lower larval weights were not considered biologically meaningful.

Within streams approach: As with the *between streams* analysis, data were combined for the three years of study in the *within streams* analysis (Tables 1 and 2). Craniofacial deformities, skeletal deformities and edema in rainbow trout embryo, as a function of selenium in egg ww, were fitted to a curve using a weighted regression and threshold sigmoidal equation from which EC_{10} values were calculated (see Figures 1, 2 and 3). EC estimates for finfold deformities, length and weight of rainbow trout embryos could not be made because of inadequate dose-response. The brook trout data were not suitable for fitting logistic curves (Figure 5).

Table 1. Rainbow trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference sites (Deerlick Creek and Wampus Creek) in northeastern Alberta over three consecutive years.

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2000	Luscar	11	6.84	7.18	13.26	1.66	4.97
2000	Luscar	12	6.66	1.48	4.43	0.74	1.85
2000	Luscar	14	11.6	14.43	23.71	7.22	85.57
2000	Deerlick	16	1.78	0.63	1.9	0.63	0.63
2000	Deerlick	17	1.39	0	0	0	0
2000	Deerlick	18	1.00	0	0.86	0	0
2000	Deerlick	15	5.01	0	0	0	0
2001	Luscar	1	5.39	7.35	6.76	3.53	2.94
2001	Luscar	3	8.39	6.29	4.97	2.98	6.95
2001	Luscar	4	6.48	22.22	22.22	33.33	26.67
2001	Luscar	8	4.47	12	9.33	2.67	10.67
2001	Luscar	14	10.4	34.55	44.85	4.24	43.64
2001	Luscar	32	5.64	8.24	5.97	3.13	9.09
2001	Luscar	33	3.88	5.26	6.58	9.21	3.95
2001	Luscar	39	5.14	1.91	3.18	0	1.27
2001	Luscar	40	3.36	11.62	7.05	5.39	6.64
2001	Luscar	41	11.7	37.67	83.41	3.59	87
2001	Deerlick	8	3.68	9.55	5.45	1.36	5.45
2001	Deerlick	9	3.08	5.39	4.98	0.41	2.07
2001	Deerlick	10	1.62	7.89	7.89	5.26	10.53
2001	Deerlick	16	2.62	24.24	48.48	3.03	12.12
2001	Deerlick	17	2.79	14.13	15.22	4.35	20.65
2001	Deerlick	21	1.96	13.27	35.71	7.14	25.51
2001	Deerlick	22	3.13	1.09	2.17	0	1.09
2001	Deerlick	23	3.03	9.65	14.04	3.51	7.89
2001	Deerlick	25	3.32	9.25	13.29	7.51	8.09
2001	Deerlick	39	2.43	11.89	9.09	7.69	14.69
2001	Gregg	2	4.57	11.97	7.75	15.49	7.04
2001	Gregg	3	4.49	5.58	9.3	2.33	4.65
2001	Gregg	5	4.05	4.95	5.45	2.48	5.94
2001	Gregg	9	5.09	20	13.85	15.38	16.15
2001	Gregg	18	5.97	16.13	19.35	41.94	35.48
2001	Wampus	9	2.66	16.07	0	1.79	7.14
2001	Wampus	13	2.04	7.84	9.8	1.31	7.84
2002	Luscar	3	5.4	60.47	27.9	93	14
2002	Luscar	8	18.3	94.12	23.5	4.4	97.1
2002	Luscar	10	22	100	64.3	3.6	100
2002	Luscar	12	15.7	82.35	47.1	66.7	52.9

Year	Site	Female #	Se in eggs, mg/kg ww	%craniofacial deformities	%skeletal deformities	%finfold deformities	%edema
2002	Luscar	22	20.5	100	42.1	2.1	100
2002	Luscar	23	6.3	5.59	6.6	1.6	2.7
2002	Luscar	24	26.8	100	100	0	100
2002	Luscar	26	6.5	1.72	1.7	4.3	0.9
2002	Deerlick	10	5.9	5.65	7.26	7.26	3.23
2002	Deerlick	18	7.8	10.77	1.54	9.23	3.08
2002	Deerlick	21	5	6.9	6.9	20.69	1.72
2002	Deerlick	24	4.3	2.88	2.88	21.58	0.72
2002	Deerlick	25	4.4	5.3	5.3	6.82	3.03
2002	Deerlick	26	6.6	2.95	1.85	1.11	1.85
2002	Gregg	1	5.8	4.76	3.81	3.81	3.81
2002	Wampus	1	3	18.84	14.49	72.46	11.59
2002	Wampus	2	4	0	0	100	100
2002	Wampus	3	4.6	4.1	3.28	7.58	0.61
2002	Wampus	4	4.7	25	20	70	12.5
2002	Luscar	28	7	19.23	0	76.9	0

Table 2. Brook trout embryo-larval parameters collected from a high Se site (Luscar Creek), an intermediate Se site (Gregg River), and reference site (Cold Creek) in northeastern Alberta over three consecutive years.

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2000	Luscar	1	4.78	15.38	0	0	15.38
2000	Luscar	2	4.83	38.06	1.49	3.73	1.49
2000	Luscar	3	5.98	7.39	3.03	0.34	0.5
2000	Luscar	5	3.86	25	5.7	8.77	4.82
2000	Luscar	12	6.06	16.77	1.83	0.7	0
2000	Luscar	13	5.8	4.06	1.42	0.2	0
2000	Luscar	14	5.17	4.13	0.49	0.36	0.12
2000	Luscar	15	9.92	16.22	0.54	0.54	0
2000	Luscar	16	5.03	5.61	0	0.27	0.27
2000	Luscar	17	6.01	9.44	5.83	0.83	1.11
2000	Luscar	18	12.7	14.34	0.72	0	0.36
2000	Cold	21	1.15	3.26	1.48	0.89	0
2000	Cold	22	1.83	4.83	1.38	1.38	0.69
2000	Cold	24	0.97	1.67	0	0.72	0
2000	Cold	25	No data	3.31	1.1	1.66	1.1
2000	Cold	26	0.59	3.45	4.83	6.9	0.69
2000	Cold	33	1.35	6.15	0	1.54	0
2000	Cold	34	2.18	6.45	0	0.81	0
2001	Cold	6	1.79	0	0	0	0
2001	Cold	7	1.36	1.61	0.69	0.46	1.38
2001	Cold	8	0.94	1.36	0	0.27	0.54
2001	Cold	21	1.07	0.43	0	0	0
2001	Cold	51	1.09	0	2.13	0	6.38
2001	Luscar	3	8.4	0	0.93	0	0.46
2001	Luscar	7	7.26	1.35	1.62	0.81	0.27
2001	Luscar	17	14.6	2.22	0.63	0.32	0
2001	Luscar	19	9.79	7.55	2.11	2.42	0.3
2001	Luscar	59	5.8	2.28	0.46	0.91	0.46
2001	Luscar	60	9.03	3.16	0	1.05	1.05
2001	Luscar	61	7.29	0	0	9.09	0
2001	Luscar	64	7.08	1.54	2.19	0	0
2001	Luscar	76	7.1	36.71	13.29	19.65	1.16
2001	Luscar	82	6.06	1.11	0.22	0.88	0.44
2001	Luscar	83	5.82	6	2	5.6	0.8
2001	Gregg	3	7.08	6.32	1.58	20.53	1.58
2001	Gregg	22	7.95	0	0	1.08	0
2001	Gregg	23	9.23	0.5	0.5	2.51	0
2001	Gregg	25	6.46	0.56	0	0.56	0

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
2001	Gregg	31	7.35	0.51	1.7	0.17	0
2001	Gregg	32	4.91	7.21	0.48	3.37	0.48
2001	Gregg	33	7.02	1.88	1.88	4.38	0
2001	Gregg	34	5.01	0	0.37	0	0
2002	Luscar	17	6.28	1.7	12.74	0.85	0.21
2002	Luscar	23	5.27	7.34	0.46	0	0.46
2002	Luscar	26	6.36	1.81	0.52	0.26	0.26
2002	Luscar	38	18.9	0.9	0.54	0	0.18
2002	Luscar	42	4.95	2.79	0.44	0.15	0.15
2002	Luscar	44	6.47	0	0.25	0	0
2002	Luscar	54	7.96	0.33	0.33	0	0
2002	Luscar	56	18.8	3.99	0.75	0.5	0.75
2002	Gregg	25	6.27	1.23	1.23	0	0
2002	Gregg	37	4.58	2.99	0	0	0
2002	Gregg	39	6.67	3.57	1.19	1.19	1.19
2002	Cold	32	0.42	0	0.6	0	0
2002	Cold	26	0.89	0	0	0	0.29
2002	Cold	2	0.94	0.96	0.32	0	0
2002	Cold	5	1	0.25	0.5	0.25	0
2002	Cold	29	1.02	0.72	1.09	0.36	0.72
2002	Cold	23	1.2	0.35	0.35	0.35	0.35
2002	Cold	48	1.25	9.52	4.76	2.38	0
2002	Cold	42	1.6	0	0	0	0
2002	Cold	22	1.74	0	0	1.09	1.09
2002	Cold	51	2.11	2.17	2.17	0	2.17

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites

% fertilization

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	77.60	25.8653	0.06336703	0.978935
Residuals	51	20817.33	408.1829		

% mortality

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3751.51	1250.504	1.848008	0.1502207
Residuals	51	34510.50	676.676		

% craniofacial deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8093.97	2697.989	4.430272	0.007732133
Residuals	50	30449.48	608.990		

% skeletal deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	3279.30	1093.101	2.773923	0.05094422
Residuals	50	19703.16	394.063		

% finfold deformities

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	6273.17	2091.056	3.888612	0.01417887
Residuals	50	26886.93	537.739		

% edema

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	8902.51	2967.502	3.449597	0.0233558
Residuals	50	43012.30	860.246		

Table 3. Results of ANOVA comparing rainbow trout endpoints among sites (continued)

Fry length

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	5.0847	1.694896	0.5694271	0.6377436
Residuals	50	148.8246	2.976493		

Fry weight

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Site	3	1721.104	573.7012	3.563888	0.02080915
Residuals	48	7726.859	160.9762		

Table 4. Rainbow trout means (standard deviation) for measurements made in eggs, embryos and larvae spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference sites (Deerlick and Wampus Creeks).

Parameter	Site			
	Luscar Cr.	Gregg Cr.	Deerlick Cr.	Wampus Cr.
egg Se, mg/kg ww	9.93 (6.77)	6.52 (4.11)	3.49 (1.90)	3.5 (1.09)
fertilization, %	77.8 (20.3)	81.2 (12.7)	77.5 (20.9)	77.5 (24.1)
mortality, %	35.0 (29.5)	34.2 (32.5)	18.1 (14.6)	37.3 (34.5)
craniofacial, %	33.3 (37.2)	10.6 (6.5)	7.1 (6.1)	12.0 (9.6)
skeletal, %	25.0 (27.9)	9.9 (5.8)	9.2 (12.3)	7.9 (8.2)
finfold, %	15.0 (27.1)	13.6 (15.2)	5.4 (6.2)	42.2 (43.7)
edema, %	34.5 (40.3)	12.2 (12.3)	6.1 (7.3)	23.3 (37.8)
larval length, mm	18.5 (2.0)	19.4 (1.6)	19.0 (1.5)	19.2 (0.9)
larval weight, mg	53.3 (16.3)	44.6 (10.4)	41.2 (9.3)	40.6 (8.4)

Table 5. Brook trout means (standard deviation) for measurements made in eggs, embryos and larva spawned from fish collected at exposed sites (Luscar and Gregg Creeks) and reference site (Cold Creek).

Parameter	Site		
	Luscar Cr.	**Gregg Cr.**	**Cold Cr.**
egg Se, mg/kg ww	7.78 (3.80)	6.59 (1.39)	1.26 (0.47)
fertilization, %	92.8 (7.2)	78.4 (18.2)	89.1 (19.6)
mortality, %	6.5 (8.9)	2.9 (2.3)	6.9 (12.1)
craniofacial, %	7.9 (10.1)	2.3 (2.5)	2.1 (2.6)
skeletal, %	2.0 (3.3)	0.8 (0.7)	1.0 (1.4)
finfold, %	1.9 (4.1)	3.1 (6.0)	0.9 (1.5)
edema, %	1.0 (2.9)	0.3 (0.6)	0.7 (1.4)
larval length, mm	17.4 (1.1)	17.9 (0.9)	18.5 (1.2)
larval weight, mg	31.7 (8.6)	31.3 (5.4)	37.8 (7.2)

Table 6. Results of ANOVA comparing brook trout endpoints among sites

% fertilization					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	1683.3	841.67	3.9128	0.0253
Residuals	60	12906.4	215.11		
% mortality					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	131.4	65.72	0.7257	0.4882
Residuals	60	5433.6	90.56		
% craniofacial deformities					
	df	**Sum of Sq**	**Mean Sq**	**F Value**	**Pr(F)**
site	2	519.1	259.54	4.9427	0.0103
Residuals	60	3150.6	52.51		

Table 6. Results of ANOVA comparing brook trout endpoints among sites (continued)

% skeletal deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	19.2	9.58	1.5631	0.2179
Residuals	60	367.6	6.13		
% finfold deformities					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	37.5	18.74	1.2562	0.2921
Residuals	60	895.1	14.92		
% edema					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	4.6	2.32	0.4966	0.6110
Residuals	60	280.6	4.68		
Fry length					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	16.1	8.04	6.5265	0.0027
Residuals	60	73.9	1.23		
Fry weight					
	df	Sum of Sq	Mean Sq	F Value	Pr(F)
site	2	546.2	273.10	4.6644	0.0131
Residuals	60	3512.9	58.55		

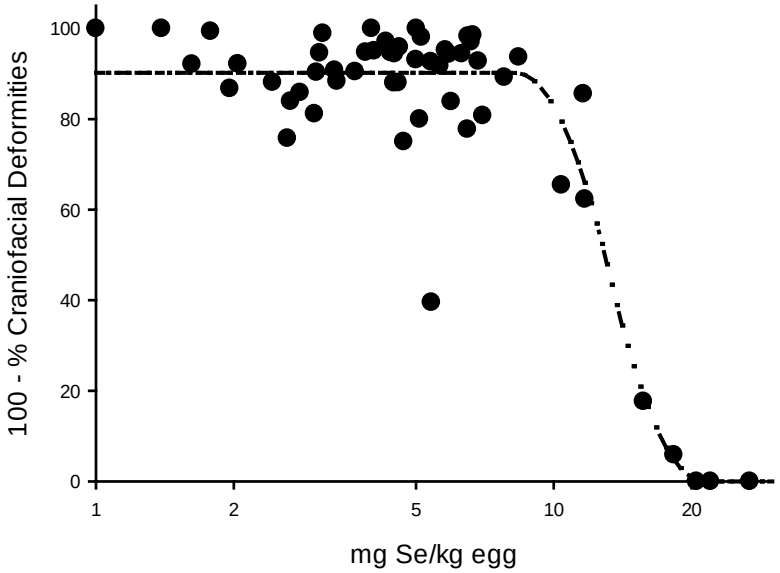


Figure 1. Rainbow trout percent normal (100 - % craniofacial deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 90.2%, the slope 4.8%,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

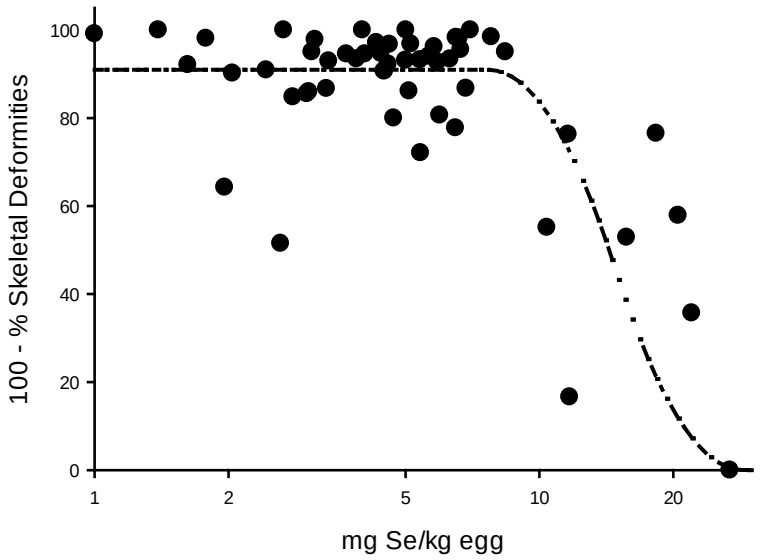


Figure 2. Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoid equation. The background value was estimated to be 91%, the slope3.5%, and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

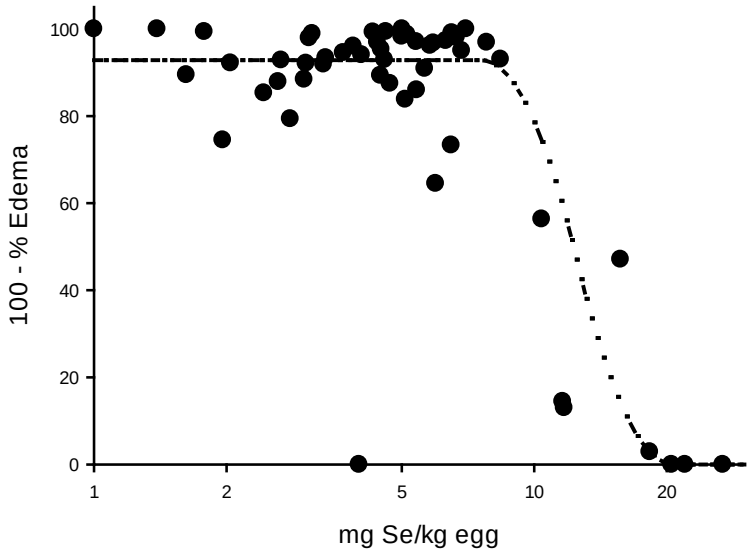


Figure 3. Rainbow trout percent normal (100 - % edema) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). TRAP weighted regression analysis using a threshold sigmoidal equation excluding the one outlier with 100% edema at 4 mg/kg. The background value was estimated to be 92.8%, the slope 4.6%, and the EC_{10} 9.5 mg Se/kg egg ww.

The previous draft used a TRAP logistic regression (Figure 4). A weighted regression using a threshold sigmoidal equation (Figures 1-3) is a better application of these data.

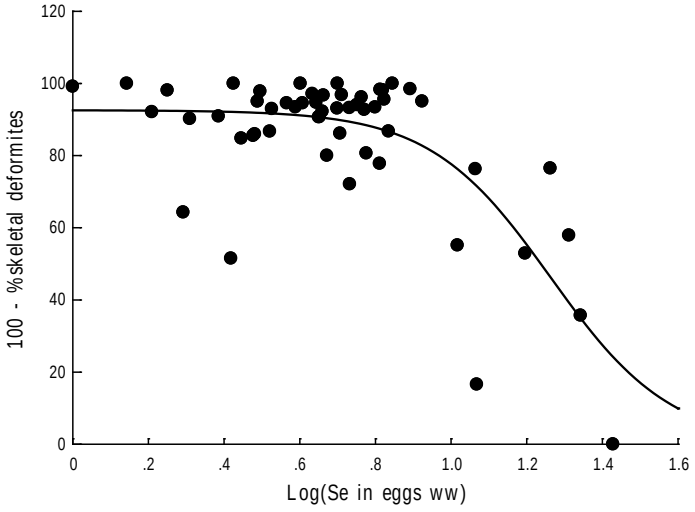
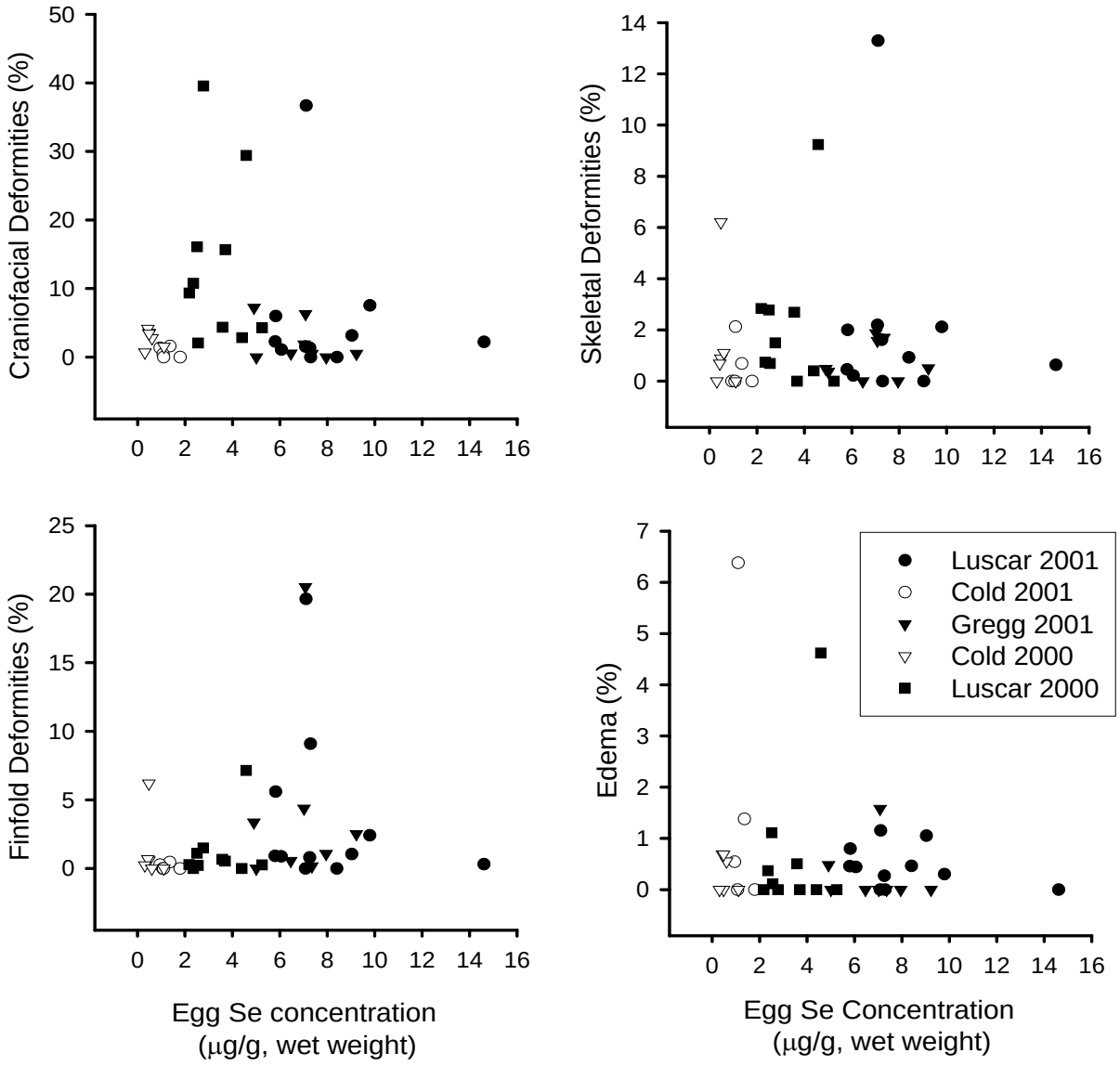


Figure 4. (From Previous Draft) Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs wet weight (Exposure Variable). EC_{10} = 8.2 mg/kg ww.

Figure 5. Plot of percent abnormal for craniofacial, skeletal and finfold deformities and edema against selenium concentration in brook trout eggs ww, 2000 and 2001 data.



The effect levels determined using the *within streams* approach resulted in values based on ww in eggs. The primary tissue for which the reproductive effect levels were based, eggs, was converted from ww to dw using the average percent moisture of 61.2% for rainbow trout eggs reported by Seilor and Skorupa (2001).

Chronic Values:

Brook trout: *Between streams approach*
No effects at EC_{10} level at 7.78 mg Se/kg eggs ww or 20.05 mg Se/kg eggs dw; egg. **Chronic value is >20.05 mg Se/kg eggs dw.** Table 3 data, converted to dry weight, suggest no effects at least up to 25-35 mg Se/kg eggs dw.

Rainbow trout: *Within streams approach*
EC_{10} value (edema) at 9.5 mg Se/kg egg ww or 24.5 mg Se/kg egg dw. **Chronic value is 24.5 mg Se/kg eggs dw.**

Kennedy, C.J., L.E. McDonald, R. Loveridge, M.M. Strosher. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarkii lewisi*). Arch. Environ. Contam. Toxicol. 39:46-52.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii lewisi*; spawning adults, 3-6 years)

Exposure Route: Dietary and waterborne - field exposure
Total selenium concentrations measured at the time the eggs were taken were <0.1 μg/L from the reference site and 13.3 to 14.5 μg/L at the exposed site.

Study Design: At reference and exposed site (Fording River, BC, Canada which receives drainage from open-pit coal mining), eggs were stripped from females (n=20 from reference site; n=17 from exposed site) and fertilized from milt from one male collected at each site. Fertilized eggs were reared in well water and examined for time to hatch, deformities (craniofacial, finfold, skeletal and yolk sac malformations), and mortalities. Inspection of deformities in eggs was performed using 40X magnification.

Effects Data : No significant correlations between the selenium concentrations in the eggs from either site and: hatching time (reference, 25.5-26.5 days; exposed, 22-25.5 days); percent deformities preponding (reference, 0-2.4%; exposed, 0-0.34%); percent deformities after ponding (reference, 0-0.26%; exposed, 0-0.09%); percent mortalities preponding (reference, 1.5-70.3%; exposed, 1-100%); percent mortalities after ponding (reference, 0.3-4.3%; exposed, 1.5-43.7%); total percent mortalities (reference, 2.8-55.8%; exposed, 3.7-100%). The average selenium residues in tissues were as follows:

Site	Adult fish liver, mg Se/kg dw	Adult fish muscle, mg Se/kg dw	eggs, mg Se/kg dw
Reference	8.2; Range: 3.4-14.6	2.4; 1.4-3.8	4.6
Exposed	36.6; Range:18.3-114	12.5; Range: 6.7-41	21.2

Chronic Value: >21.2 mg Se/kg dw in eggs
>12.5 mg Se/kg dw in muscle

Hardy, R.W. 2005. Effects of dietary selenium on cutthroat trout (*Oncorhynchus clarkii*) growth and reproductive performance. Report for Montgomery Watson Harza. December 14, 2005.

Test Organism: Cutthroat trout (*Oncorhynchus clarkii*, 0.9 g)

Exposure Route: Dietary only
Six experimental dietary treatments were produced by cold extrusion. The formulation of the diet was designed to be similar to commercial trout diets and had a proximate composition of 45% protein and 16% lipid. Seleno-methionine diluted in distilled water (100 µg/L) was added in appropriate volumes to each batch of feed to facilitate pelleting. Measured dietary selenium concentrations were 1.2 (control), 3.8, 6.4, 9.0, 11.5, and 12 mg Se/kg dw. Fry were fed initially at a rate of 10 times per day 6 days each week to apparent satiation. Feeding frequency decreased as fish grew.

Test Duration: 124 weeks (865 days, 2.5 yrs)

Study Design: Groups of 50 fish were placed into triplicate tanks (145 L) receiving 4-15 L/min of hatchery water at 14.5EC and fed one of the six experimental diets. The fish in each tank were bulk-weighed and counted every 14 days for the first 12 weeks of the experiment, and then every 4 weeks until 48 weeks. Samples of fish for whole-body selenium analysis were taken at each sampling date for the first 12 weeks followed by every 3 months thereafter. After six months of feeding, the fish were transferred to 575 L tanks and the number of replicate tanks per dietary treatment was reduced to two. After 80 weeks of feeding, the fish were transferred to 1050 L outdoor tanks each supplied with 70 L/min of constant temperature (14.5°C) spring (hatchery) water. After 2.5 years of the feeding trial, fish were spawned and whole body selenium level , egg selenium level, % eyed eggs, % hatched eggs, and % deformed larvae were examined.

Effects Data: No signs of toxicity (reduced growth or survival relative to controls) were observed in fish fed the highest dietary selenium treatment (12 mg Se/kg dw) after the first 80 weeks of exposure just prior to transfer outdoors. No signs of clinical disease were evident, and no relationship was found between feed conversion ratios and the level of selenium added to the feed. Average whole body selenium levels of female Henry's Lake cutthroat trout at spawning at 2.5 to 3 years of age were 5.87, 9.10, 11.37 and 5.61 mg Se/kg dw in the four highest dietary treatments. Average egg selenium levels in the same four dietary treatments were 6.61, 5.05, 5.18, and 16.04 mg Se/kg dw. Percent survival from the eyed stage to hatching varied among treatment groups, with the control and the highest Se dietary treatment having the second highest survival (85%) and the fifth dietary treatment group the highest (93%). Percent deformed larvae ranged from a low of 5.6% in controls to a high of 20.2% in the 6.4 mg Se/kg dw dietary treatment group; larvae in the two highest dietary treatment groups only exhibited 7 and 6.8 %, respectively.

Chronic Value: The chronic value for embryo/larval deformity is a NOAEC of >11.37 mg Se/kg dw whole-body parent tissue and >16.04 mg Se/kg dw egg.

Rudolph, B-L, I. Andreller, CJ. Kennedy. 2008. Reproductive success, early life stage development, and survival of Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) exposed to elevated selenium in an area of active coal mining. Environ. Sci. Technol. 42: 3109-3114.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)

Exposure Route: Field collected.
In June, 2005, eggs were collected from 12 females from Clode Pond (exposed site) and 16 females from O'Rourke Lake (reference site). Milt was obtained from 3-5 males at each site. Clode Pond is on the property of Fording River Coal Operations in Southeast British Columbia with reported selenium concentrations of 93 µg/L. O'Rourke Lake is an isolated water body into which Westslope cutthroat trout were stocked in 1985, 1989 and 1992 and has selenium levels reported <1 µg/L.

Test duration: Through the end of yolk sac absorption (at swim-up) by the alevins.

Study Design: Individual batches of eggs were fertilized in the field with 2 ml composites of milt. Water-hardened eggs were transported to the rearing laboratory. Eggs and alevins were monitored daily for fertilization, hatching and mortality. After the yolk sacs were absorbed, alevins were sacrificed and preserved in Davidson's solution.

All viable fry (n = 4,922) after yolk absorption were observed for the frequency and severity of skeletal (lordosis, kyphosis, and scoliosis), craniofacial (head, eyes or jaw), and fin malformations as well as edema. The authors used a graduated severity index (GSI) for deformities in which fry were scored 0 (normal) to 3 (severe) based on the level of defect.

Effects Data: Eggs with the four highest Se concentrations (86.3 to 140 mg/kg dw) collected from Clode Pond fish died before reaching the laboratory (Table 1). Excluding the eggs that died from females CP1, CP3, CP4 and CP5, fertilization (total eggs reaching the eyed stage/total eggs x 100) was not related to Se concentrations in the eggs. The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs (Table 1). Note: The data used to estimate the EC_{10} value excluded the variable from OL1 and OL2 (shaded areas in Table 1). These are data from the reference lake in which only 57% of the larvae survived (OL1) or where the % dead eggs plus % hatch did not add up to %100. Alevin survival was meaningfully higher in the other 15 clutches of eggs from the reference site (85.1 to 99.8%). Because there were insufficient partial effects, a TRAP model was not used to estimate the EC_{10} value. The data consist of a cluster background data and a cluster of 100% mortality (Figure 1). With no way to fit a credible curve, the interpolation method is applied here with the EC_0 set to 20.6 mg/kg with background % survival of 95.75% (not including the one low outlier) and the second extrapolation point being 46.8 mg/kg with 0.3% survival. The resultant slope is 5.6 (similar to slopes in other datasets where it was estimated) and the EC_{10} is 24.7 mg/kg. Note: TRAP was used in the previous draft to derive a similar EC_{10} of 24.1 mg/kg, however as stated above, it

was determined that the data are not amenable to a TRAP model because of insufficient partial effects.

An EC_{10} based on Se in maternal muscle was estimated using the same approach as was used for Se in eggs, that is, by interpolation between an EC_0 and a high EC_P. An EC_{10} of 16.6 mg Se/kg muscle dw was interpolated from an EC_0 (HNOEC) of 13.4 mg/kg and the average background survival of 95.75 and the EC_{100} set to 34.7 mg/kg muscle (Figure 2).

Deformity analysis was not performed on the alevins that died prior to the swim-up stage. Therefore, due either to dead eggs or dead alevins, the occurrence and severity of deformities were assessed on four clutches of eggs from Clode Pond (CP2, CP6, CP11 and CP12) with a range of 11.8 to 20.6 :g Se/g dw and 15 of the 16 clutches (all eggs died in OL8) from O'Rourke Lake (Table 1). There was no correlation between egg Se concentration and frequency of deformity or edema. Statistical differences between sites were observed ($p < 0.05$) for skeletal deformities and edema for both the frequency of the occurrence and the severity score (Table 2). Note: the percent and severity score of skeletal deformities were greater in the reference site than in the exposed site.

The effect level for this study was based on the alevin mortality data and not the deformity measurements. Although edema occurred statistically more often at the exposed site (87.7% at Clode Pond, 61.2% at O'Rourke Lake), it was not correlated with selenium levels in the eggs. Also the greater occurrence of skeletal malformations in the reference site confounded the use of statistical differences between sites to determine effect levels for this study.

Effect Concentration: 24.7 mg Se/kg dw in eggs; 16.6 mg Se/kg dw in muscle.

Table 1. Fertilization, egg mortality and alevin mortality for offspring from individual fish collected in Clode Pond and O'Rourke Lake.

Fish ID	Muscle [Se] mg/kg dw	Egg [Se] mg/kg dw	Hatch %	Dead eggs, %	Dead alevins, %	% Survival[1]
Clode Pond (exposed site)						
CP1	38.8	88.3	0	100	NA	
CP2	11.8	16.1	98.2	1.8	0.9	99.1
CP3	40.4	86.3	0	100	NA	
CP4	46.1	121	0	100	NA	
CP5	50.4	140	0	100	NA	
CP6	34.7	51	92.6	7.4	92.6	0.0
CP7	39	65.3	91.1	8.9	91.1	0.0
CP8	7	11.8	63.9	36.1	0.8	98.7
CP9	35.4	46.8	63.4	36.6	63.2	0.3
CP10	35.5	75.4	82.4	17.6	82.4	0.0
CP11	11.3	16.9	77.9	22.1	1.3	98.3
CP12	13.4	20.6	97	3	5.1	94.7
avg	30.3	61.6	55.5	44	42	20.0
SD	15.1	42.4	42.5	42	44	0.0
O'Rourke Lake (reference site)						
OL1	8.28	12.9	71.4	28.6	42.9	39.9
OL2	7.7	13.9	27.7	53.1	6.9	75.1
OL3	8.16	12.5	96.1	3.9	2.4	97.5
OL4	8.03	15	85.5	14.5	12.7	85.1
OL5	8.12	14.9	80.7	19.3	5.3	93.4
OL6	6.61	15.2	68	32	4	94.1
OL7	8.52	12.9	97.9	2.1	0.2	99.8
OL8	7.22	12.3	0	100	NA	
OL9	7.25	16.7	87.2	12.8	4.5	94.8
OL10	7.64	13.1	79.6	2.5	5.5	93.1
OL11	8.74	15.6	89.2	10.8	2.4	97.3
OL12	8.2	13.9	83.6	16.4	3	96.4
OL13	7.86	15.1	74.1	25.9	2.8	96.2
OL14	8.5	13.1	77.8	22.2	0.5	99.4
OL15	7.62	12.3	88.2	11.8	2.6	97.1
OL16	8.13	12.7	54.8	45.2	4.8	91.2
avg	7.9	13.9	72.6	25	7	
SD	0.6	1.4	25.8	25	10	

[1] % Survival based on % hatch

Table 2. Deformity results (frequency and severity) for offspring from O'Rourke Lake and Clode Pond. Values are presented as mean ± SE. * indicates a significant difference ($p < 0.05$) between means from the two sites.

Frequency of deformity, %	**O'Rourke Lake**	**Clode Pond**
Skeletal*	37.4 ± 3.6	16.5 ± 2.2
Craniofacial	10.2 ± 2.0	5.7 ± 1.0
Finfold	10.6 ±3.1	7.5 ± 3.84
Edema*	61.2 ± 4.9	87.7 ± 2.0
Severity of deformity, score		
Skeletal*	0.47 ± 0.07	0.18 ± 0.02
Craniofacial	0.12 ± 0.03	0.06 ± 0.01
Finfold	0.15 ± 0.05	0.09 ± 0.05
Edema*	0.61 ± 0.05	0.88 ± 0.02

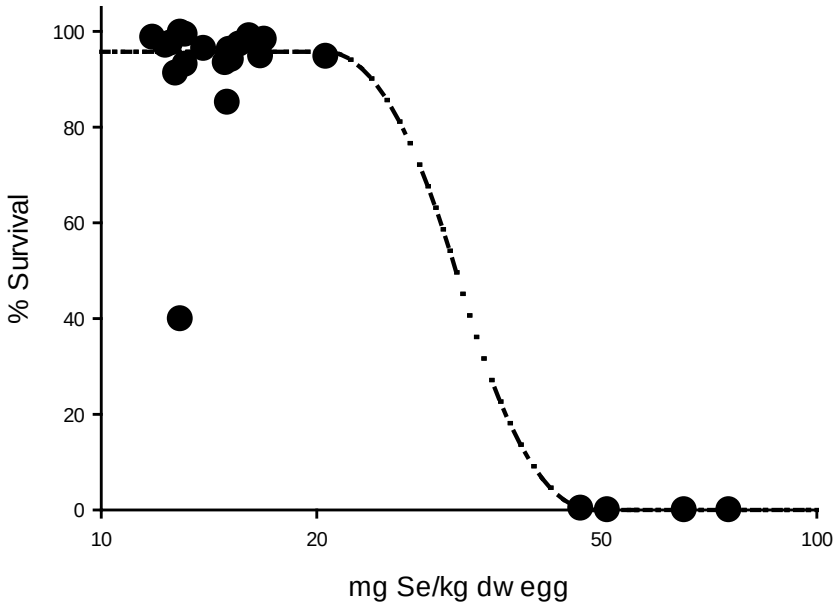


Figure 1. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in eggs.

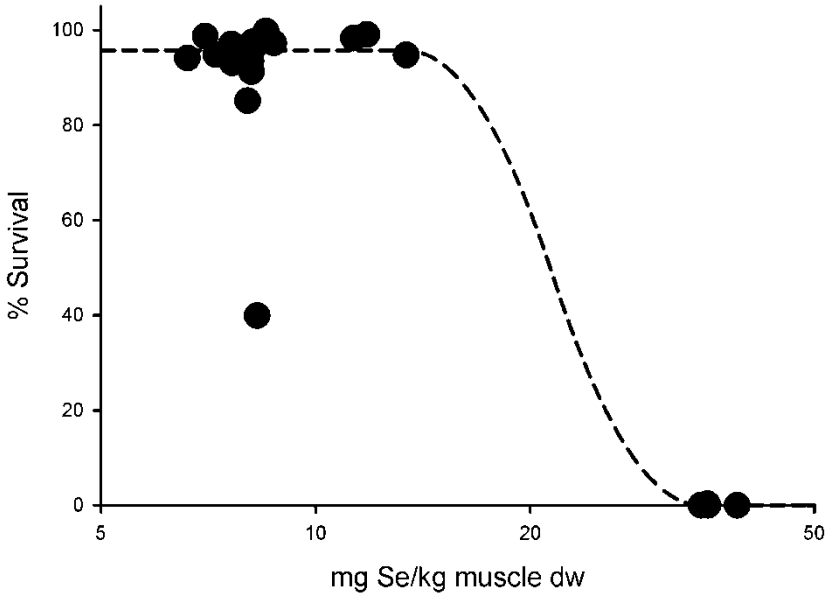


Figure 2. Post-hatch survival of Westslope cutthroat trout alevin as a function of the logarithm of the selenium concentration in maternal muscle.

Nautilus Environmental. 2011. Evaluation of the Effects of Selenium on Early Life Stage Development of Westslope Cutthroat Trout from the Elk Valley, BC. Report to Elk Valley Selenium Task Force, November 24, 2011.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarkii lewisi)*

Exposure Route: Field collected. Adult fish were collected and spawned from lentic and lotic environments in areas proximate to Teck Coal's Fording River Operations. Eggs were also obtained from fish collected from Connor Lake, a lake located within the Elk valley watershed not exposed to mine discharges and considered a reference site and a methodological control.

Test Duration: Fertilized eggs were reared in the laboratory until they reached swim-up fry stage. A subset of fry surviving at swim-up were reared for an additional 28 days.

Study Design: Gametes were stripped from the ripe adults in the field during June and July 2008 and transported immediately to the laboratory in coolers containing wet ice. Eggs were fertilized in the laboratory. After stripping the eggs, female fish were sacrificed and the whole body stored on ice for later Se analysis. For a given female, approximately 240 fertilized eggs were divided into four replicates of 60 eggs. In cases when fewer eggs were available three replicates of 60 eggs were used. If less than 180 eggs were available, either 3 or 4 replicates of 30 were used. Females with less than 90 eggs were not used. The fertilized eggs were maintained in the laboratory until the fry reached swim-up at which point deformities were assessed. Survival was also assessed up to swim-up. In test chambers in which there were at least 40 surviving fish at swim-up, one-half of the surviving fish were maintained for an additional 28 days. Survival, length, weight and deformities were assessed in the 28-day post swim-up test.

The number, type and severity of deformities were measured at swim-up and at the end of the 28-day post swim-up test. Deformity assessments were conducted on recently killed fresh fish to avoid artifacts caused by preservation. A graduated severity index (GSI) was assigned to each of four types of deformity/abnormality: skeletal, craniofacial, finfold and edema. Graduated Severity Index (GSI) methods followed those described in Holm et al. (2003) and Rudolph et al (2006; 2008).

Effects Data: Survival of the larvae from hatch through swim-up spawned from the four fish collected from the reference site, Connor Lake, ranged from 73 to 92% (egg Se 4.32 to 7.31 mg/kg dw) (Table 1). Larval survival at swim-up was also generally high for fish collected in the Se exposed sites up to egg Se concentration 29.6 mg/kg dw (Table 1, Figure 1). Larvae exposed above this egg Se concentration had poor to no survival. Larvae from one fish (P00811) below this threshold did have poor survival (11.7%). The authors noted that the many of the eggs from this fish displayed an unusual distribution of lipid vesicles which resulted in greater than 50% mortality in the first 24 hours due to egg breakage. The remaining eggs may have been compromised due to the organic material released during the egg breakage.

The rate of deformities in larvae at swim-up showed no relationship with Se in egg through 29.6 mg/kg dw (Table 2).

The results of the 28-day post swim-up test showed no relationships between larval survival or deformities and egg Se (Table 3). The authors also measured the length and weight of larvae at the end of the 28 day test; neither of which showed a relationship with egg Se concentration.

Se Tissue Concentrations. Two analytical laboratories (A and B) measured Se in the eggs. The mean difference in egg Se concentrations between the two laboratories was 34.2%. To better understand the difference between the two laboratories, five egg samples (i.e., from five different fish) from this study were sent to both laboratories in 2010. Both laboratories digested the eggs using the methods they used in their own 2008 original analysis. The respective digestates were split and then shared between laboratories. Both labs then measured selenium in their own digestates and the digestate received from the other lab. The results of this follow-up study showed that when each lab used their own digestion procedures Laboratory A had on average 43% higher measurements in the 2008 analysis and 23% higher in the follow-up 2010 analysis. When each lab measured selenium using the same digestate the difference in the Se measurements between labs was on average only 1 to 8%. The authors concluded that although both laboratories employed acceptable and approved practices, Laboratory A used a more efficient digestion process resulting in higher Se measurements. To compensate for the reduced Se measurements in Laboratory B, its values were increased by 34.2%. The measurements made by Laboratory A are marked in Table 1; unmarked values are Laboratory B measurements increased by 34.2%.

Effect Concentration: The most sensitive endpoint determined by TRAP was larval survival at swim-up. Interpolation was used to estimate an effect concentration for larval survival with the entire egg Se dataset that included egg Se measurements from Laboratory A and adjusted measurements from Laboratory B (EC_{10} = 31.1 mg/kg egg dw; Figure 1) and using only the egg Se measurements from Laboratory A (Figure 2). Because the Laboratory A dataset estimated slightly lower EC values, the EC_{10} of 27.7 mg/kg egg dw is the selected effect concentration for this study. Note: In the previous draft, a TRAP model was used to estimate the EC_{10}. However, because of insufficient partial effects, TRAP was determined not appropriate so the EC_{10} was estimated using an interpolation between the HNOEC and the LOEC (see Figure 3 for the TRAP analysis used in the previous draft).

Table 1. Summary of westslope cutthroat trout larvae surviving to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

				Proportion surviving				
Fish ID	Location	Se egg, mg/kg dw	Replicates	Replicate mean	Replicate min	Replicate max	Number survivors	Total number
YO93	Lentic	3.88*	4	0.8125	0.6667	0.9167	195	240
CL1	Reference	4.32	4	0.9167	0.8833	1	220	240
R082	Lotic	5.21	3	0.9056	0.8333	0.95	163	180
CL4	Reference	5.96*	4	0.7333	0.6	0.8	176	240
CL2	Reference	6.82	4	0.8333	0.7	0.9167	200	240
CL3	Reference	7.31	4	0.8542	0.8167	0.8833	205	240
P00815	Lotic	7.6	3	0.8222	0.7167	0.95	148	180
R026	Lotic	12.53	4	0.5792	0.5	0.65	139	240
P00823	Lotic	12.71	4	0.8875	0.85	0.95	213	240
R039	Lotic	12.9	4	0.6042	0.55	0.65	145	240
R086	Lotic	13.4*	4	0.9417	0.85	0.9833	226	240
R077	Lotic	14.29	3	0.6444	0.6167	0.6667	116	180
R042	Lotic	16.44	3	0.8	0.7	0.9	72	90
R055	Lotic	16.5	4	0.8792	0.7833	0.9667	211	240
R043	Lotic	16.85	4	0.8667	0.7667	0.9667	104	120
R074	Lotic	17.8*	4	0.9375	0.8833	0.9833	225	240
P00811	Lotic	19.25	1	0.1167	0.1167	0.1167	7	60
P00809	Lotic	19.72	4	0.7667	0.65	0.8833	184	240
P00803	Lotic	24.8*	4	0.9375	0.9333	0.95	225	240
R078	Lotic	29.61	4	0.8825	0.8333	0.9333	105	119
GO99	Lotic	34.2*	4	0.2083	0.1667	0.2667	50	240
O087	Lentic	54.7*	4	0.07083	0.01667	0.2	17	240
O085	Lentic	56.8*	4	0	0	0	0	240
WO52	Lentic	61.1*	4	0	0	0	0	240
R069	Lotic	65.61	4	0	0	0	0	240
R071	Lotic	72.9	4	0	0	0	0	240
WO94	Lentic	73.1	4	0	0	0	0	240
UT101	Lentic	74.67	4	0	0	0	0	240

*Laboratory A dataset

Table 2. Summary of westslope cutthroat trout larval deformities to swim-up per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Se egg, mg/kg dw	Skeletal combined	Craniofacial combined	Finfold combined	Edema combined	Deformities combined
YO93	Lentic	3.88*	4.5%	0.9%	4.4%	1.9%	7.7%
CL1	Lentic	4.32	7.6%	1.9%	1.0%	1.0%	9.5%
R082	Lotic	5.21	1.2%	1.3%	2.5%	0.0%	3.7%
CL4	Lentic	5.96*	4.3%	7.3%	1.7%	0.7%	12.6%
CL2	Lentic	6.82	11.1%	3.7%	0.8%	3.0%	15.9%
CL3	Lentic	7.31	5.0%	2.0%	1.0%	0.0%	7.0%
P00815	Lotic	7.6	0.0%	2.7%	0.0%	2.9%	5.6%
R026	Lotic	12.53	2.1%	2.1%	0.7%	1.4%	2.1%
P00823	Lotic	12.71	1.9%	2.9%	1.8%	5.6%	7.4%
R039	Lotic	12.9	2.1%	1.9%	2.9%	4.9%	9.9%
R086	Lotic	13.4*	2.7%	1.0%	0.0%	0.0%	2.7%
R077	Lotic	14.29	1.7%	10.4%	0.9%	12.2%	15.5%
R042	Lotic	16.44	1.2%	0.0%	0.0%	2.6%	2.6%
R055	Lotic	16.5	0.0%	2.8%	1.0%	2.9%	4.7%
R043	Lotic	16.85	0.9%	2.6%	1.8%	1.7%	4.4%
R074	Lotic	17.8*	2.7%	1.8%	0.9%	0.9%	3.6%
P00809	Lotic	19.72	3.9%	2.8%	3.3%	4.7%	9.0%
P00803	Lotic	24.8*	2.7%	0.9%	0.0%	0.9%	4.5%
GO92	Lotic	26.1	0.0%	1.9%	1.9%	4.4%	4.4%
R078	Lotic	29.61	1.8%	0.0%	1.0%	2.9%	5.7%
GO99	Lotic	34.2*	14.5%	53.9%	6.8%	28.2%	64.7%

*Laboratory A dataset

Table 3. Summary of larval survival and rates deformities after the 28-day post swim-up test per parent female (fish ID) including location of collection of parent female and concentration of selenium in the eggs.

Fish ID	Location	Sample size (n)	Egg Se (mg/kg dw)	Survival (%)	Skeletal (%)	Craniofacial (%)	Finfold (%)	Total (%)
CL1	Reference	112	4.3	99.1	0	0	0	0
CL2	Reference	93	6.8	99	0	0	0	0
CL3	Reference	96	7.3	91.7	0	1	1	2
CL4	Reference	68	6	98.6	0	0	4.3	4.3
Y093	Lentic	93	3.9	95.6	0	0	2	2
R082	Lotic	71	5.2	87.4	0	2.9	0	2.9
P00815	Lotic	69	7.6	91.1	0	1.2	1.4	2
P00823	Lotic	105	12.7	96.3	0	0	0	0
R086	Lotic	112	13.4	97.2	0	0.9	0	0.9
R077	Lotic	36	14.3	92.4	2.8	2.8	2.8	4.2
R055	Lotic	101	16.5	95.9	0	4.6	0	4.6
R074	Lotic	106	17.8	93.1	0	0	0	0
P00809	Lotic	65	19.7	91.7	0	0	0	0
P00803	Lotic	108	24.8	95.7	0	0	1	1

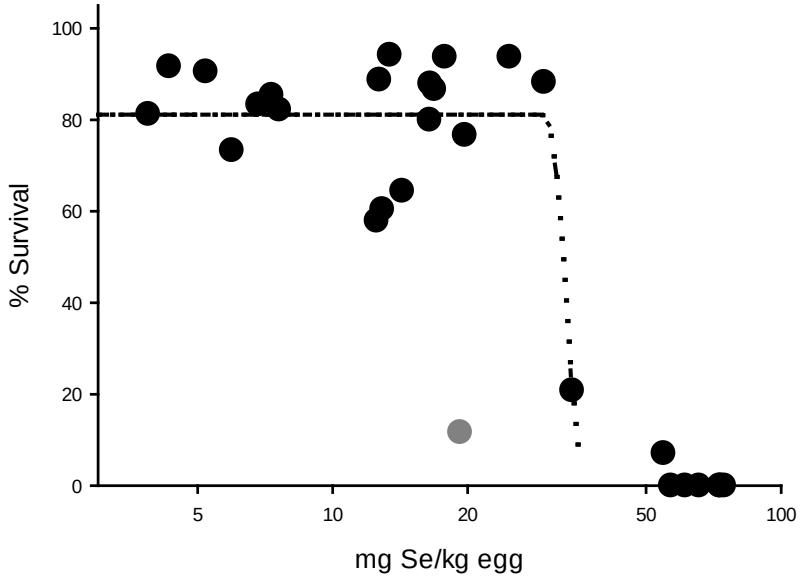


Figure 1. Labs A and B datasets. EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (29.6 mg/kg and 81.1%). The slope is 20.5 and the EC10 is 31.1 mg/kg. Note: the gray point denotes egg batch with quality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

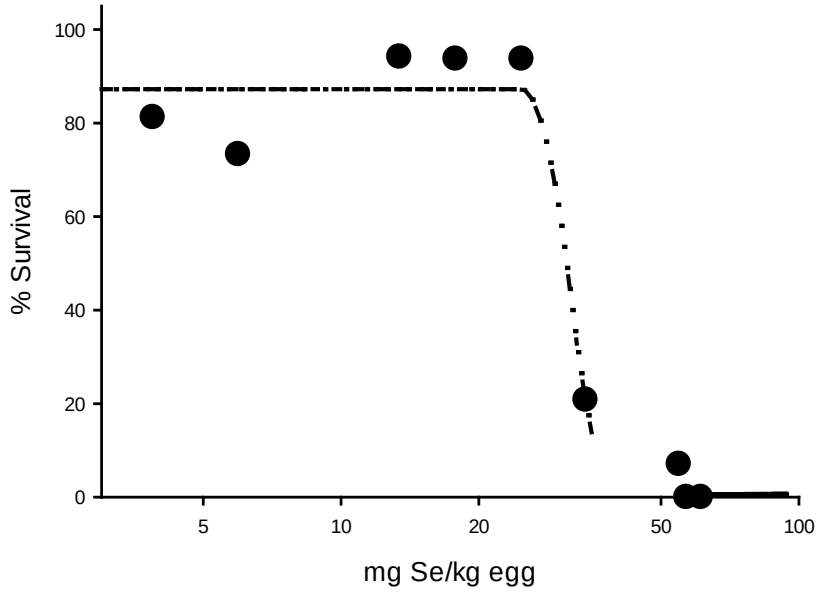


Figure 2. Lab A dataset.* EC10 based on interpolation between the one partial effect (34.2 mg/kg, 20.8%) and an EC0 set at the HNOEC and the average % survival for all the NOECs (24.8 mg/kg and 87.25%). The slope is 9.4 and the EC10 is 27.7 mg/kg.

*Although some scientists have attempted to explain certain occurrences of improved response with increasing concentration in terms of nutrient selenium sufficiency-deficiency, the concentrations involved in this study are too high to for selenium deficiency to be an explanation. The figure's apparent bi-phasic measured response is thus best explained as being a chance outcome of noise.

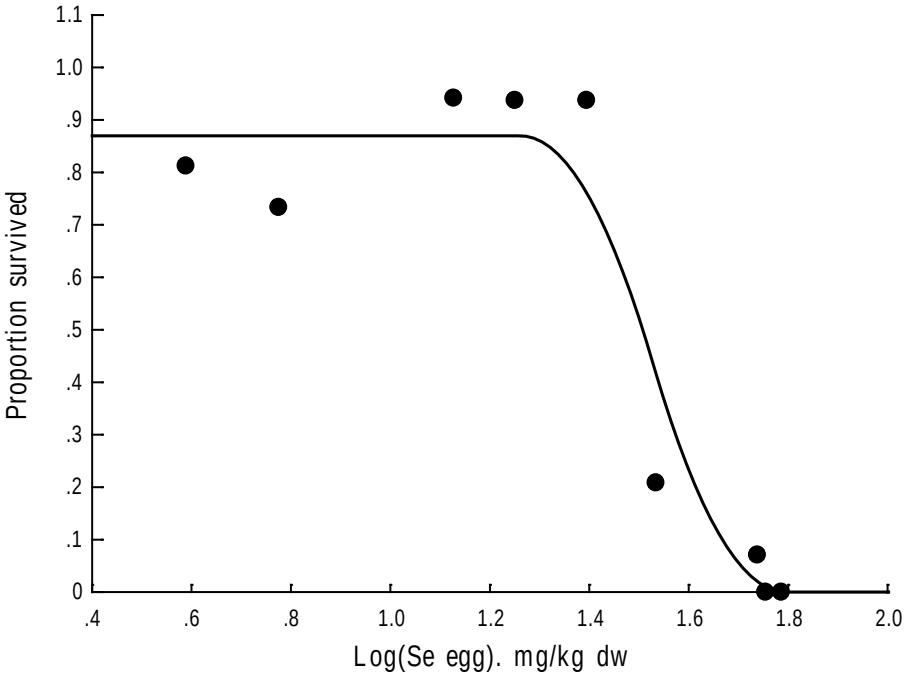


Figure 3. (From previous draft) Tolerance distribution; Model option – Triangular distribution (3 parameter). Includes Laboratory "A" dataset only TRAP EC_{10} estimate = 24.0 mg/kg.

Golder Associates. 2009. Development of a Site-specific Selenium Toxicity Threshold for Dolly Varden Char. Report to Northgate Minerals Corporation, PO Box 3519, Smithers, British Columbia. Report Number 04-1421-101/2000.

Test Organism: Dolly Varden (*Salvelinus malma*)

Exposure Route: Field collected.

Adult Dolly Varden char were collected from reference (North Kemess Creek), high Se exposure (Upper Waste Rock Ponds and Creek) and moderate Se exposure (lower Waste Rock Creek) sites during September 22 to 24, 2008. Eggs were stripped from females and fertilized with milt from males collected from the reference site. Fertilized eggs were taken to the laboratory for testing.

Test duration: The test was terminated when 90% of the larvae reached swim-up, approximately 5 months after fertilization.

Study Design: Approximately 30 fertilized eggs were added to each replicate rearing container. The number of replicates per female parent ranged from one to four depending on the number of eggs available. Embryos were maintained in 4 L containers with 3.5 L dechlorinated tap water in a static-renewal system (3 renewals times/week) at 5°C. The condition of the embryos and alevins were observed daily and any dead individuals were counted and removed. Test termination occurred over a 3-day period during February 11 to 13, 2009. The hatched larvae were sacrificed using an overdose of the anesthetic, clove oil. Individual length and weight were measured on each fry, and deformity analysis was performed on fresh unpreserved larval fish using 40X magnification.

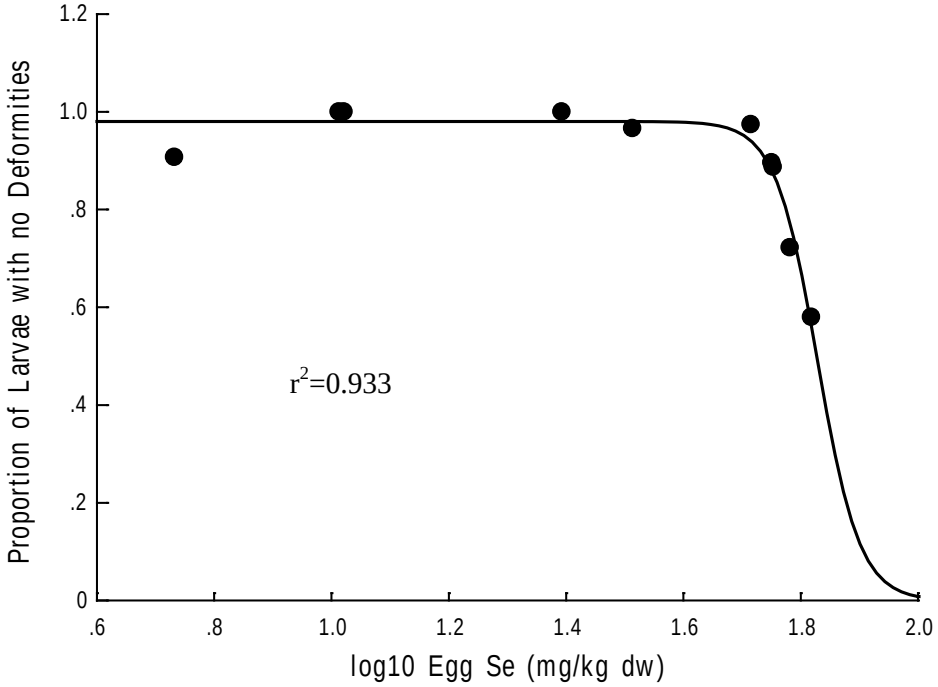
A graduated severity index (GSI) was used for deformity assessment (skeletal, craniofacial, and finfold as well as edema). The narrative criteria were the same as used by Holm et al. (2005) and Rudolph et al. (2008).

Effects Data: Alevin survival was not related to Se concentration in the eggs (Table 1). Almost all of the mortality occurred during the egg stage. Only 4 alevins died during the study, 1 from Fish #19 and 3 from Fish #2, both females collected at an exposed site. The prevalence of deformities increased sharply after the selenium egg concentration exceeded 50 mg/kg dw (Table 1, Figure 1). The proportion of Dolly Varden larvae with any type of deformity (skeletal, craniofacial, and finfold as well as edema) as a function of the log of the selenium concentration in the eggs using TRAP (logistic equation) produced an EC_{10} value of 56.22 mg/kg dw eggs (Figure 1).

Table 1. Selenium concentration in the eggs of Dolly Varden char and the survival of alevins to the swim-up stage and the proportion of larvae without any type of deformity.

Fish #	Sample ID	Location	[Se] eggs mg/kg dw	Survival of eggs to swim-up			Proportion of larvae without any type of deformity
				Initial	End	%	
1	WRC-F105	Waste Rock Creek	56.6	120	71	59	0.89
2	WRC-F61	Waste Rock Creek	65.8	120	81	68	0.58
5	WRC-F103	Waste Rock Creek	32.6	29	29	100	0.97
6	WRC-F83	Waste Rock Creek	51.9	120	115	96	0.97
15	WRC-F104	Waste Rock Creek	56.3	60	48	80	0.90
19	WRC-F86	Waste Rock Creek	60.5	120	115	96	0.72
9	NK-F30	North Kemess Creek	11	30	1	3	a
12	NK-F29	North Kemess Creek	10.5	46	15	33	1.00
17	NK-F21	North Kemess Creek	5.4	90	86	96	0.91
SCD1	Redd #1	Southern Collection Ditch	10.3	30	18	60	1.00
SCD2	Redd #2	Southern Collection Ditch	24.7	40	32	80	1.00

Figure C-1. Proportion of Dolly Varden alevin without any type of deformity as a logistic function of the logarithm of the selenium concentration in eggs (TRAP).



	Guess	Final	SE	95% LCL	95% UCL
LogX50	1.844	1.829	0.007	1.812	1.845
Slope	4.152	6.963	1.252	4.003	9.924
Y0	0.975	0.980	0.017	0.939	1.021

ECx	EC	95% LCL	95% UCL
50	67.42	64.92	70.01
20	60.12	57.96	62.35
10	56.22	53.00	59.64
5	52.85	48.64	57.43
1	46.11	40.13	52.99

	DF	SS	MS	F	P
Total	9	1.74E-01	1.93E-02		
Model	2	1.63E-01	8.13E-02	51.429	0.99993
Error	7	1.11E-02	1.58E-03		

AECOM. 2012. Reproductive success study with brown trout (*Salmo trutta*). Data quality assurance report. Final. December 2012.

Formation Environmental. 2011. Brown Trout Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion. Prepared for J.R. Simplot Company by Formation Environmental. Revised October 2011.

Test Organism: Brown trout (*Salmo trutta*)

Exposure Route: Field collected.

Adult female and male brown trout were collected at three field sites from two streams downstream of the Smokey Canyon mine. In addition, brown trout eggs were obtained from two hatcheries as method controls.

Test duration: Embryo-larval monitoring to 15 days post swim-up.

Study Design: Eggs were collected from 26 ripe female brown trout at three field sites downstream of the Smokey Canyon mine. These included one site on the highly impacted Sage Creek (LSV2C) as well as two sites along Crow Creek (CC-150 and CC-350) downstream of the conflux with Sage Creek. The downstream – most station along Crow Creek (CC-150) was intended to be a field control. Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. Selenium was measured in adult fish (whole body) and in eggs of field collected females. In addition, eggs were collected from 8 ripe females obtained from the Saratoga National Fish Hatchery (SC) to serve as method controls. Similar to field-caught fish, SC hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. As a result of lower than expected hatch rates and fungal contamination in some SC hatchery samples, additional hatchery fish were obtained (as already fertilized eyed embryos) from the Spring Creek Trout Hatchery (SPC), which were divided into four treatments.

Approximately 600 fertilized eggs from each female (or 600 eyed embryos for SPC treatments) were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15-day post swim up feeding trial. Test termination ranged from 83 to 88 days after hatch for all but the Spring Creek Hatchery egg treatments, which occurred 50 days after the arrival of fertilized, eyed embryos from that hatchery.

Endpoints measured in the laboratory study were fecundity, hatch, growth, survival/mortality, and feeding success (growth) post swim up. Larval brown trout were also evaluated for deformities (craniofacial, vertebral, fin) and edema. For this study, deformities were combined and assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Se concentrations in eggs ranged from 6.2-12.8 mg Se/kg dw at CC150, 6.9-14.0 mg Se/kg dw at CC350, and 11.2-40.3 mg Se/kg dw at LSV2C. Se concentrations in hatchery eggs ranged from 0.76-1.2 mg Se/kg dw at the SC hatchery, and were 0.73 mg Se/kg dw at the SPC hatchery. The Se whole body concentration in field collected fish ranged from 7.2-22.6 mg/kg dw at LSV2C, 4.7-8.4 mg/kg dw at CC150, and 5.5-9.2 mg/kg dw at CC350. Se whole body concentrations in SC hatchery fish ranged from 2.5-4.3 mg/kg dw. Hatchery data were combined with field data and included in all analyses.

Three endpoints were considered for purposes of calculating an EC_{10}. These were percent survival, percent fully free from deformities, and percent surviving and normal. Initially, data for these endpoints were combined and analyzed for both portions of the test: hatch through swim up and the 15-day post swim feeding trial. Data for these endpoints over both portions of the test are shown in Tables 1-3.

A U.S. Fish and Wildlife (2012) review of the Formation Environmental (2011) report suggested that fish lost due to an overflow even resulting from a drain the became clogged with food during the 15-day post swim up portion of the test were more likely to have been dead or deformed, and proposed that all treatments that lost fish to the overflow event should be excluded from the EC_{10} calculation. In the 2014 and 2015 draft Se documents, endpoints assessed for the hatch through 15-day post swim up test were analyzed using two scenarios. In the "worst-case" scenarios, the hypothesis from the USFWS review was examined, by treating all fish lost to overflow as either dead or deformed, rather than excluding those treatments altogether. In the "optimistic" scenario, the overflow event was treated as a random technician error unrelated to selenium toxicity, and any lost fish were removed from the calculation. In other words, fish lost to overflow were assumed to be equally likely to have been dead or deformed compared to fish that were not lost.

Because of the importance of these data for the numeric criterion calculation, and because of several experimental factors that resulted in the calculation of several reasonable EC_{10}s, such as the loss of fish due to an overflow event described above, EPA conducted a careful and thorough reanalysis of the study data and subjected the reanalysis to independent, external peer review (ERG 2012) to confirm the validity and scientific robustness of the approach taken by EPA in the reanalysis and use of the reanalyzed data. Those assessments were then superseded by a reanalysis of a more complete enumeration of the deformity counts provided by AECOM (2012). All analyses reported in the 2014 and 2015 draft Se documents and the current Se document used values from the updated dataset provided by AECOM (2012).

Hatch Through 15-Day Post Swim Up Combined Data

In the 2014 and 2015 draft Se documents, data for three endpoints, survival, deformities, and combined survival+deformities were considered for both portions of the test. The first portion of the test was from hatch through swim up, lasting 88 days (on average). The second portion was the 15-day post-swim up

feeding trial. None of the fry from the five treatments with Se concentrations of 26.8 mg/kg and higher reached swim-up. However, surviving fry from those treatments were included in the post-swim up feeding trial.

Combined Survival and Deformity Endpoint

Selenium concentrations and counts of total larvae, and counts of proportions of fully normal larvae (alive and normal) are included in Table 1. Background percentages of live and normal individuals were extremely variable and often low (Figure 1). In the 2014 draft document, EC_{10}s for the optimistic (21.16 mg/kg) and worst case (20.65 mg/kg) scenarios were calculated, and these were also reported in the 2015 draft document. Although there is a clear demarcation between treatments equal to or less than 20.5 µg/L and treatments equal to or greater than 26.8 µg/L, suggesting an effect level between these concentrations, a careful reanalysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} cannot be calculated because of the high background variability.

Table 1. Brown trout selenium concentrations and survival + deformity data (combined endpoint) from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
SC-001	3.6	0.76	63		63	115	8		123	0.512
SC-002	4.1	0.94	72		72	113	4		117	0.615
SC-003	3.7	0.83	131		131	302	7	9	309	0.424
SC-004	4.3	0.92	46		46	140	28		168	0.274
SC-005	3	1.2	23		23	42	6		48	0.479
SC-006	3.1	1.2	457		457	535	8		543	0.842
SC-007	2.7	1	93		93	137	30		167	0.557
SC-008	2.5	0.96	283		283	359	6	10	365	0.775
SPC-001[c]		0.73	427		427	570	8		578	0.739
SPC-002[c]		0.73	371		371	545	20		565	0.657
SPC-005[c]		0.73	400		400	561	8		569	0.703
SPC-006[c]		0.73	427		427	556	17		573	0.745
CC-150-009	8.4	12.8	106		106	142	11		153	0.693
CC-150-011	5.6	8.4	87		87	266	2		268	0.325
CC-150-012	6.7	8.5	156		156	282	12		294	0.531
CC-150-013	5.9	8.4	137		137	310	46	26	356	0.385
CC-150-015	6	9.1	210		210	445	14		459	0.458
CC-150-016	7	7.5	13		13	23	3	43	26	0.500
CC-150-017	5.6	6.6	99		99	163	7	33	170	0.582
CC-150-018	4.7	6.9	195		195	486	16		502	0.388
CC-150-020	7.2	6.2	453		453	558	6		564	0.803
CC-350-006	9.2	14	120		120	386	26		412	0.291
CC-350-007	5.5	6.9	68		68	131	10	20	141	0.482
CC-350-008	8.5	9.5	269		269	338	21	28	359	0.749
LSV2C-002	8.9	12.8	483		483	544	4	16	548	0.881
LSV2C-003	13.8	40.3	2	2	0	0	395		395	0.000
LSV2C-004	17.9	36	16	16	0	0	289		289	0.000
LSV2C-005	13.6	26.8	8	8	0	0	267		267	0.000
LSV2C-008	9.6	17.7	147		147	194	4	45	198	0.742

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Normal that were dead at assessment	# Normal and alive	# Live fish assessed for deformities	# Fish died during test	# Fish lost to overflow during post swim up test	# Live fish assessed + # died during test.	Prop. Live fish assessed + # died during test.
LSV2C-010	22.6	38.8	5	5	0	0	97		97	0.000
LSV2C-012	7.2	13.2	217		217	554	17		571	0.380
LSV2C-016	9.2	13.4	440		440	530	20		550	0.800
LSV2C-017	13.2	20.5	110		110	150	28	19	178	0.618
LSV2C-019	8.6	12.5	267		267	390	22	39	412	0.648
LSV2C-020	11.3	11.2	240		240	296	5	36	301	0.797
LSV2C-021	20	28.1	8	8	0	0	404		404	0.000

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek
[b] Test end was 15 days after swim up.
[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

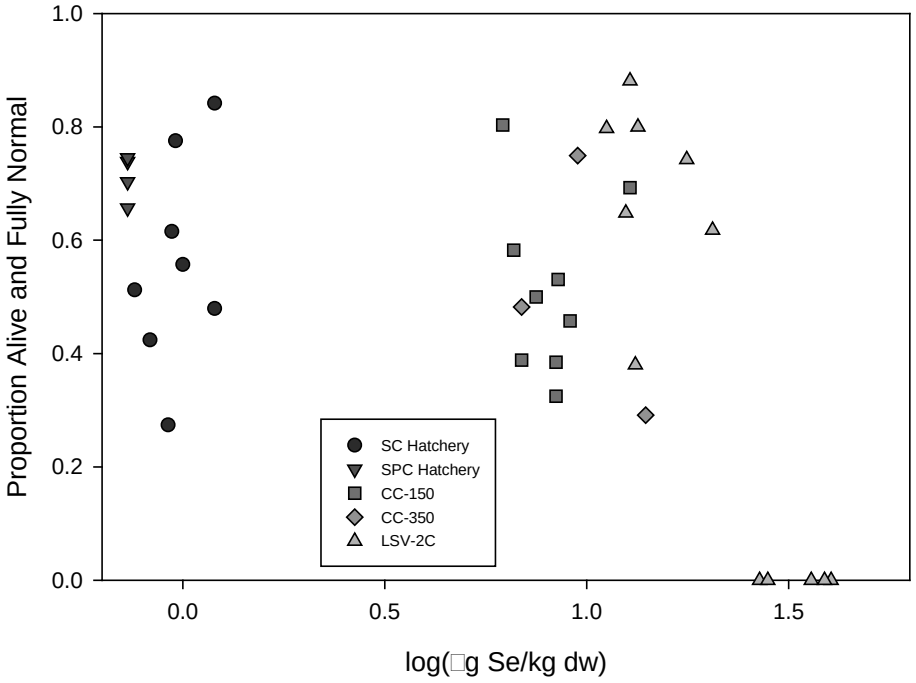


Figure 1. Proportion of alive and normal larvae plotted against Se concentrations in eggs. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for this endpoint.

Deformity Endpoint

Selenium concentrations, counts of larvae assessed for deformities, and counts and proportions of normal larvae are included in Table 2. As with the combined endpoint, background (at or below 20.5 mg/kg) proportions of deformities were highly variable (Figure 2). In the 2014 draft document, EC_{10}s were calculated for both the optimistic and worst case scenarios, and the EC_{10} of 15.91 mg/kg for the worst case scenario was used as the EC_{10} for Salmo. During the review phase following the release of the 2014 draft Se document, several public commenters noted that because of the high variability, more than one EC_{10} could be calculated by TRAP for both the optimistic and the worst case scenarios depending on the initial model conditions, in particular the slope of the falling limb of the concentration-response curve. For the optimistic scenario, EC_{10}s based on initial conditions ranged from 16.36-21.95 mg/kg, and for the worst case scenario, EC_{10}s based on initial conditions ranged from 15.91-21.58 mg/kg. In order to evaluate the most appropriate EC_{10} for the deformity endpoints, models were evaluated based on residual sum of squares, and the EC_{10} for the model with the lowest residual sum of squares was selected as the most appropriate. For the worst case scenario deformity endpoint, the model with the lowest residual sum

of squares was the EC_{10}=21.58 mg/kg model, and for the optimistic deformity endpoint, the model with the lowest residual sum of squares was the EC_{10}=21.94 mg/kg model.

These variable EC_{10}s were the result of large variability in background concentration, with several treatments at low Se concentrations experiencing greater than 60% deformities (Figure 2). Although there is clear evidence of an effect between the 20.5 and 26.8 mg/kg concentrations, because of this high background variability, a careful re-analysis of these data following the release of the 2015 draft Se document determined that a meaningful EC_{10} could be calculated for the deformity endpoint.

Some of the background variability in deformities appears to be the result of differences among field sites. For example, deformity rates among field samples appear to be greater for fish hatched from eggs collected in the two Crow Creek sites (CC-150, CC-350) compared to Sage Creek (LSV-2C) (Figure 2). If the result of higher background deformities among Crow Creek sites is not a random artifact, it suggests a confounding factor, unrelated to selenium exposure. Whether the higher deformity rates represent random variation, population differences, other environmental quality differences (unrelated to Se), or methodological issues is unclear**.**

Table 2. Brown trout selenium concentrations and deformity data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
SC-001	3.6	0.76	63	115		0.548
SC-002	4.1	0.94	72	113		0.637
SC-003	3.7	0.83	131	302	9	0.434
SC-004	4.3	0.92	46	140		0.329
SC-005	3	1.2	23	42		0.548
SC-006	3.1	1.2	457	535		0.854
SC-007	2.7	1	93	137		0.679
SC-008	2.5	0.96	283	359	10	0.788
SPC-001[c]		0.73	427	570		0.749
SPC-002[c]		0.73	371	545		0.681
SPC-005[c]		0.73	400	561		0.713
SPC-006[c]		0.73	427	556		0.768
CC-150-009	8.4	12.8	106	142		0.746
CC-150-011	5.6	8.4	87	266		0.327
CC-150-012	6.7	8.5	156	282		0.553
CC-150-013	5.9	8.4	137	310	26	0.442
CC-150-	6	9.1	210	445		0.472

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Normal	# Assessed for deformities. "Optimistic Case"	# Lost to overflow during post swim up test	Prop. Assessed for deformities plus # lost.
015						
CC-150-016	7	7.5	13	23	43	0.565
CC-150-017	5.6	6.6	99	163	33	0.607
CC-150-018	4.7	6.9	195	486		0.401
CC-150-020	7.2	6.2	453	558		0.812
CC-350-006	9.2	14	120	386		0.311
CC-350-007	5.5	6.9	68	131	20	0.519
CC-350-008	8.5	9.5	269	338	28	0.796
LSV2C-002	8.9	12.8	483	544	16	0.888
LSV2C-003	13.8	40.3	2	100		0.020
LSV2C-004	17.9	36	16	142		0.113
LSV2C-005	13.6	26.8	8	149		0.054
LSV2C-008	9.6	17.7	147	194	45	0.758
LSV2C-010	22.6	38.8	5	80		0.063
LSV2C-012	7.2	13.2	217	554		0.392
LSV2C-016	9.2	13.4	440	530		0.830
LSV2C-017	13.2	20.5	110	150	19	0.733
LSV2C-019	8.6	12.5	267	390	39	0.685
LSV2C-020	11.3	11.2	240	296	36	0.811
LSV2C-021	20	28.1	8	172		0.047

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

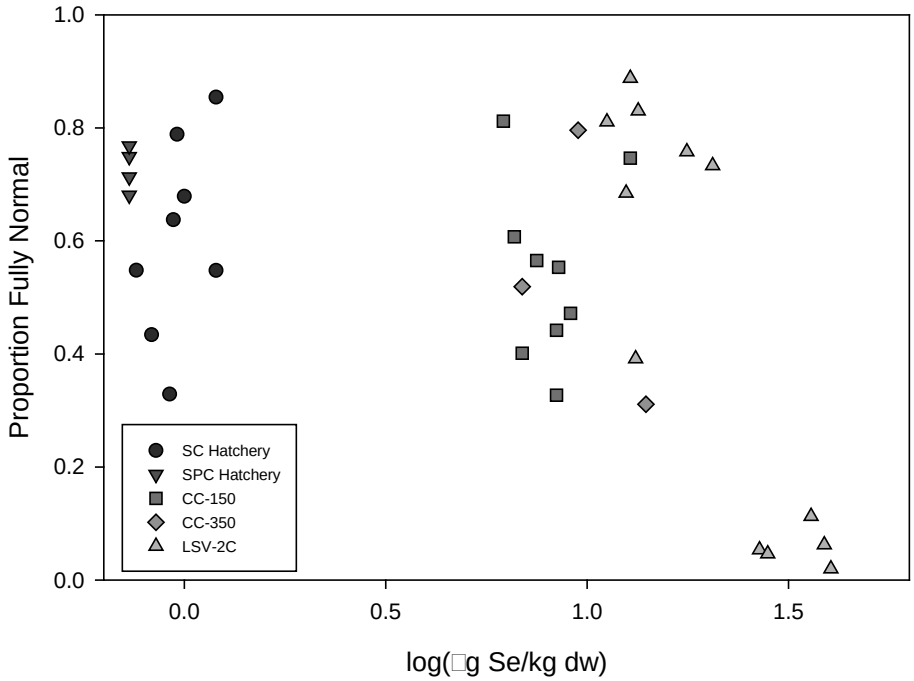


Figure 2. Proportion of normal (free from deformities) larvae plotted against Se concentrations in eggs, hatch through 15-days post swim up. Effects were highly variable across the entire background concentration range (20.5 mg/kg and lower), such that a meaningful EC_{10} could not be calculated for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

Survival Endpoint

Selenium concentrations and estimated counts and proportions of larvae surviving from hatch through 15 days post swim up are included in Table 3. Estimated counts and proportions were reported for survival through the 15-day post swim up test because larvae were thinned to a target of 100 individuals/treatment prior to the onset of the post swim up test, and final full test survival is calculated as the product of survival from hatch to swim up and survival during the 15-day post swim up test. In the 2014 draft document, EC_{10}s were calculated for the worst case (16.78 mg/kg) and optimistic (20.40 mg/kg) survival scenarios, and these were also reported in the 2015 draft document. For both scenarios, the assumption was made that fry that failed to swim up would not have survived, and so the survival for the post swim up portion of the test in the 5 treatments with the highest selenium concentrations (26.8 mg/kg and above) was set to zero. The EC_{10} of 16.78 mg/kg for the optimistic is nearly identical to the EC_{10} for the worst case survival scenario of 16.76 mg/kg presented in the response to the FWS review of the Formation Environmental study (Taulbee et al. 2012), peer reviewed by ERG (2012).

In contrast to the deformity and combined deformity+survival endpoints, background survival (concentrations up to and including 20.5 mg/kg) was much less variable. Despite the lower variability among background effect levels, a careful re-examination of these data following the release of the 2015 draft Se

document determined that a meaningful EC_{10} cannot be calculated by TRAP so long as the assumption is made that fry failing to reach swim up are assumed to be dead. This is because TRAP requires at least 2 partial effects to calculate an EC_{10}, and this dataset has no partial effects, but rather, a background range with high and relatively stable survival through 20.5 mg/kg, and then no survival at concentrations of 26.8 mg/kg and above (Figure 3). In order to calculate an EC_{10} for survival, the assumption regarding fry that failed to swim up was removed. In addition, in order to remove the uncertainty introduced by the clogged drain leading to the overflow and loss of fish from some of the treatments in the post swim up test, the EC_{10} for larval survival was calculated for the much longer hatch through swim up portion of the test, as described below.

Table 3. Brown trout selenium concentrations and survival data from hatch to test end (15 days post swim up).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
SC-001	3.6	0.76	144	0.951	0.990	0.942
SC-002	4.1	0.94	138	0.978	0.990	0.968
SC-003	3.7	0.83	340	0.982	0.989	0.971
SC-004	4.3	0.92	189	0.868	0.971	0.842
SC-005	3	1.2	70	0.914	1.000	0.914
SC-006	3.1	1.2	564	0.988	0.990	0.978
SC-007	2.7	1	188	0.856	0.970	0.830
SC-008	2.5	0.96	396	0.985	1.000	0.985
SPC-001[c]		0.73	598	0.987	1.000	0.987
SPC-002[c]		0.73	20	1.000	1.000	1.000
SPC-003[c]		0.73	585	0.966	1.000	0.966
SPC-004[c]		0.73	21	1.000	1.000	1.000
SPC-005[c]		0.73	589	0.986	1.000	0.986
SPC-006[c]		0.73	593	0.971	1.000	0.971
CC-150-009	8.4	12.8	173	0.942	0.990	0.933
CC-150-011	5.6	8.4	288	0.993	1.000	0.993
CC-150-012	6.7	8.5	314	0.965	0.990	0.955
CC-150-013	5.9	8.4	402	0.891	0.973	0.866
CC-150-015	6	9.1	479	0.971	1.000	0.971
CC-150-016	7	7.5	89	0.966	1.000	0.966
CC-150-017	5.6	6.6	223	0.969	1.000	0.969
CC-150-018	4.7	6.9	522	0.969	1.000	0.969
CC-150-020	7.2	6.2	584	0.990	1.000	0.990
CC-350-006	9.2	14	432	0.944	0.980	0.926
CC-350-007	5.5	6.9	181	0.950	0.988	0.938
CC-350-008	8.5	9.5	407	0.951	0.986	0.938
LSV2C-002	8.9	12.8	584	0.993	1.000	0.993
LSV2C-003[d]	13.8	40.3	404	0.079	0.281	0.022
LSV2C-004[d]	17.9	36	309	0.414	0.477	0.197
LSV2C-005[d]	13.6	26.8	287	0.387	0.622	0.240
LSV2C-008	9.6	17.7	263	0.989	0.982	0.971
LSV2C-010[d]	22.6	38.8	108	0.231	0.440	0.102
LSV2C-012	7.2	13.2	591	0.971	1.000	0.971

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Eggs Hatched	Prop. Survival. Hatch to swim up	Prop survival. Post swim up."	Prop survival. Hatch to end[b].
LSV2C-016	9.2	13.4	570	0.965	1.000	0.965
LSV2C-017	13.2	20.5	217	0.885	0.963	0.852
LSV2C-019	8.6	12.5	471	0.953	1.000	0.953
LSV2C-020	11.3	11.2	357	0.986	1.000	0.986
LSV2C-021[d]	20	28.1	424	0.288	0.730	0.210

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Test end was 15 days after swim up.

[c] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.[d] Survived but failed to reach swim up. Assumed dead in all hatch to 15-day post swim up analysis.

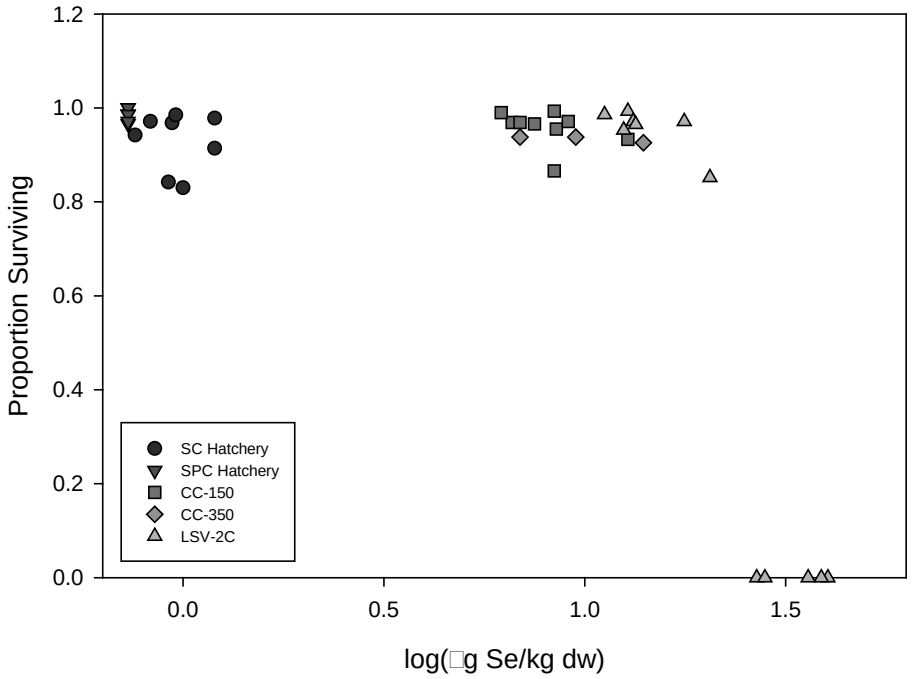


Figure 3. Proportion of larval survival plotted against log transformed Se concentrations in eggs, hatch through 15-day post swim up. Larvae from the five highest Se concentration treatments failed to reach swim up and were assumed to not have survived in the wild.

<u>Assessment of Overflow Loss During 15-day Post Swim Up Feeding Trial</u>

In the 2015 draft Se document, an assessment was made to determine whether the loss of fish from the overflow event during the 15-day post swim up portion of the test was related to survival or to Se treatment concentration measured during the first portion of the test. In this assessment, data were examined from the perspective of whether the overflow loss of brown trout during the second stage of the test could reflect dead, dying, or weak organisms. This was done to examine the hypothesis proposed in the U.S. FWS review that fish lost to overflow were either dead or dying.

First, the relationship between larval survival in the first and second stages of the test (hatch to swim up, 15 days post swim up) were compared for all treatments where larvae successfully reached the swim up stage (Figure 4). Overall, survival in the second stage tracks survival in the first stage (r^2=0.6), but survival in the second stage was noticeably higher in than in the first stage. This result is consistent with the following statement made by the principle scientist of the brown trout study in the public comments to the 2014 selenium draft document submitted for external peer review: "escaped fry were observed swimming in the water bath where the treatment containers were being held. These fry congregated near the treatment cells. Dead or dying fish were not observed."

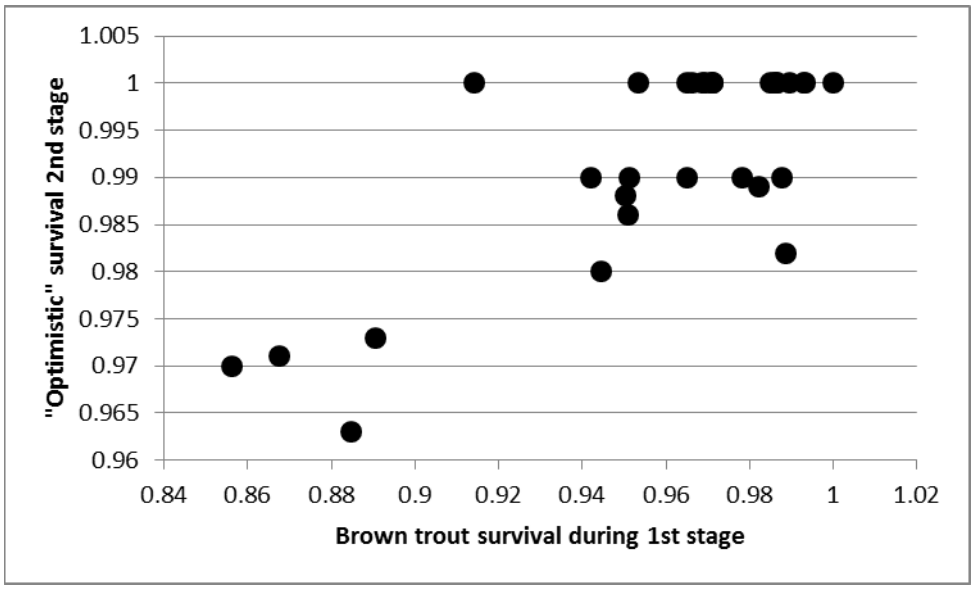


Figure 4. Relationship between survival during the first and second portions of the test. All treatments where larvae successfully reached swim up (Se concentrations of 20.5 mg/kg and lower).

Second, the relationship between larval mortality in the first stage and overflow loss in the second stages of the test (hatch to swim up, 15 days post swim up) were compared separately for all treatments (field and hatchery) and for all field collected treatments (Figure 5). As with figure 4, these correlations were made for treatments where larvae successfully reached the swim up stage. In these instances, there is no apparent relationship between health, as reflected by mortality in the first stage, and overflow loss in the second stage, whether considering all individuals or wild-only: r^2 for both graphs is 0.0. The lack of a relationship in these correlations suggests that overflow loss has a likelihood of being a random noise variable.

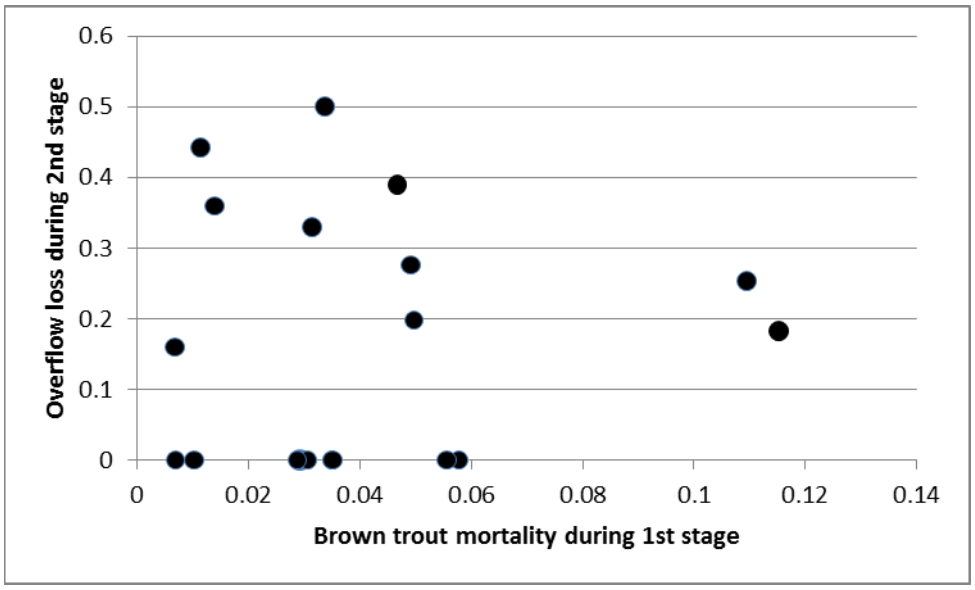


Figure 5. Relationship between mortality during the first stage of the test and overflow loss during the second stage of the test. Upper figure – all hatchery and field treatments. Lower figure – field treatments only. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

Finally, the relationship between overflow loss and selenium concentrations in eggs was examined (Figure 6). As with previous correlations, only larvae from treatments where individuals reached swim up were considered.

Figure 6 shows a clear difference between hatchery (far left) and field treatments, but across the concentration range for the offspring of field collected fish there is no apparent relationship between overflow loss and Se concentration. Within the field treatments, the r^2 of the correlation between Se concentration and overflow loss is 0.01. Although there are no known genetic differences between hatchery

and wild fish, if leaving the aquarium required swimming over the rim, one might speculate that previous generations of hatchery fish might have developed a tolerance to remaining in conditions that might seem crowded to wild organisms. (That is, however, purely speculative.) Otherwise, the difference between hatchery and wild fish would seem only to reflect a random artifact, since the Se concentrations at which the wild fish displayed high overflow losses are low.

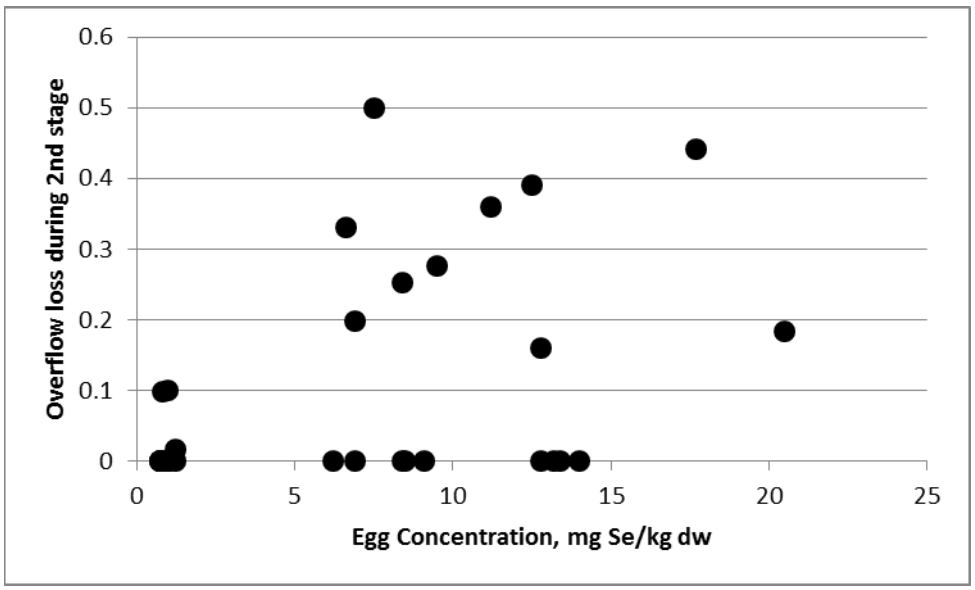


Figure 6. Relationship between egg Se concentration and overflow loss during the second stage of the test. Larvae from treatment levels 26.8 mg/kg and higher, which failed to swim up, were excluded.

In summary, the positive correlation between survival during the hatch to swim up portion of the test and survival during the 15-day post swim up portion of the test, combined with the lack of a correlation between mortality during the hatch to swim up portion of the test and overflow loss during the second stage of the test, suggests that the overflow loss likely represents a random technician error not related to the health of the individuals lost. The relationship between selenium egg concentrations and overflow loss was lower for the larvae hatched from hatchery fish compared to the larvae hatched from field collected fish; however, among field treatments ranging from 6.0-20.5 mg/kg there was no correlation, further supporting the hypothesis that the overflow event was a random occurrence unrelated to the health of larval fish.

The results of the above assessment of the overflow event strongly suggest that the overflow event was a random technician error unrelated to selenium toxicity, and that the "optimistic" scenario is also likely more realistic.

Survival Endpoint – EC_{10} for the first portion of the test

Because larval survival was measured at the end of the first portion of the test (hatch to swim up), an alternative approach to measuring survival would be to

calculate the brown trout EC_{10} for survival for only the first portion of the test. Selenium concentrations and counts of total larvae and larvae that survived the first portion of the test are included in Table 4. The hatch to swim up portion of the test was much longer than the second portion (88 days on average compared to 15 days), and more importantly, it avoids the experimental confound introduced by the loss of fish during the overflow event. With this approach, the second portion of the test would be rejected as inconclusive due to the laboratory accident.

Unlike survival, deformities could not be analyzed for the first portion of the test because of a bias introduced during the thinning process prior to the initiation of the 15-day post swim up portion of the test. During the thinning process, visibly deformed larvae were selectively removed, so that the fish used in the 15-day post swim up test were less likely to have been deformed. Because of this selection bias, only survival could be evaluated from hatch to swim up. Nevertheless, survival appears to be as sensitive an endpoint as deformities or survival+deformities, as all endpoints exhibit background effects (with differing levels of variability) through 20.5 mg/kg, and severe effects at concentrations between 26.8-40.3 mg/kg.

In contrast to survival endpoints measured from hatch through 15 days post swim up, survival for all treatments were included, including larvae from the five treatments of 26.8 mg/kg and higher, where larvae failed to reach swim up. This avoids any potential inconsistency stemming from not knowing whether small percentages of individuals did not swim up in other treatments. In contrast to the previous EC_{10} calculations, this approach is free from all assumptions about individuals lost in the lab accident. In the 2015 draft document, an EC_{10} of 18.09 mg/kg was calculated for this endpoint in TRAP, and this EC_{10} was used as the GMCV for Salmo. During a subsequent review, this EC_{10} was determined to be inappropriate, because it is lower than the 20.5 mg/kg concentration, which with 88.5% survival falls within the variability of the 32 data points at lower concentrations. Compared to the average survival for all 33 background concentration treatments, the survival at 20.5 mg/kg represents an approximately 8% effect.

In order to calculate an EC_{10} that would not fall below the background concentration of 20.5 mg/kg, a weighted least squares linear regression was calculated in TRAP, using a threshold sigmoid model (Figure 7). The model was weighted using the standard deviation of the 33 background concentrations (all concentrations between 0.73-20.5 mg/kg), and the residual standard deviation of the five concentrations between 26.8-40.3 mg/kg. This was done to provide less weight to the more variable, and more uncertain, high Se treatments relative to the less variable background treatments. The EC_{10} for survival using the weighted regression model is 21.0 mg/kg.

One issue with the above TRAP analysis is that to fit the 5 higher effects data well, the EC_0 estimate is pushed down to 16.4 mg/kg, below two of the points in the background range. Also, the fitted curve goes through the data point at 20.5 mg/kg, so that this point is considered to be an EC_8. This is not unreasonable because the response is so steep at concentrations above this point that some effect at this point is plausible. Nevertheless, this point is within the range of the

background and there are insufficient data to say that this concentration is an effect level. Thus, to accept this analysis and use the EC_{10} from this curve requires making a slightly conservative risk management decision that the point at 20.5 mg/kg should be treated as having some effect.

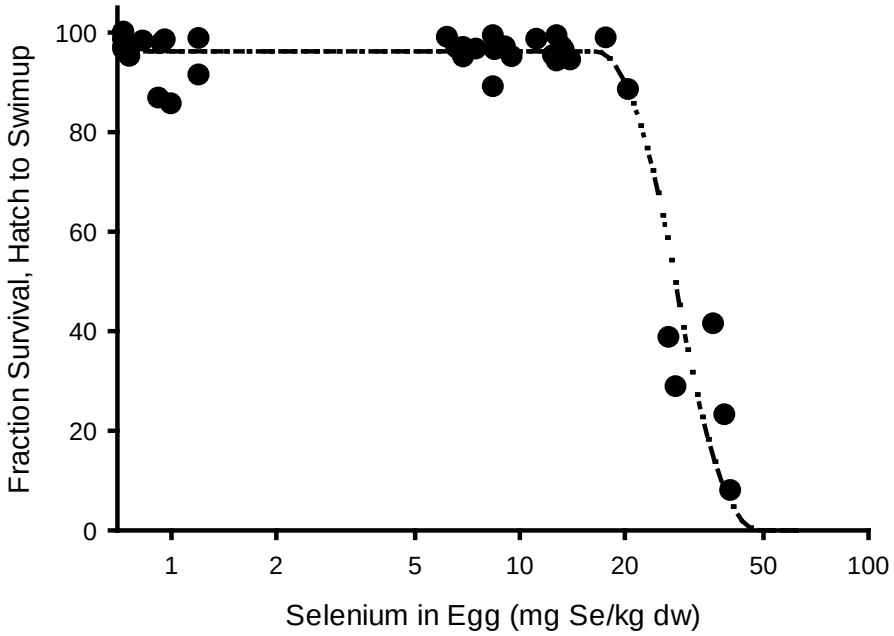


Figure 7. Brown trout survival, hatch to swim up. EC_{10} of 21.0 mg/kg calculated using a weighted nonlinear regression model.

Table 4. Brown trout selenium concentrations and survival data from hatch to swim up (first portion of the test).

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SC-001	3.6	0.76	144	137	95.1
SC-002	4.1	0.94	138	135	97.8
SC-003	3.7	0.83	340	334	98.2
SC-004	4.3	0.92	189	164	86.8
SC-005	3	1.2	70	64	91.4
SC-006	3.1	1.2	564	557	98.8
SC-007	2.7	1	188	161	85.6
SC-008	2.5	0.96	396	390	98.5
SPC-001[b]		0.73	598	590	98.7
SPC-002[b]		0.73	20	20	100
SPC-003[b]		0.73	585	565	96.6
SPC-004[b]		0.73	21	21	100
SPC-005[b]		0.73	589	581	98.6

Sample ID[a]	Whole body Se, mg/kg dw	Egg Se mg/kg dw	# Larvae Hatched	# Larvae Survived – Hatch to Swim Up	% Larvae Survived – Hatch to Swim Up
SPC-006[b]		0.73	593	576	97.1
CC-150-009	8.4	12.8	173	163	94.2
CC-150-011	5.6	8.4	288	286	99.3
CC-150-012	6.7	8.5	314	303	96.5
CC-150-013	5.9	8.4	402	358	89.1
CC-150-015	6	9.1	479	465	97.1
CC-150-016	7	7.5	89	86	96.6
CC-150-017	5.6	6.6	223	216	96.9
CC-150-018	4.7	6.9	522	506	96.9
CC-150-020	7.2	6.2	584	578	99
CC-350-006	9.2	14	432	408	94.4
CC-350-007	5.5	6.9	181	172	95
CC-350-008	8.5	9.5	407	387	95.1
LSV2C-002	8.9	12.8	584	580	99.3
LSV2C-003	13.8	40.3	404	32[c]	7.9
LSV2C-004	17.9	36	309	128[c]	41.4
LSV2C-005	13.6	26.8	287	111[c]	38.7
LSV2C-008	9.6	17.7	263	260	98.9
LSV2C-010	22.6	38.8	108	25[c]	23.1
LSV2C-012	7.2	13.2	591	574	97.1
LSV2C-016	9.2	13.4	570	550	96.5
LSV2C-017	13.2	20.5	217	192	88.5
LSV2C-019	8.6	12.5	471	449	95.3
LSV2C-020	11.3	11.2	357	352	98.6
LSV2C-021	20	28.1	424	122[c]	28.8

[a] SC – Saratoga National Fish Hatchery; SPC – Spring Creek Trout Hatchery; CC – Crow Creek; LSV – Sage Creek

[b] Arrived as fertilized, eyed-eggs. No whole body Se measurement possible.

[c] Survived, but failed to reach swim up.

Whole Body Concentration

The whole-body concentration response curve for survival, hatch to swim up is shown in Figure 8. These data are not amenable to TRAP modeling, and Figure 8 shows the interpolation procedure, the first interpolation point being an EC_0 at 13.2 mg/kg and 96% survival and the second point an LOEC at 13.6 mg/kg and 39% survival. Because the HNOEC (13.2 mg/kg) and LOEC (13.6 mg/kg) are so close, the chronic value for whole body selenium is the HNOEC of 13.2 mg/kg dw.

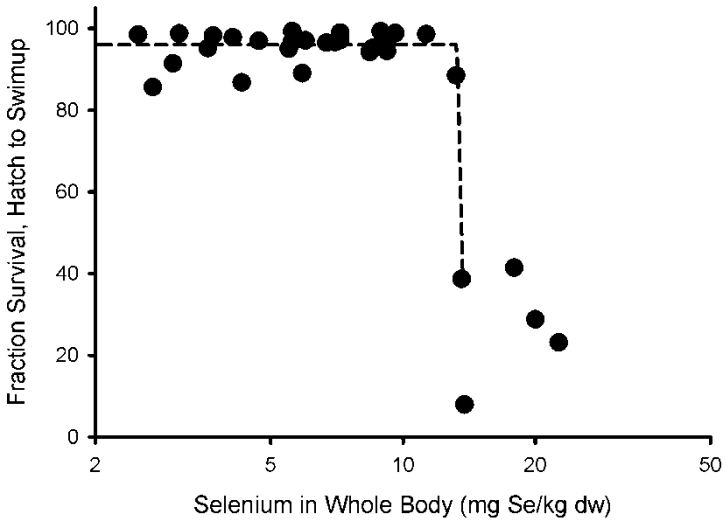


Figure 8. Fraction survival of brown trout larvae as a function of selenium in eggs.

Effect Concentration: For this study the most appropriate, least confounded endpoint is survival, hatch to swim up. For egg selenium, EC_{10} is 21.0 mg Se/kg egg dw, calculated for survival from hatch to swim up using a weighted nonlinear regression model. Expressed as whole body, the chronic value is 13.2 mg Se/kg WB dw.

Besser, J.M., W.G. Brumbaugh, D.M. Papoulias, C.D. Ivey, J.L. Kunz, M. Annis, and C.G. Ingersoll. 2012. Bioaccumulation and toxicity of selenium during a life-cycle exposure with desert pupfish (*Cyprinodon macularius*): U.S. Geological Survey Scientific Investigations Report 2012–5033, 30 p. with appendixes.

Test Organism: Desert pupfish (*Cyprinodon macularius*)

Exposure Route: Dietary and waterborne. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, which had been grown on a diet of selenized yeast.

Test Duration: 180 days life cycle, 21 days F1 larvae, 58 days F1 juveniles and adults.

Study Design: Desert pupfish (*Cyprinodon macularius*), a federally-listed endangered species, were exposed simultaneously to waterborne and dietary selenium at six exposure levels (controls and five selenium treatments) in a three-phase life cycle exposure study. Aqueous exposures were prepared using sodium selenate and sodium selenite salts at an 85%-15% proportion, respectively. Pupfish were fed the oligochaete, *Lumbriculus variegatus*, daily to satiation (25 to 30% rations based on wet weights). Prior to being fed to the pupfish, the oligochaetes were exposed to aqueous selenium and fed selenized yeast at appropriate concentrations to attain the target dietary tissue concentrations. The measured concentrations in water, oligochaetes (pupfish diet), and pupfish tissues for the control and five treatments during the life cycle exposures.

Treatment	water µg/L	oligochaetes mg/kg dw	pupfish, mg/kg dw		
			F_0 WB	eggs	F_1 WB
Control	nd	1.6	0.75	1	1.2
Se–1	3.4	5.1	2.5	3	3.4
Se–2	6.2	7.3	3.4	4.4	3.7
Se–3	14	14	6.7	8	6.7
Se–4	26	24	12	13	12
Se–5	53	52	24	27	31

The 85-day Phase 1 exposure was initiated with approximately five week old juvenile pupfish (F_0). Phase 1 consisted of two separate groups with one group (started two weeks prior to the second group) used for determining survival, growth and whole body selenium concentrations, and the other group used for survival assessment and to provide adults for the main reproduction exposure. Both groups in Phase 1 were similarly exposed to all six treatments, with each treatment having 8 replicates and 10 fish in each replicate.

At the end of the 85-day Phase 1 exposure, the pupfish were reproductively mature and were used for the Phase 2 exposure, the main reproduction study. A preliminary reproduction study was conducted with adults from the first exposure group of F_0 pupfish. These fish were divided into two spawning groups and eggs were collected on four dates during a 9-day period. The main purpose of the preliminary study was to confirm the reproductive maturity of the pupfish, but samples of larvae from this study were used for assessment of deformities. The main reproduction study in Phase 2 was started with adults from the second F_0 exposure. These fish were sorted into spawning groups (1 male and 3 females) in

7-L exposure chambers, with eight replicate spawning groups per selenium treatment. Spawning activity was monitored by removing (and replacing) spawning substrates from each chamber three times a week (Monday-Wednesday-Friday). There were 23 egg collection dates during a 60-day period. All eggs were counted and eggs collected from eight Wednesdays were used for hatching success, deformities and F_1 larval and juvenile growth and survival in the 58-day Phase 3 exposure. Larvae were examined for developmental endpoints including edema, delayed development, and skeletal, eye, craniofacial, and fin deformities.

Effects Data: A summary of the endpoints by each treatment level is shown below.

Table 1. Summary of pupfish toxicity endpoints by exposure treatment (average across all replicates). There were no statistically significant differences across controls and selenium amendment treatments for any of the endpoints shown here (1-way ANOVA, α=0.05).

Endpoint[a]	Control	Se-1	Se-2	Se-3	Se-4	Se-5
F0 survival, day 28	100	100	100	100	100	98
F0 survival, day 56	100	100	100	100	100	100
F0 survival, day 85	100	100	100	100	100	100
F0 survival, day 150	91	94	94	94	91	97
F0 growth, day 28	213	206	204	198	213	203
F0 growth, day 56	535	526	486	469	509	447
F0 growth, day 85	935	998	941	934	914	1053
F0 growth, day 150	1718	1763	1776	1755	1673	1606
F1 survival, day 30	100	100	100	100	98	98
F1 survival, day 58	100	100	93	90	95	88
F1 growth, day 30	73	73	76	78	77	58
F1 growth, day 58	260	264	286	286	288	255
total number eggs	6845	6331	4143	4386	3337	5225
% reduction eggs	NA	8	39	36	51	24
avg % deformities, main	5.3	2.7	4.9	2.4	11.4	8.1
avg % deformities, preliminary	4.4	8.8	11.6	14.3	10.7	21

[a] Endpoint units: survival, %; growth, mg wet weight; % reduction eggs is relative to the control.

The authors observed no significant differences in pupfish survival or growth among treatments. The authors hypothesized the lack of statistically significant acute effects was because the pupfish in this study were near their chronic toxicity threshold, as suggested by the (non-significant) mean reductions in growth (7% in F_0 day 150) and survival (12% in F_1 day 58) in the highest selenium treatment (Se-5), relative to controls (Table 1).

Egg hatching and larval survival in all selenium treatments (not listed in Table 2) were within 10 percent of control means, and differences among treatments were

not related to selenium exposure. The authors noted that the highest selenium treatment, Se-5, did have the lowest larval survival (84%) and lowest combined egg hatching and larval survival (76 percent). The means frequencies of deformities were higher in the two highest Se treatments (Se-4 and Se-5, Table 1); however % deformities across treatment levels were not statistically significant (1-way ANOVA, p=0.13; Beckon et al. (2012). However, overall deformity rates were statistically significantly higher in a preliminary reproduction than in the main reproduction test. Beckon et al. (2012) hypothesized that the reason for the difference in deformity rates between the two tests was related to the time the eggs were collected relative to the time the respective spawning groups were isolated. Eggs were collected in the preliminary reproductive study 1 - 9 days after the spawning groups were isolated, whereas spawns used to characterize deformities in the main reproduction test were collected at least 14 days after the onset of spawning. The larvae produced from the earlier collected eggs may have been exposed to higher selenium concentrations in the egg. The pattern of a gradual decrease in egg selenium concentration over time was observed in the life cycle study.

Egg production varied considerably over the 23 collection dates (Table 2 and Figure 1). Although each of the selenium treatments had a lower total number of eggs relative to the control, one-way ANOVAs of cumulative egg production did not indicate significant differences among treatments on either a per-replicate basis (p=0.34) or on a per-female basis (p=0.20). Similarly, repeated measures ANOVA indicated no differences between treatments, but the authors indicated significant differences among sampling dates and significant interactions of treatment and date. Because of the lower number of eggs in the selenium treatments and the significance of the interaction of treatment and time, the authors concluded that pupfish egg production was adversely affected by elevated selenium exposure and reported significant reductions in egg production at treatment levels Se-2 through Se-5 (4.4 to 27 mg/kg dw Se in eggs). The authors recognized that typically larval survival and deformities are the most sensitive reproductive endpoint for selenium toxicity and not egg production and suggested more study is needed to confirm the unusual sensitivity of pupfish egg production to selenium.

Table 2. Number of pupfish collected on each sampling date throughout the study, by treatment level. Values represent the sum of all eggs collected on a given date for a given Se treatment.

Day	Control	Se-1	Se-2	Se-3	Se-4	Se-5
2	136	112	90	67	122	94
4	275	173	123	142	188	162
7	307	273	301	283	160	432
9	265	252	226	169	271	283
11	401	136	424	319	265	380
14	417	359	333	246	198	401
17	448	456	206	163	145	232
21	303	664	404	204	163	400
23	287	205	141	143	177	175
25	340	308	94	143	150	228
28	366	273	103	101	95	181
30	130	164	104	52	82	132
32	323	304	271	78	75	151
35	320	427	81	150	74	223
37	236	176	41	113	38	38
39	326	151	159	184	113	140
42	507	140	55	193	101	140
44	251	133	66	152	69	137
51	380	359	227	338	305	370
53	278	63	38	197	56	188
56	199	478	138	195	238	222
58	202	329	331	410	143	320
60	148	396	187	344	109	196

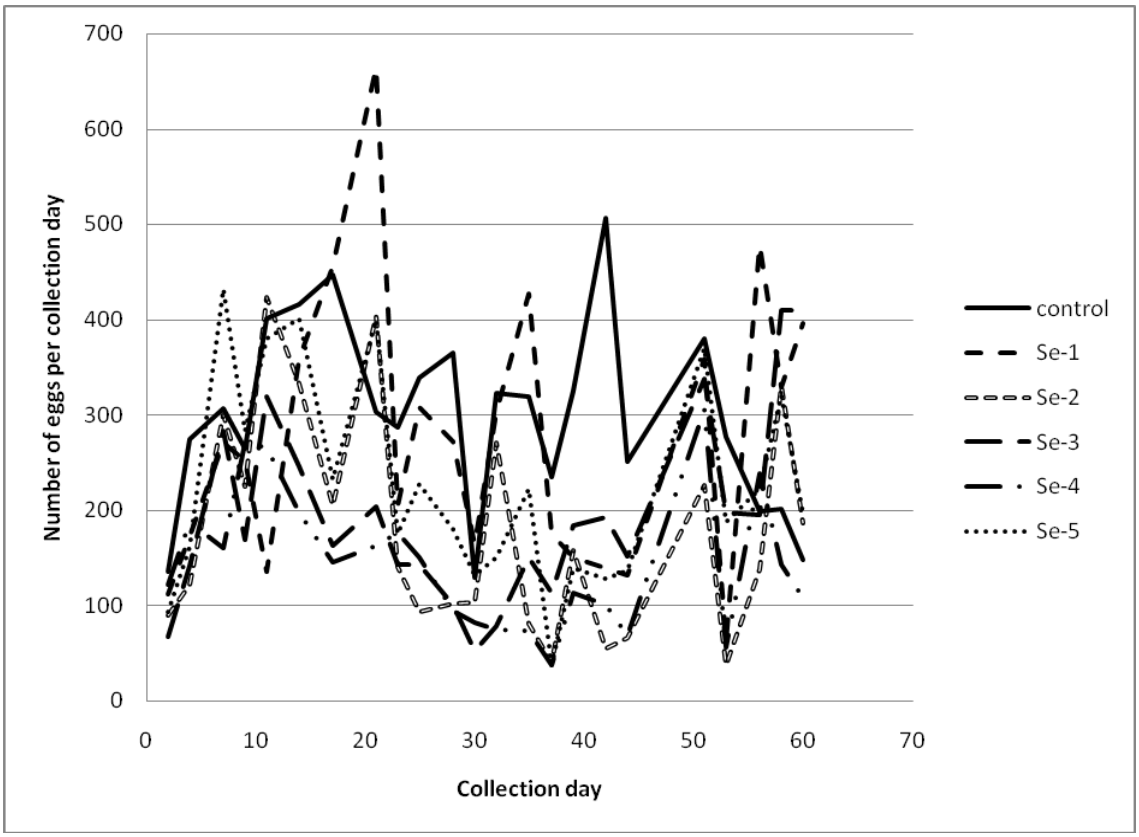


Figure 1. Pupfish egg production by sampling date

Several findings from the pupfish study put a clear demonstration of effect due to selenium in question. The fact that the typical sensitive endpoints for selenium, larval survival and deformities, were not demonstratively responsive to selenium through the highest treatment level, the fact that the egg production data did not show significance among treatments alone, and the fact that egg production increased at the highest selenium treatment level provide sufficient doubt of a clear effect due to selenium. These issues are discussed below.

Examination of the Repeated Measures Analysis:

Analysis Using the Full Dataset: The effects of selenium treatment and sampling date on pupfish egg production (eggs per female per day) were reanalyzed. First, the data were reanalyzed using repeated measures ANOVA. Results of the repeated measures ANOVA analysis were qualitatively similar to those reported in Besser et al. (2012) and are shown in the following table.

Between Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Se treatment	2,202.6	5	440,5	1.755	0.143
Error	10,543.5	42	251.0		

Within Subjects

Source	Sum of Sq.	df	Mean Sq.	F-rat.	p-value
Sampling Date	1,867.5	22	84.89	4.973	<0.001
Se Treatment x Sampling Date	2,566.3	110	23.33	1.367	0.010
Error	15,771.8	924	17.07		

As with the results reported in Table 7 of Besser et al. (2012), there was no main effect of Se treatment (note – for purposes of these analyses and associated text, "Se treatment" is defined as the control plus the 5 treatments that received Se amendments), but there was a statistically significant ($p \leq 0.05$) effect of sampling date and a significant date by Se treatment interaction. Results were qualitatively similar because the p-values for Se treatment and sampling day were identical in both analyses, yet the p-values for the day by Se treatment interaction term were nearly identical.

A statistically significant sampling date effect means that there were significant differences in overall egg production on different sampling dates. Daily egg production per female ranged from 2.176 on day 2 to a high of 7.294 on day 11, and was variable throughout the study. Of greater interest is the statistically significant day x Se treatment interaction. What this means is, although there was not an overall significant effect of Se treatment on egg production per female, there was a significant Se treatment effect ($p<0.05$) on egg production per female on at least one of the 23 sampling dates.

Analysis after Removal of Control Replicate Outlier: Repeated measures ANOVA analysis confirmed the results reported in Besser et al. (2012). However, as shown on Figure 8b of Besser et al. (2012), one replicate chamber (replicate g) within the control treatment had only one surviving female pupfish from day 7 through the end of the test (day 60), and that replicate also had the highest overall egg production per female of any test chamber. All replicate chambers in all treatments began with three female pupfish, and the replicate described above was the only one with only one surviving female. All three females survived the 60 day test in the majority of the replicate chambers. In order to determine whether the significant date by Se treatment interaction was an artifact of this one test chamber, data were reanalyzed after removing this replicate.

One requirement of repeated measures ANOVA is that the model cannot contain any missing values. An alternative to repeated measures ANOVA when data are missing, and the most commonly followed procedure under these circumstances, is to analyze the data using a mixed model. This was the procedure followed here.

The results of a fully balanced mixed model (no missing data) should be identical to repeated measures ANOVA. As an initial check, the full dataset was reanalyzed as a mixed model. Sample chamber was the random effect parameter, and Se treatment, sampling date, and Se treatment by sampling date were the fixed effect parameters. As expected, the F-ratios for the effects of selenium treatment, sampling date, and the sampling date by Se treatment interaction were identical. Next, the data were reanalyzed after removing data from control replicate g from all sampling dates. Results of this analysis are reported in the table below.

Mixed Model – Fixed Effect

Effect	Numerator df	Denominator df	F-ratio	p-Value
Se Treatment	5	902	1.087	0.366
Sampling Date	22	902	6.042	<0.001
Se Treatment x Sampling Date	110	902	1.310	0.023

T

The statistically significant interaction between Se Treatment and Sampling Date persisted after removal of the potentially anomalous control treatment chamber with one female pupfish. In other words, even after removing the one potentially anomalous control replicate, there were still some individual sampling dates where the effects of Se treatment were statistically significant ($p<0.05$).

Se Treatment x Sampling Date Interaction: When a significant interaction is observed in a repeated measures ANOVA, the next recommended step in the process is to examine each of the repeated measures (sampling dates) separately to identify those dates where the significant difference in Se treatment level occurred. When individual dates for the full dataset (including the replicate with one surviving female) were analyzed separately, there were significant ($p<0.05$) effects of Se treatment level on egg production on days 28, 35, 37, 42, and 53 (1-way ANOVA, $df_{5,42}$). There were no significant Se treatment effects on the remaining 18 sampling dates. ANOVA results are summarized in the table below.

Sampling Date	F-ratio	p-value
28	2.501	0.045
35	2.704	0.033
37	3.351	0.012
42	4.294	0.003
53	3.352	0.012

Because of the large number of comparisons (23 individual ANOVA models for each sampling date), an alpha of 0.05 is inappropriate for this particular analysis. This is because an alpha of $p<0.05$ means that a statistically significant result will be observed 5% of the time due to chance alone (Type I error). In order to control for the increased likelihood of a Type I error when making multiple comparisons, the alpha level of 0.05 was adjusted using Sidak's correction (Abdi 2007). For 23 comparisons and an alpha of 0.05 for one comparison, the adjusted alpha using Sidak's correction is as follows:

$$1 - (1 - 0.05)^{\frac{1}{23}} = 0.0027$$

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As a result, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Each of the 23 sampling dates for the dataset where the replicate chamber from the control treatment with one surviving female pupfish was excluded were also analyzed using one-way ANOVA to determine which sampling dates had significant Se treatment effects. Significant differences among Se treatment levels at alpha 0.05 are shown in the table below.

Sampling Date	F-ratio	p-value
35	2.839	0.027
42	3.164	0.017
53	2.549	0.042

After adjusting alpha to account for the 23 separate sampling dates, there were no sampling dates with a significant Se treatment effect ($p \leq 0.0027$). As with the full dataset, it was not necessary to perform *post hoc* means comparisons tests for any of the individual sampling dates to determine which Se treatment levels were significantly different from each other.

Summary of Repeated Measures Analysis: This analysis demonstrated that although there was a significant Se treatment by sampling date interaction, regardless of whether or not the control treatment chamber with one female pupfish was excluded, differences among Se treatment levels were only observed for a small subset of the 23 sampling dates. Furthermore, after adjusting alpha to account for multiple comparisons, one-way ANOVA analyses conducted separately for each sampling date to locate the source of the Se Treatment x Sampling Date interaction determined that there were no statistically significant differences among Se treatment levels on any sampling date, precluding the need to perform *post hoc* comparison of means tests to identify significant differences among individual Se treatments.

Combining Effect Metrics Using a Population Model: To improve the certainty of any conclusions to be made about the sensitivity of pupfish to selenium, it is also worthwhile to consider the biological (as opposed to statistical) significance of the observations. But for total egg production, survival, and deformities, the concentration-response curves did not show a sufficient concentration-related effect to calculate an EC10. Nevertheless, because Besser et al. (2012) raised the issue of an interaction of egg production with time, there is a particular concern that there could be a delay in egg production that would reduce population growth rate, even while total numbers of eggs were not significantly affected. This question was evaluated by constructing a population model corresponding to data available from the test.

This modeling approach allows for combining and properly weighting effects on egg production, timing of egg production, and survival. Percent hatch and percent deformities were also considered in alternate calculations. Because the model is only intended for combining the lab data into a unified concentration-response curve, it cannot be interpreted as making real-world population predictions. The relevant data were taken from spreadsheets Besser et al. (2012b and 2012c), which were provided by Besser.

The reproduction and larval endpoints spreadsheet, Besser et al. (2012b), presents egg production at 23 time points. This information thus allows for 23 adult life stages, each assigned its own fecundity. Another page of this spreadsheet provides larval survival data, thus defining survival of the early life stage. The juvenile and adult survival spreadsheet, Besser et al. (2012b), defines a survival rate shared by these life stages.

For each treatment, the data from the test thus provide *all* the needed input for 25 life stages: (1) an embryo-larval stage with its own daily survival probability (along with hatching and deformity percentages, when considered in alternative calculations), (2) a non-reproducing juvenile stage sharing its treatment's daily survival probability with the adult stages, and (3 – 25) 23 short-duration adult stages each with its own egg production, but sharing its treatment's daily survival probability with the treatment's other adult stages. Use of the data is detailed below.

Egg Production: Egg production at the test's 23 observation time points is from the spreadsheet Besser et al. (2012b), expressed as eggs per female per day. The intent of Besser et al. (2012) was for each treatment to have eight replicates, and each replicate was to have one male and three females. Only replicates matching that design were used. Early in the test Control Replicate "g" ended up with only one female, and was therefore not used here. Se-1 Replicate "h" and Se-3 Replicates "d" and "h" had been inadvertently stocked with two males and two females, and were likewise not used here. Table 3 shows the time course of egg production incorporated into the population model. For each treatment, model fecundity, m_i, for life stages i = 3 – 25, is the observed egg production *divided by 2*, in order to provide *female eggs* per female per day.

Percent Hatch: The spreadsheet Besser et al. (2012b) presents percent hatch for eggs collected at selected time points. Within each treatment these were averaged. In selenium reproductive studies percent hatch is often treated as a noise variable unrelated to selenium exposure. Consequently, the population growth calculations were run with and without including percent hatch. When hatch was incorporated into the calculation, daily fecundity was reduced by multiplying by percent hatch.

Deformities: The Besser et al. (2012b) spreadsheet also provides deformity counts for the study's preliminary test and for its main test. Only the main test results were used here. Counts were totaled for each treatment, and a percentage calculated. Population growth calculations were performed both with and without consideration of deformity percentage. For simplicity when considered, a worst case assumption was made that deformed individuals do not contribute to the

population. Percent deformity was thereby handled in manner parallel to percent hatch, by multiplying daily fecundity by percent free of deformity.

Table 3. Life stage durations, and observed eggs per female per day at observation time points for control and selenium treatments, *only with replicates having the design three females and one male*. Model fecundity, m, is set at one-half the observed, to yield female eggs per female.

Repro Study Observation Day	Assigned Life Stage Number	Life Stage Duration	Observed Eggs/Female/Day					
			Control	Se-1	Se-2	Se-3	Se-4	Se-5
-	1	35	-	-	-	-	-	-
-	2	85	-	-	-	-	-	-
2	3	2	2.690	2.571	1.875	1.319	2.542	1.958
4	4	2	5.548	4.048	2.563	2.153	3.917	3.375
7	5	3	4.333	4.302	4.181	3.185	2.222	6.000
9	6	2	5.762	5.524	4.708	3.639	5.646	5.896
11	7	2	8.024	3.238	8.833	4.528	5.521	7.917
14	8	3	6.540	4.905	4.625	2.296	2.750	5.569
17	9	3	6.429	7.143	2.861	1.481	2.014	3.222
21	10	4	3.345	7.881	4.208	1.764	1.698	4.167
23	11	2	5.786	4.643	2.938	3.806	3.688	3.646
25	12	2	6.905	7.286	1.958	2.792	3.125	4.750
28	13	3	4.794	4.317	1.431	1.306	1.319	2.514
30	14	2	1.881	3.881	2.167	1.403	1.708	2.750
32	15	2	5.464	7.286	5.646	1.444	1.563	3.146
35	16	3	4.373	7.310	1.132	2.880	1.028	3.097
37	17	2	5.631	4.417	0.927	1.556	0.792	0.792
39	18	2	6.119	3.917	4.240	3.556	2.354	2.917
42	19	3	7.349	2.222	1.056	2.500	1.403	1.944
44	20	2	4.798	3.274	1.719	3.194	1.438	2.854
51	21	7	1.847	2.139	1.571	2.532	2.060	2.202
53	22	2	6.310	1.512	0.823	5.403	1.333	3.917
56	23	3	3.183	7.317	2.076	2.491	3.528	3.083
58	24	2	3.405	7.810	8.469	9.597	3.104	7.656
60	25	2	3.810	8.226	4.115	6.347	2.271	4.271
Total as $\sum$ (duration · eggs/f/d) =			281.6	294.3	181.9	174.7	142.0	220.1

Larval Survival: The Besser et al. (2012b) spreadsheet also has data for larval survival after 14 and 21 days for eggs collected at three time points. The fraction surviving 21 days was used here. For each treatment, the probability of the early life stage (i=1) surviving each day equals the fraction surviving for 21 days, raised to the 1/21 power: $\sigma_1 = \sigma_L = (21\text{-d Surv})^{1/21}$, shown in Table 4.

Juvenile and Adult Survival: A second spreadsheet, Besser et al. (2012c), has data on juvenile and adult survival after 30 and 58 days. The fraction surviving 58 days was used (Table 4). Parallel to the handling of larval survival, for each treatment the juvenile-adult daily survival probability, σ_{JA} = (58-d Surv)$^{1/58}$, as shown in the table. This value applies to life stages i=2-25 (σ_2 through σ_{25}).

Table 4. Pupfish observed survival and modeled daily survival; fraction hatching and fraction free of deformity.

Treat-ment	Conc	21-d Larval Surv	Larval Daily Surv (σ_L)	58-d Juv+Adlt Surv	Juv+Adlt Daily Surv (σ_{JA})	Fraction Hatch	Fraction Free of Deformity
Control	1	0.9038	0.9952	1.0000	1.0000	0.9023	0.9489
Se-1	3	0.9770	0.9989	1.0000	1.0000	0.9026	0.9727
Se-2	4.4	0.9109	0.9956	0.9250	0.9987	0.8197	0.9563
Se-3	8	0.9600	0.9981	0.9000	0.9982	0.8922	0.9750
Se-4	13	0.9586	0.9980	0.9500	0.9991	0.8988	0.9048
Se-5	27	0.8396	0.9917	0.8750	0.9977	0.9104	0.9174

Formulation of the Population Model: The population growth equation is shown below, in abbreviated form.

$$\begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_t = \begin{bmatrix} \sigma_1(1-\gamma_1) & 0 & \sigma_3 m_3 & \cdots & \sigma_{25} m_{25} \\ \sigma_1\gamma_1 & \sigma_2(1-\gamma_2) & 0 & \cdots & 0 \\ 0 & \sigma_2\gamma_2 & \sigma_3(1-\gamma_3) & \cdots & 0 \\ \vdots & \vdots & \vdots & \cdots & 0 \\ 0 & 0 & 0 & \cdots & \sigma_{25}(1-\gamma_{25}) \end{bmatrix} \begin{bmatrix} N_1 \\ N_2 \\ N_3 \\ \vdots \\ N_{25} \end{bmatrix}_{t-1}$$

The diagonal of the 25x25 projection matrix has $\sigma_i(1-\gamma_i)$, the sub-diagonal has $\sigma_i\gamma_i$, and the top row has $\sigma_i m_i$. All other elements are 0. For life stage i, σ_i is the daily survival probability, γ_i is the daily probability of graduating to the next life stage, and m_i is the fecundity expressed as number of female eggs produced per female per day, set at one-half the observed eggs/female/day.

The graduation probability, γ_i, for individuals in each life stage was calculated as follows:

$$\gamma_i = \frac{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - \left(\frac{\sigma_i}{\lambda}\right)^{(Dur_i - 1)}}{\left(\frac{\sigma_i}{\lambda}\right)^{Dur_i} - 1}$$

where λ is the population growth rate and Dur_i is the duration of the life stage. In a 2-day duration life stage, were survival 100% (σ=1) and were the population not growing (λ=1), exactly one half (1/*Dur*) would graduate each day from the 2-day life stage. However, with σ<1 and λ>1, there would be a slight youthful bias

within the life stage, such that slightly more than half would be only 1 day into the life stage and not ready to graduate, and slightly less than half would be in their second day and ready to graduate. The above function adjusts for that.[1] The projected population growth rate for each treatment was calculated as follows. The 25x25 projection matrix was placed on an Excel spreadsheet. Each cell in the diagonal was then modified to subtract the eigenvalue, λ, which represents the population growth rate. That is, each cell in the diagonal was rewritten as $\sigma_i(1-\gamma_i) - \lambda$. The determinant of the 25x25 matrix was then calculated by function MDETERM. To obtain the population growth rate, Excel's Solver was then tasked with finding a value for λ that yielded a value of zero for the matrix determinant. In this case,-10^{-18} < MDETERM < $+10^{-18}$ was deemed sufficiently close to zero. Introducing the constraint to look for λ values between 1.01 and 1.04 was found helpful for Solver to find the dominant eigenvalue. When Solver occasionally could not get the determinant within 10^{-18} of zero, probably due to a solution oscillation that can occur because the input values γ_i are expressed as a function of the solution output λ, digits were removed from Solver's best estimate for λ, to provide a new starting value with which Solver could complete the solution.

Effects on Projected Population Growth Rates: Table 5 and Figure 2 show the model results. Figures 2-B, -C, and -D are almost indistinguishable from Figure 2-A, because hatch and deformity rates varied so little across treatments. Although population growth rates at 4.4 – 27 mg Se/kg are less than at 1 – 3 mg Se/kg, the 6-fold increase in concentration from 4.4 – 27 mg Se/kg yields no change in response. Consequently, the results do not suggest a selenium-related effect, and no EC_{10} can be calculated. Based on the combined influences of egg production and timing, and survival (with or without percentage hatch and deformities), pupfish does not appear to be among the most sensitive species.

[1] The formula for γ is undefined (0/0) under the condition σ=1 and λ=1, so it is not obvious from inspection how it behaves. This function addresses a model artifact that is called numerical dispersion when it occurs in pollutant transport models. It prevents overoptimistic rates of moving through the life stages, particularly in the 35-day and 85-day larval and juvenile stages, and allows a 25-stage model of life duration 180 days to yield precisely the same growth rate as a 180-stage (one day per stage) model, which was also constructed and checked for comparison. However, in this application where absolute growth rates have no particular meaning and only relative differences between treatments are of interest, the function does not change the overall perspective.

Table 5. Model output: daily population growth rates as λ (factor increase) and r (=ln λ), for models that account for survival, fecundity and its timing, and optionally also hatch and/or deformities. Because λ is responding to all the treatment parameters included in the model, its treatment-to-treatment variations do not exactly track the variations in any single input.

Treat-ment	Conc	Factors included in model:							
		All account for survival (σ_L , σ_{JA}) and fecundity (m) and its timing							
		-		Hatch		deformity		hatch & deform.	
		λ	r	λ	R	λ	r	λ	r
Control	1	1.0337	0.0332	1.0330	0.0324	1.0334	0.0328	1.0326	0.0321
Se-1	3	1.0346	0.0340	1.0338	0.0333	1.0344	0.0338	1.0336	0.0331
Se-2	4.4	1.0299	0.0294	1.0284	0.0280	1.0295	0.0291	1.0281	0.0277
Se-3	8	1.0285	0.0281	1.0277	0.0273	1.0283	0.0279	1.0275	0.0271
Se-4	13	1.0291	0.0287	1.0283	0.0279	1.0283	0.0279	1.0276	0.0272
Se-5	27	1.0294	0.0290	1.0288	0.0283	1.0288	0.0284	1.0281	0.0277

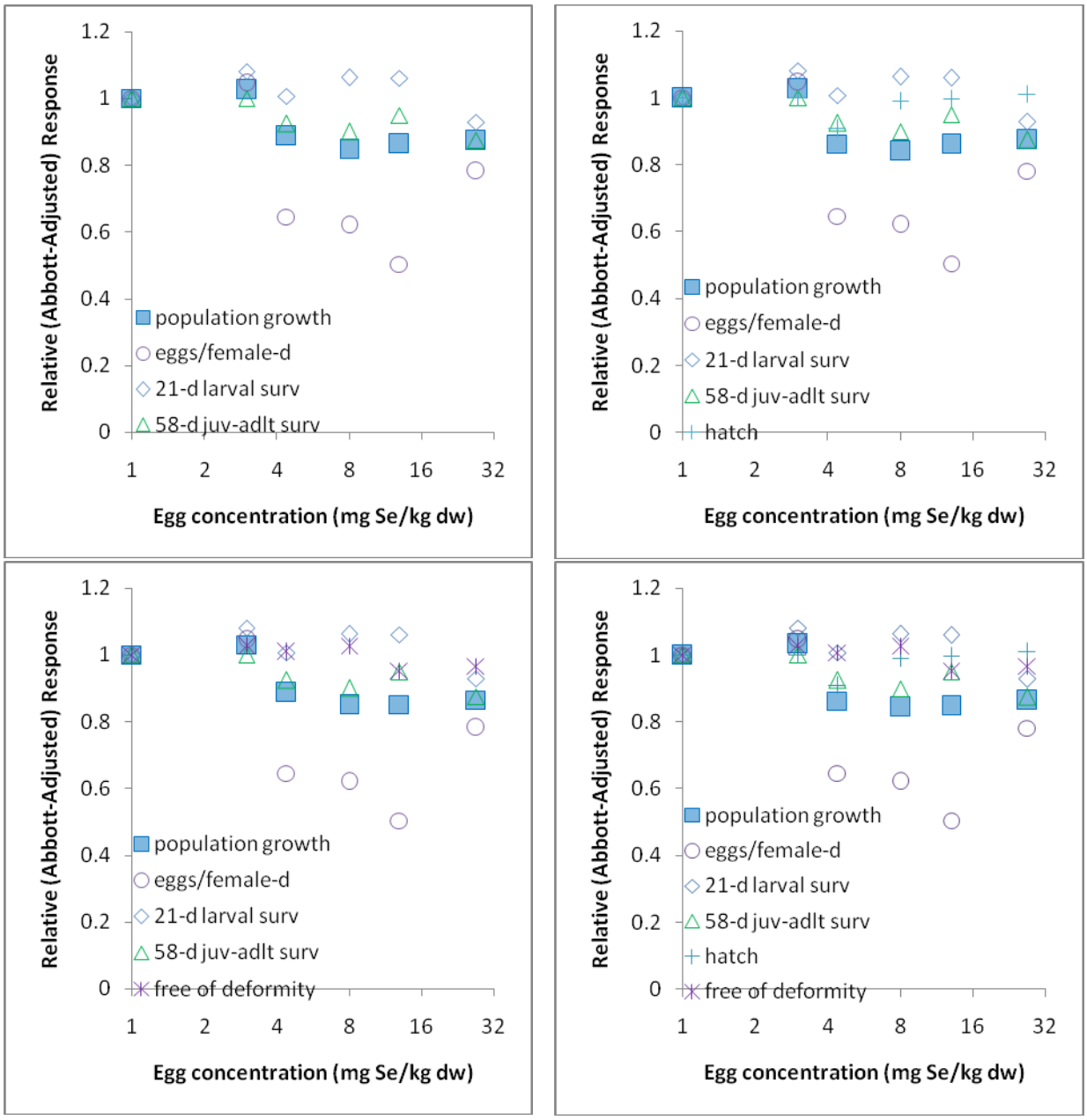


Figure 2. Abbott-adjusted pupfish response as modeled population growth rate (solid-filled symbols) and observed eggs per female per day, larval survival, and juvenile and adult survival (open symbols). Where used in the population model (to modify fecundity), hatch and deformity are shown as open symbols. Some open-symbol points are obscured beneath solid-symbol points. (A) Upper left, egg production and survival only, (B) upper right, adds in influence of percent hatch, (C) lower left, adds in influence of deformities, and (D) lower right, adds in influence of percent hatch and deformities.

Isolating the Influence of Timing of Egg Production: By combining survival with egg production and its timing in the above analysis, the assessment obscures the influence of timing: the issue that was the main reason for undertaking population modeling in the first place. The concern is whether selenium exposure could delay reproduction, thereby yielding reduced population growth. To help isolate the influence on the timing of egg production, two population model runs were performed where all treatments were assigned one of two daily survival rates (0.99 or 0.999) spanning the full range of daily survival rates observed in the 21 and 58 day survival calculations. That is, with survival held constant, the only factors varying across treatments were egg production and timing.

The results are shown in the table below. The Abbott-adjusted results are plotted in Figure 3. Although the relative differences in Figure 3 population growth rates are subdued compared to the wider variation in egg production, this is merely a consequence of the predicted population growth rate being more responsive to survival than to reproduction. It is still apparent that the variations in total egg production are affecting growth rate. The question to be addressed here is whether increasing selenium concentration yields a decline in growth rate beyond the pattern reflecting total egg production.

Population growth rates, as influenced only by differences in egg production and timing					
		With only egg production (*m*) and its timing variable across treatments			
		σ=0.999		**σ=0.99**	
Treat-ment	**Conc**	**λ**	**r**	**λ**	**r**
Control	1	1.0339	0.0334	1.0246	0.0243
Se-1	3	1.0338	0.0333	1.0245	0.0242
Se-2	4.4	1.0310	0.0306	1.0217	0.0215
Se-3	8	1.0293	0.0289	1.0201	0.0199
Se-4	13	1.0293	0.0288	1.0200	0.0198
Se-5	27	1.0324	0.0318	1.0231	0.0228

Inspection of Figure 3 indicates that when survival is assigned a constant value across treatments, the pattern of population growth differences across treatments does not suggest an additional selenium-accentuated factor depressing population growth rate. Population growth at 13 and 27 mg Se/kg is slightly higher than might be expected from total egg production, when compared to lower concentrations. The lack of influence of selenium exposure on timing of egg production is also illustrated by comparing each treatment's cumulative proportion of egg production over the course of the test, as shown in Figure 4. Although the treatments differ somewhat in the temporal pattern of their egg production, there is no consistent relationship with selenium exposure.

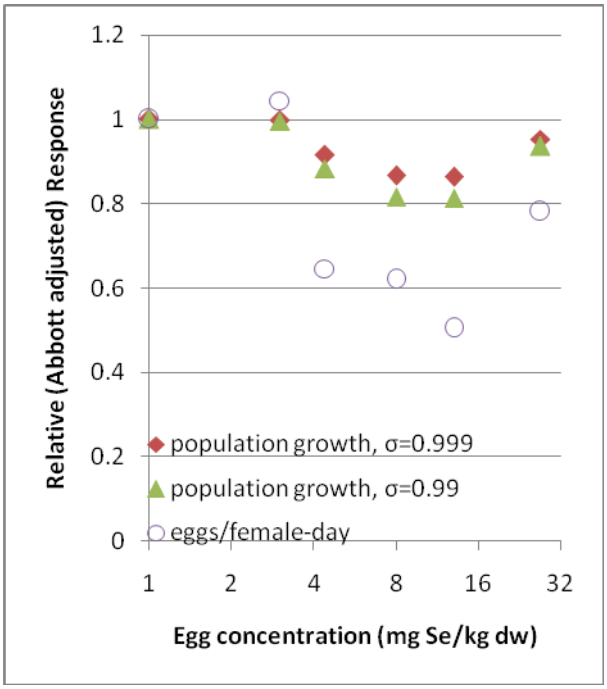


Figure 3. Predicted population growth rate calculated considering differences only in egg production and timing (having assigned uniform survival rates across treatments).

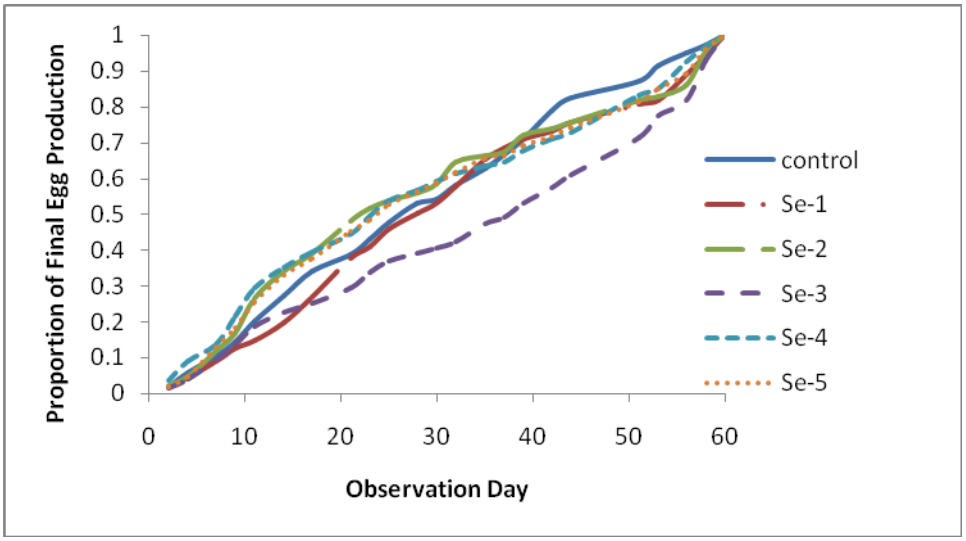


Figure 4. Cumulative pattern of egg production over time. (Control: continuous line. Se-1: dot, dot, long dash. Se-2: long dashes. Se-3: medium dashes. Se-4: short dashes. Se-5: dots.)

Chronic Value: In other selenium studies, egg production and percent hatch have not generally been thought to be related to selenium exposure. Although Besser et al. (2012) noted that repeated measures ANOVA indicated a potential interaction between selenium treatment and egg production on particular sampling dates, a thorough examination of the study data from multiple perspectives indicates no statistically significant or biologically apparent effect of selenium on egg production, timing of egg production, or percent hatch at or below the highest tested concentration of 27 mg Se/kg (dw). Likewise there was no discernible effect on deformity rates.

In the separate tests of F1 larval survival at 21 days and of F1 juvenile-adult survival at 58 days, the highest treatment, 27 mg Se/kg (dw), displayed lower survival than any other treatment. Although the reduction was not sufficient to be statistically significant, Besser et al. (2012) suggest that this is indicative of a threshold. Note that among toxicity tests in general, the 10% effect level of the EC_{10} might or might not be statistically significant from the perspective of hypothesis testing.

Shown below are the survival rates for the 27 mg Se/kg treatment adjusted to the control (Abbott-adjusted), or similarly adjusted to the average survival at all lower treatments (some of which had better survival than the controls). Either way the adjustment is done, results are similar. (These survival data, Abbott-adjusted, are included in Figure 2.)

27 mg Se/kg treatment:	**Larval Surv at 21 days**	**Juv-Adlt Surv at 58 days**
adjusted to control	92.9%	87.5%
adjusted to all lower treatments	89.1%	91.6%

The effect level at 27 mg Se/kg was thus 7% – 13% in the above comparisons. While the concentration response curve is not sufficiently defined to allow confident assignment of an EC_{10}, the data suggest a chronic value in the general neighborhood of 27 mg Se/kg.

An effect level of 27 mg Se/kg egg for the pupfish in this study is consistent with the findings of Saiki et al. (2012a) who evaluated selenium in two related species in the Salton Sea, California. These authors measured 3.09 to 30.4 mg/kg whole body Se levels in mosquitofish and sailfin mollies and based on a lack of a negative relationship with the catch-per-unit-effort deduced these species were not adversely affected by selenium. They extrapolated the finding of selenium tolerance to the pupfish based on the results of another study (Saiki et al 2012b) in which mosquitofish and sailfin mollies accumulated similar levels of selenium to the pupfish. Note: the ratio of selenium in whole body to egg tissues in the pupfish was approximately 1:1 in the Besser study (see first table in the pupfish study summary above).

Staub, B.P. W.A. Hopkins, J. Novak, J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Arch. Environ. Contamin. Toxicol. 46:96-101.

Test Organism: Eastern mosquitofish (*Gambusia holbrooki*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from a contaminated ash basin (ASH) and a reference pond (REF)

Study Design: In July 1999, male eastern mosquitofish were collected from ASH and REF (n=26, n=20, respectively) for measurement of standard metabolic rate (SMR). In July 1999, gravid female eastern mosquitofish were collected from ASH and REF and transported to a laboratory for testing. To ensure all females were fertilized in the field, all offspring used in testing were limited to three weeks after collection. (Eastern mosquitofish are live-bearers with a four week gestation period.) Response variables compared between ASH and REF were (1) SMR of males, (2) brood size of females, (3) percent of live offspring at parturition, and (4) trace element concentration in females and offspring.

Effects Data: SMRs of males, brood size of females, and offspring viability were not significantly different between sites. Average (n=5) concentrations of selenium in females were 11.85 and 0.61 mg/kg dw in ASH and REF sites respectively. The average concentrations of selenium in offspring were 15.87 mg/kg dw and below detection in ASH and REF sites, respectively. The authors point out that the selenium concentrations are an under-estimate of the field levels since the females were allowed to depurate during their time in the laboratory prior to parturition.

Chronic Value: >11.85 mg Se/kg dw whole body

Saiki, M.K., B.A. Martin, and T.M. May. 2004. Reproductive status of western mosquitofish inhabiting selenium-contaminated waters in the grassland water district, Merced County, California. Arch. Environ. Contamin. Toxicol. 47:363-369.

Test Organism: Western mosquitofish (*Gambusia affinis*)

Exposure Route: Waterborne and Dietary - field exposed
Fish were collected from selenium-contaminated sites and reference sites in the San Joaquin River watershed.

Study Design: Western mosquitofish were collected in June and July 2001 from San Luis Drain (SLD) at Gun Club Road (Se-contaminated site), North Mud Slough at Gun Club Road (MSN1; reference site); North Mud Slough at State Highway 140 (MSNs; Se-contaminated site); San Joaquin River at Lander Avenue (SJR; reference site). 20 gravid females from each site were held in the laboratory for two weeks to quantify live and dead births and to make other measurements. Only 17 females from SLD were collected. Live and dead fry were visually examined under low magnification with a binocular microscope for evidence of external abnormalities (teratogenic symptoms such as spinal curvature, missing or deformed fins, eyes and mouths and edema).

Effects Data: The percentage of live births was high at both Se-contaminated sites (96.6 to 99.9%) and reference sites (98.8 to 99.2%). There were no obvious anomalies (e.g., deformities, edema) observed during the study. The concentration of selenium in 4 postpartum females from the site with the highest selenium concentration, SLD, ranged from 13.0 to 17.5 mg Se/kg dw (geometric mean of the high and low is 15.1 mg Se/kg dw. The concentration of selenium of western mosquitofish collected at each site is in Table D-8.

Chronic Value: >15.1 mg Se/kg dw whole body

Table D-8. Selenium in whole body samples of western mosquitofish from study sites

Site	N	[Se], mg/kg dw
SLD	8	18.1
MSN2	24	9.31
MSN1	20	2.72
SJR	22	0.907

Coughlan, D.J. and J.S. Velte. 1989. Dietary toxicity of selenium-contaminated red shiners to striped bass. Trans. Am. Fish Soc. 118:400-408.

Test Organism: Striped bass (*Morone saxitilis*; adults from Lake Norman, NC, approximately 250 g each)

Exposure Route: dietary only
Treated fish were fed selenium contaminated red shiners (1 g) from Belews Lake, NC (9.6 mg Se/kg ww or 38.6 mg Se/kg dw based on a mean reported moisture content of 75.1 percent). Control fish were fed golden shiners from a local bait dealer (0.3 mg Se/kg ww or 1.3 mg Se/kg dw based on a mean reported moisture content of 76.3 percent).

Test Treatments: Test treatments were as described above. Two tanks contained treated fish (n = 20 fish total), and one tank of fish served as the control (n = 10 fish). Each tank received a continuous flow of soft well water (hardness and alkalinity approx. 30 mg/L as $CaCO_3$) throughout the exposure.

Test Duration: 80 days

Study Design: During the experiment, all striped bass (n = 10 per tank) were fed to satiation three times per day. Pre-weighed rations of live red shiners (treated fish) and golden shiners (controls) were added to the tanks and allowed 5 hours to feed. Uneaten prey was removed and weighed. Composite whole-body samples of each prey fish were collected at regular intervals throughout the study for whole-body tissue selenium analysis. The final selenium concentration in epaxial white muscle was determined for surviving striped bass at the end of the test. Moribund striped bass were sacrificed so as to obtain muscle tissue samples for selenium analysis. Samples of liver and trunk kidney of these and the surviving striped bass were dissected for observations of histopathology.

Effects Data: Striped bass fed selenium-laden red shiners exhibited changes in behavior (lethargy, reduced appetite), negligible weight gain, elevated selenium concentrations in muscle, histological damage, and death. Control fish ate and grew well, and behaved normally. Average selenium ingestion was between 60 and 140 Φg Se/fish per day until day 30. Appetite of the treated fish appeared to be significantly reduced beyond this point compared to the appetite of the control group. By day 78, all striped bass fed the Se-laden red shiners either had died or were moribund and sacrificed for analysis. The final selenium concentration in muscle of treated striped bass averaged from 3.5 (tank 1) and 4.0 (tank 2) mg/kg ww, or 16.2 and 18.5 mg/kg dw, respectively, assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species. The final selenium concentration in muscle of control striped bass fed uncontaminated golden shiners averaged 1.1 mg/kg ww, or 5.09 mg/kg dw (assuming 78.4 percent moisture content in muscle tissue; default May et al (2000) value for all species).

Chronic Value: The chronic value for percent survival of striped bass relative to final selenium in muscle tissue after being fed Se-laden red shiners is <16.2 mg/kg dw.
An EC_{20} value could not be calculated for this data set because the data did not meet the assumptions required for analysis.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1984. Roxboro Steam Electric Plant 1982 Environmental Monitoring Studies, Volume II, Hyco Reservoir Bioassay Studies. Environmental Technology Section. Carolina Power & Light Company.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* embryos and larvae)

Exposure Route: dietary and waterborne - field exposure
Native adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. No selenium values were given for Hyco Reservoir, total selenium was not detected in the control lake (<1 µg/L). A mean selenium for the ash pond effluent from a previous study was 53 µg/L (N=59; range 35-80 µg/L).

Study Design: All combinations of crosses between the Hyco and control fish were made using gametes from the collected fish. Fertilized eggs were exposed in egg cups to 0, 20 and 50 percent ash pond effluent under flow-through conditions. Percent hatch and swim-up successes were measured. Swim-up larvae were released to exposure tanks where there were fed zooplankton collected from Hyco and the control lake. Larvae were observed for 28 days at which time survival and weight were measured.

Effects Data: Survival to the swim-up stage was different between larvae from Hyco females fertilized with either male type and those larvae from control females fertilized with either male type. All crosses involving a Hyco female resulted in larvae exhibiting 100 percent mortality prior to reaching swim-up. Percent survival from hatch to 28 days for larvae from control females exposed to control water and fed control lake zooplankton was only 5 and 12 percent for the two replicates so no meaningful comparisons can be made to the different dilution exposures or diet exposure. The mean concentrations of selenium in the ovaries, female liver and female muscle were 49, 130, and 84 mg/kg dw, respectively.

Effect level: <49, <130 and <84 mg Se/kg dw in adult ovaries, liver and muscle, respectively

Chronic Value: <49.65 mg Se/kg dw in whole body using the muscle to whole body equation
<84 mg Se/kg dw maternal muscle
<49 mg Se/kg dw ovary

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; 30-day old larvae)

Exposure Route: Dietary and waterborne - field exposed adults
Juvenile bluegill from crosses with females in 0, 20 and 50 percent ash pond effluent were transferred to control water and fed zooplankton from either Hyco or the control lake. Selenium in Hyco and control zooplankton was 45 and 1.9 mg/kg dw, respectively. Duration was not given.

Study Design: Survival and observations on pathology and morphology were made in the two diet treatments.

Effects Data: Mortality in larvae fed control zooplankton was 23.7 percent, whereas mortality in larvae fed Hyco zooplankton was 97.3 percent. There were no differences in survival (for two diet treatments) in larvae that were raised for the 30 days prior to the test in different effluent concentrations (0, 20 50 percent). The average selenium concentrations in the larvae fed control and Hyco zooplankton were 1.9 and 24.7 mg/kg dw, respectively.

Effect level for larval survival: <24.7 mg Se/kg dw in larvae

Chronic Value: None recommended for larval tissue.

Bryson, W.T., W.R.Garrett, M.A. Mallin, K.A. MacPherson, W.E. Partin, and S.E. Woock. 1985a. Roxboro Steam Electric Plant Hyco Reservoir 1983 Bioassay Report. Environmental Services Section. Carolina Power & Light Company. September 1985.

28-day Embryo/Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; embryos and larvae)

Exposure Route: dietary and waterborne - field exposed
Resident adult bluegill were collected from Hyco Reservoir in Person County, North Carolina and from a nearby control lake (Roxboro City Lake). Hyco Reservoir is a cooling lake for Carolina Power & Light and receives the discharge from the ash storage pond. For embryo/larval study up to swim-up stage, control fish were collected from the unaffected portion of Hyco.

Study Design: Repeat of 1982 28-day Embryo/Larval Study. Three crosses between: Hyco female and Hyco male; control female with Hyco male; and control female with control male. Gametes were fertilized and maintained for the 28-day test in ash pond effluent dilutions of 0, 20 and 50 percent. Percent hatch, percent swim-up success and survival were measured to 28 days post hatch. Two treatments were replicated and fed zooplankton collected from Hyco-affected and Hyco-unaffected (control). Larvae were observed for 28 days at which time survival and weight were measured.

Embryo/Larval Study up to Swim-up Stage. Five crosses were made between fish collected from the affected and unaffected areas. Percent hatch, percent swim-up and survival were measured until swim-up (approximately 3-4 days after hatch).

Effects Data: 28-day Embryo/Larval Study. All larvae that hatched from eggs obtained from Hyco females died prior to completing swim-up (see table below).

Effect level (larval survival): <30, <33 and <59 mg Se/kg dw for adult female bluegill in ovaries, liver and muscle, respectively

Summary of 28-day embryo larval study										
% effluent	Parent source in cross M X F	% hatch	% swim-up	% survival, 28-days	Adult tissue, mg Se/kg dw					
					Gonad		Liver		Muscle	
					M	F	M	F	M	F
0	H X H	92	0	0	33	30	43	33	62	59
20	H X H	98	0	0	33	30	43	33	62	59
20	H X H	92	0	0	33	30	43	33	62	59
50	H X H	97	0	0	33	30	43	33	62	59
0	H X C	89	87	18	33	2.2	43	4.4	62	2.7
20	H X C	96	96	34	33	2.2	43	4.4	62	2.7
50	H X C	60	84	58	33	2.2	43	4.4	62	2.7
0	C X C	79	95	40	nd	2.2	37	4.4	27	2.7
20	C X C	90	96	36	nd	2.2	37	4.4	27	2.7
20	C X C	88	97	25	nd	2.2	37	4.4	27	2.7
50	C X C	72	92	42	nd	2.2	37	4.4	27	2.7

Chronic Value: <36.49 mg Se/kg dw in whole-body using the muscle to whole body equation.
<59 mg Se/kg dw muscle
<30 mg Se/kg dw ovary

Embryo/larval study to swim-up. Percent swim-up of larvae from parents collected in non-affected Hyco averaged 93 percent, whereas percent swim-up from larvae collected from affected Hyco was 12 percent. Effect levels were determined for adult female and larval tissues. Larval tissues were averaged across effluent concentrations (geometric mean).

Effect level (percent swim-up):
Adult female ovaries: >9.1 mg/kg dw; <30 mg/kg dw
Adult female liver: >26 mg/kg dw, <33 mg/kg dw
Adult female muscle: >25 mg/kg dw, <59 mg/kg dw
Larvae: >12.8 mg/kg dw; < 165 mg/kg dw

Summary of Embryo/Larval Study up to Swim-up - Affected vs Unaffected Hyco											
date of fert.	Parents' capture location in Hyco	Percent hatch			Percent swim-up			Selenium in tissue, mg/kg dw			
		at % effluent			at % effluent			Adult female			
		0	20	50	0	20	50	Ovary	Liver	Musc	Larvae
6-24	affected	93	98	94	0	0	0	30	33	59	0: 130 20: 120
6-27	affected	99	88	77	0	0	0	30	33	59	0: 130 20: 120
6-28	affected	29	34	35	25	14	3	30	33	59	0: 130 20: 120
6-28	affected	98	86	91	5	0	0	30	33	59	0: 130 20: 120
6-29	affected	88	93	85	59	42	25	30	33	59	0: 130 20: 120
7-14	unaffected	92	80	84	79	92	89	9.1	26	25	0: 19 20: 11 50: 10
7-26	unaffected	99	94	93	100	98	98	9.1	26	25	0: 19 20: 11 50: 10
7-27	unaffected	76	84	86	100	89	91	9.1	26	25	0: 19 20: 11 50: 10

Chronic Value: The chronic value estimated for the percentage larvae reaching the swim-up stage is presented as a range:
>25 mg Se/kg dw (unaffected area) and <59 mg Se/kg dw muscle (affected area)
>30 mg Se/kg dw (unaffected area) and <9.1 mg Se/kg dw ovary (affected area)

Bryson, W.T., K.A. MacPherson, M.A. Mallin, W.E. Partin, and S.E. Woock. 1985b. Roxboro Steam Electric Plant Hyco Reservoir 1984 Bioassay Report. Environmental Services Section. Carolina Power & Light Company

Ingestion Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile- hatchery raised)

Exposure Route: Dietary only

Test Treatments: 5 diets: Se form (nominal selenium concentration in base diet)
seleno-DL-cystine (5 mg/kg)
seleno-DL-cystine (10 mg/kg)
seleno-DL-methionine (5 mg/kg)
sodium selenite (5 mg/kg)
Hyco zooplankton (5 mg/kg)

Test Duration: 60 days

Study Design: Each treatment contained 40 fish which were maintained in a flow-through system. Fish were fed at 3 percent of their body weight. Length and weight were measured on days 30 and 60. Total selenium was measured in liver and whole-body.

Effects Data: No decreased length or weight in any of the Se-diets relative to the control.

Chronic Value: all values are whole-body
seleno-DL-cysteine: >2.16 mg Se/kg dw
seleno-DL-cysteine-2X: >3.74 mg Se/kg dw
seleno-DL-methionine: >2.46 mg Se/kg dw
sodium selenite : >1.21 mg Se/kg dw
Hyco zooplankton: >2.35 mg Se/kg dw

Because none of the selenium-spiked diet formulations affected growth of juvenile fish at the concentrations tested, the chronic value selected for this study is >3.74 mg Se/kg dw for the seleno-DL-cysteine-2X formulation.

Source and Exposure Embryo-Larval Study

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* Adults from Hyco and a control lake)

Exposure Route: Dietary and waterborne - field exposure

Test Treatments: Four treatments:
Hyco–collected fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.
Control fish exposed to Hyco water in flow through spawning tanks.
Hyco-collected fish in control water in flow through spawning tanks.

Test Duration: Adult fish were in spawning tanks 4-7 months

Study Design: Eggs from each treatment were observed for percent hatch and percent swim-up.

Effects Data: Fish collected from the control lake did not spawn. Percent hatch and percent swim-up from Hyco fish in Hyco and control water are given in the table below. The percent hatch and percent swim-up were >83 and >83 for all the Hyco fish suggesting no effect for these endpoints.

Source of parents	Se in parental liver tissue, mg/kg dw	Water type for eggs and larvae	N	Percent hatch	Percent swim-up
Hyco	18.6	Hyco	16	86.6	91.1
Hyco	18.6	well water	10	83.8	95.5
Control	13.8	Hyco	a	a	83.3
Control	13.8	well water	12	86.0	97.4

[a] percent hatch unknown.

Chronic Value: The chronic value for this study is >18.6 mg Se/kg dw liver tissue.

Gillespie, R.B. and P.C. Baumann. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans. Am. Fish. Soc. 115:208-213.

Test Organism: Bluegill sunfish, wild-caught (*Lepomis macrochirus*; adults; embryos and larvae)

Exposure Route: dietary and waterborne - field exposure

Test Treatments: High selenium adult fish were collected (electrofishing and with Fyke nets) from Hyco Reservoir. Low selenium adult fish were collected from Roxboro City Lake, Roxboro, NC.

Study Design: All possible combinations of bluegill parents from Hyco Reservoir and Roxboro City Lake were artificially crossed in June and July, 1982 and 1983, respectively. Fertilization success was assessed by stripping subsamples of 100 to 500 eggs per female and combining them with 2 ml of sperm. All zygotes were reared in Roxboro City Lake water and percent fertilization was estimated 2-3 hours later as the proportion of mitotically active zygotes. To estimate hatching success, gametes were combined as before and subsamples of 100 to 300 embryos per cross were transferred to egg cups and maintained in closed aquaria receiving re-circulated Roxboro City Lake water. Percent hatch (approx. 2d at 22 to 25^{o}C) was based on the number of yolk-sac larvae. In 1982, about 200 embryos from 8 crosses were observed and preserved at intervals up to 40 h after fertilization, and about 450 larvae were preserved at intervals of 40 to 180 h after fertilization. In 1983, about 1,800 larvae were observed and preserved from 40 to 150 hr from crosses involving females from Hyco Reservoir, and about 40-300 hr for crosses involving females from Roxboro City Lake (10 crosses total).

Effects Data: No significant differences were found in percent fertilization or in percent hatch among parent combinations from the 18 crosses made in June 1982 and July 1983. In contrast, larvae from all crosses involving a Hyco female were edematous; 100 percent of the larvae were abnormal in 7 of 8 crosses. Note: This outcome was observed when the same female from Hyco Reservoir was crossed with males from either Hyco Reservoir or Roxboro City Lake. The range of selenium concentrations in the ovaries of Hyco Reservoir females used for the cross experiments was from 5.79 to 8.00 (GM = 6.945 mg/kg ww; n=7). The reported concentrations of selenium in ovaries and carcasses of females collected from Hyco Reservoir in 1982 and 1983 were 6.96 and 5.91 mg/kg ww (n=22 and 28, respectively). The reported concentrations of selenium in ovaries and carcasses of females collected from Roxboro City Lake in 1982 and 1983 were 0.66 and 0.37 mg/kg ww (n=14 and 19, respectively). The mean selenium concentration in bluegill larvae (n=222) from artificial crosses of parents from Hyco Reservoir was 28.20 mg Se/kg dw.

Chronic Value: <46.30 mg Se/kg dw ovary using 85 percent moisture for ovaries measured in study.

Doroshov, S., J. Van Eenennaam, C. Alexander, E. Hallen, H. Bailey, K. Kroll, and C. Restrepo. 1992. Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River; Part II, Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (*Lepomis macrochirus*). Final Report to State Water Resources Control Board, State of California. Contract Number 7-197-250-0.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); Population A: selenium bioaccumulation observations used 113 g (range 30-220 g) obtained from Rainbow Ranch Fish Farm, California. Population B: spawning performance observations used 106 g (range 65-220 g) females and 164 g (range 80-289 g) males obtained from Chico Game Fish Farm.

Exposure Route: Dietary only
Dietary
Seleno-L-methionine added to trout chow; the three nominal dietary concentrations of 8, 18 and 28 mg/kg seleno-L-methionine were measured at 5.5, 13.9, and 21.4 mg/kg Se (moisture content 13 to 16%).

Test Duration: 140 days

Study Design: Population A fish and Population B females were fed nominal dietary treatments 8, 18 and 28 mg/kg seleno-L-methionine; Population B males were fed untreated diets until the start of spawning. Population A fish were sampled on days 0, 30, 58, 86 and 114 for Se measurement. At least 3 females were sampled each event. Fish remaining after day 114 were transferred to an outdoor pond fed untreated diet and sampled on day 144 for depuration analysis.

On day 120 Population B males and females were paired for natural spawning which had limited success. Fish were maintained in treatment tanks and females were monitored for egg ripeness. When ripe, females were induced to ovulate and ova were fertilized *in vitro* with semen stripped from males. Fertilized eggs were sampled for fertilization success, Se content, and two live sub-samples for bioassay, one a 30-day embryo-larval test and another for larval development during first 5 days after hatching.

Larval development: after hatching, 100 larvae were transferred to beakers and samples were examined daily for normal, abnormal and dead were recorded.

Larval bioassay: 90 fertilized eggs from each female were placed in groups of approximately 30 eggs. Larvae and fry were fed rotifers and brine shrimp nauplii through the 30 day observation.

Effects Data: Selenium concentrations in parental tissues for Populations A and B are given in Tables 1 and 2, respectively. Treatment effects were only observed on early development bioassays. In the 5-day larval bioassay, systemic edema and underdeveloped lower jaw were apparent in all larvae in the 28 mg/kg dietary treatment by day 3 and complete mortality by day 5, except for two progenies where 10% of the larvae appeared normal. No abnormalities were observed in control and 8 mg/kg treatment. 3 of the 6 progenies in the 18 mg/kg treatment exhibited 10 to 20% larvae with similar abnormalities (Table 3). The average proportion of larvae with edema were 5% in 18 and 95% in 28 mg/kg, both of

these were statistically different from the control (0% edema).

For analysis of the effect level determination, 4-day edema observations were used (Table 4) rather the 5-day data because the latter were difficult to interpret relative to edema because of almost complete mortality at the highest concentration (although the 4-day and 5-day edema observations were almost identical). Of the 33 edema measurements, only 15 could be used because not all the individual-replicate egg concentrations were reported. Table 4 also shows the treatment averages, which are only slightly different than the 5-day edema data. These averages do not match the average of the individual replicates in this table because they are for all the replicates, not just those with which concentrations could be paired.

The Se egg and edema data from Table 4 are plotted on Figure 1. The individual replicates are analyzed using TRAP. TRAP warns about inadequate partial responses because the partial responses are less than 10% or greater than 90%, and there are no data between 10 and 90%. However, for this dataset, these partial responses at both ends, albeit small, are sufficiently informative based on multiple lines of evidence (e.g., same response on both days 4 and 5, other endpoints that show effects at treatment 18, and several instances of edema at treatment 18 in contrast to absolutely none for many observations at any lower concentration). And because treatment 18 does have an effect of several percent or so, estimating the EC_{10} near these points is defensible; the EC_{10} is 22.6 mg/kg egg. The EC_{10} of 22.6 mg Se/kg egg dw was selected for the chronic value because it was determined using the individual replicates rather than treatment averages as was done in the previous draft document. The EC_{10} of 22.6 mg Se/kg egg is slightly higher than that in the previous draft which used means rather than replicate data (Figure 3).

In the 30-day larval survival bioassay, statistical difference was only in the highest test treatment for survival and growth measurements, length and weight (Table 5). The proportion of abnormal larvae was higher in the selenium-treated diets but was not significantly different from the control. The percent of abnormal larvae in the 18 mg/kg treatment (7.2%) was only slightly higher than the control (6.3%).

Authors present the effect level for bluegill at the 18 mg/kg dietary treatment (NOEC 8 mg/kg) based on proportions of edema and delayed resorption of the yolk sac. The latter endpoint is based on significantly greater yolk area and oil globule area in the 18 and 28 mg/kg treatments.

The most sensitive endpoint, percent edema, as a function of selenium in maternal muscle dw, was fitted to a TRAP tolerance distribution analysis using the individual replicates (Figure 2). The response is steep and the EC_{10} estimate is 15.7 mg/kg. This basically is setting the EC_{10} to the average of the two replicates with nominally 10% edema (15.4 and 16.6 mg/kg), with 90% edema occurring at only a slightly higher concentration (17.3 mg/kg).

Chronic Value: EC_{10} value (edema) at 22.6 mg Se/kg egg dw or 15.7 mg Se/kg muscle dw Chronic Value is 22.6 mg Se/kg eggs dw.

Table 1. Selenium Concentrations (mg/kg dw) in Bluegills from Population A Day 113 of				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Ovary	2.17 (0.05)	10.89 (1.83)	26.17 (0.07)	40.32 (2.44)
Female liver	2.51 (0.32)	NA	22.75 (2.96)	40.68 (2.14)
Testis	2.65 (0.21)	9.87	16.38 (0.71)	29.70 (5.02)
Male liver	4.10 (0.37)	14.32	24.28 (4.54)	52.47 (5.23)

Table 2. Selenium Concentrations (mg/kg dw) in Bluegill Parents (Population B) Used in Larval Toxicity Tests				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Male liver	4.07 (0.23)	6.94 (1.58)	20.46 (3.46)	31.63 (1.75)
Testis	1.87 (0.11)	3.64 (0.47)	9.96 (0.45)	15.25 (0.45)
Female liver	4.00 (0.26)	12.33 (1.09)	25.98 (4.28)	47.60 (4.11)
Female muscle	**1.47 (0.14)**	**5.80 (0.79)**	**10.41 (2.02)**	**23.64 (2.04)**
Ovary	2.23 (0.11)	6.34 (0.47)	14.10 (2.62)	30.63 (3.23)
Eggs	**2.81 (0.14)**	**8.33 (0.63)**	**19.46 (3.83)**	**38.39 (3.14)**
Larvae	NA	NA	NA	35.30 (4.16)
Fry	1.48 (0.11)	1.25 (0.02)	1.37 (0.06)	1.46 (0.03)

Table 3. 5-day Larval Development Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Free of Edema, %	100	100	95 (2)*	4.3(2.7)*

Table 4. 4-day Edema Observations by Replicate from 5-day Larval Toxicity Test			
Treatment/Replicate ID	**Se egg, mg/kg dw**	**Se muscle, mg/kg dw**	**Percent edema (n=10)**
08-2C	3.54	2.25	0
18-4C	3.25	0.95	0
8-1S	11.49	7.07	0
8-2S	8.31	5.80	0
8-6S	6.18	1.41	0
18-1S	8.55	2.75	0
18-3S	22.06	15.44	10
18-6S	30.20	16.58	10
28-1S	44.02	NA	100
28-2S	36.31	31.10	100
28-3S	25.21	17.28	90
28-4S	52.18	27.40	100
28-5S	42.40	24.00	100
28-6S	38.47	24.66	100
28-7S	30.12	17.42	90
Treatment	**Se egg, mg/kg dw treatment avg**		**Percent edema treatment avg**
C	2.81	--	0 (n=140)
8	8.33	--	0 (n=50)
18	19.5	--	6.67 (n=60)
28	38.4	--	97.1 (n=70)

Table 5. Results from 30-day Embryo-larval Toxicity Test, average (SD)				
Dietary treatment	**Control**	**8 mg/kg dw**	**18 mg/kg dw**	**28 mg/kg dw**
Larval survival, %	71 (8.5)	51.9 (26.5)	64.4 (3.4)	2.5 (3.5)*
Larval length, mm	19.1 (1.2)	19.9 (1.2)	19.3 (0.8)	16.6 (2.5)*
Larval weight, mg	114 (24)	133 (27)	119 (16)	81 (37)*
Abnormalities in larvae, %	6.3 (7.9)	15.0 (5.8)	7.2 (3.1)	25.0 (43.3)

* Statistically significantly different from control

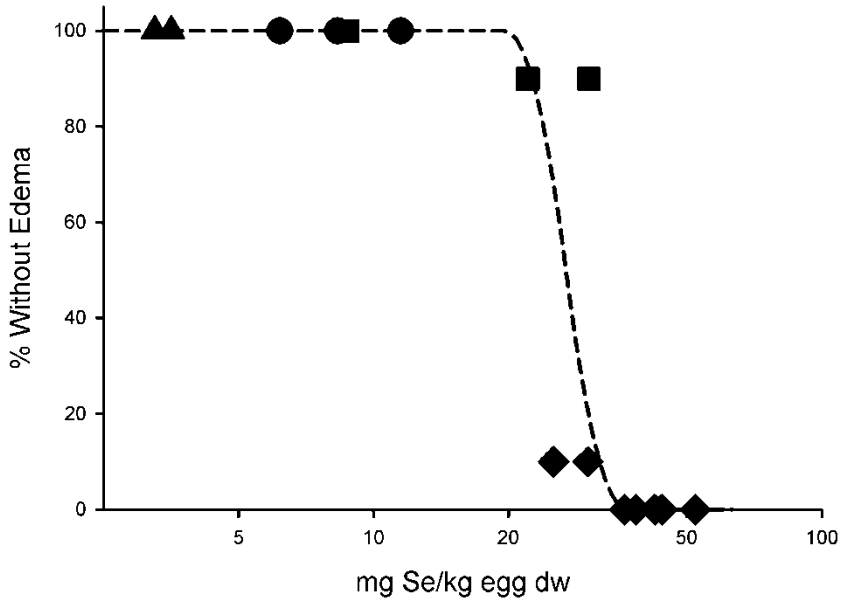


Figure 1. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance distribution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

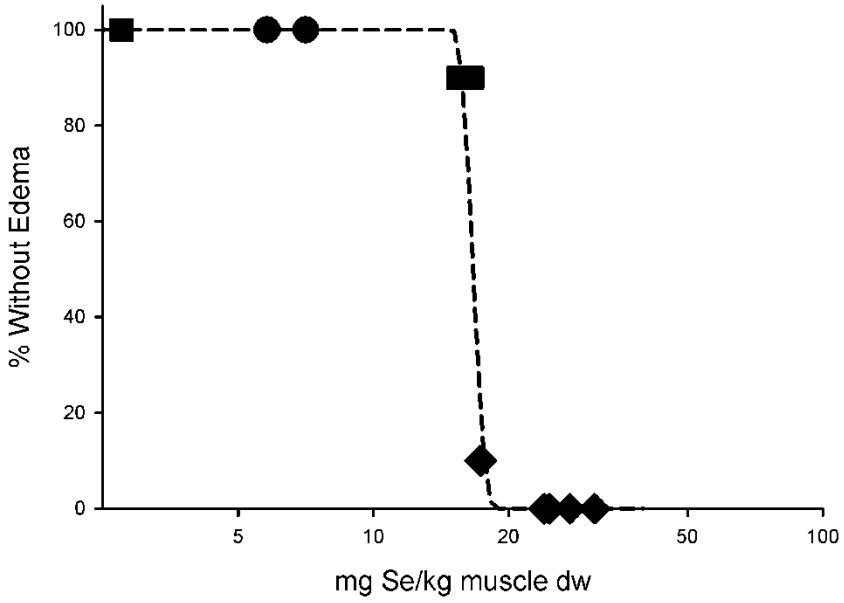


Figure 2. Bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in maternal muscle. Triangles denote control, circles treatment 8, squares treatment 18, diamonds treatment 28. The line denotes TRAP fits based on the individual replicates using the tolerance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

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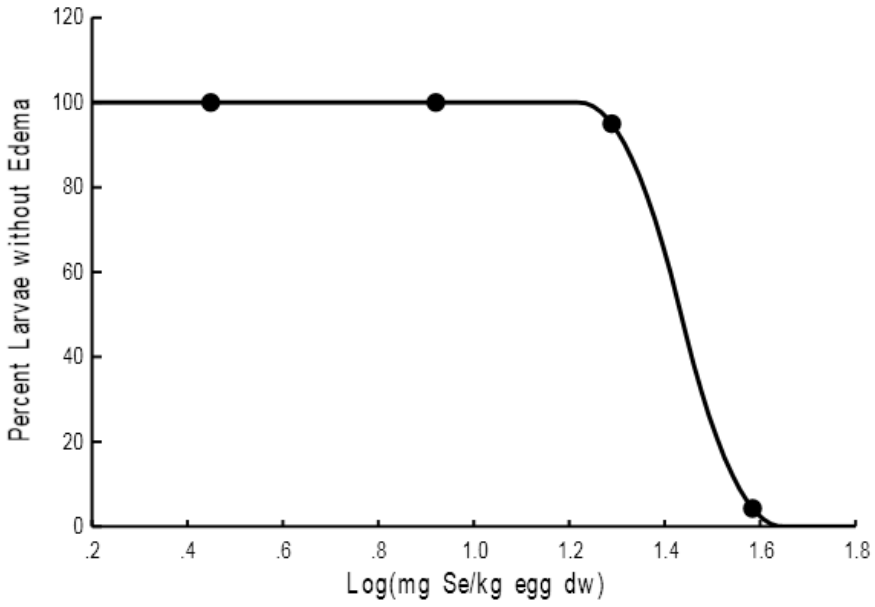

Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4428	1.4342	0.0000	1.4342	1.4342
S	5.452	4.712	0.000	4.712	4.712
Y0	98.33	100.00	0.00	100.00	100.00

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	27.18		
20.0	22.71		
10.0	20.75		
5.0	19.460		

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Figure 3. (From previous draft document) TRAP analysis of bluegill larvae without edema (percent) as a function of the logarithm of selenium concentrations in eggs.

Hermanutz et al. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ. Tox. & Chem. 11: 217-224

Hermanutz et al. 1996. Exposure of bluegill (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. EPA Report. Mid-Continent Ecology Division. Duluth, MN.

Tao, J., P. Kellar and W. Warren-Hicks. 1999. Statistical Analysis of Selenium Toxicity Data. Report submitted for U.S. EPA, Health and Ecological Criteria Div. The Cadmus Group.

Test Organism: Bluegill (*Lepomis macrochirus*; 3 to 4-year old adults)

Exposure Route: Dietary and waterborne followed by dietary only

Dietary and waterborne

Selenite was added to artificial streams which entered the food web; thus, fish were also exposed to selenium in the diet.

Dietary only

Recovering streams exposed bluegill to selenium in prey organisms. Selenite addition to water was ceased (selenium in water was below detection level).

Study Design: Eight Monticello artificial streams were used for three separate studies between 1987 and 1990.

Table 1. Study Design.

Stream	**Study I**	**Study II**	**Study III**
Dates **BG[a] put in station 0-2** **BG transferred to sta. 6 End of study**	**9-1-87** **5-16-88** **8-22-88**	**10-88** **5-89** **8-89**	**11-89** **5-90** **7-90**
1	Unused	Control	Control
2	Unused	2.5 µg/L	Recovering
3	10 µg/L	10 µg/L	Recovering
4	30 µg/L	Recovering	Recovering
5	Control	Control	Control
6	30 µg/L	Recovering	Recovering
7	Control	2.5 µg/L	Recovering
8	10 µg/L	10 µg/L	Recovering

[a] BG = Bluegill

The design of the three Hermanutz et al. studies is included in Table 1 and a schematic diagram of an artificial stream is provided below (Figure 1). For each study, a random sample of 22-50 adult bluegill were transferred from stations 0-2 (provided temperatures above 4°C during winter) to station 6 (most suitable for nests) during mid-May for spawning. Spawning activity was monitored in the streams. Embryo and larval observations were made *in situ* and in the laboratory from fertilized eggs taken from the streams and incubated in the lab.

Figure 1. Schematic Design of One of the Artificial Streams in the Monticello Study

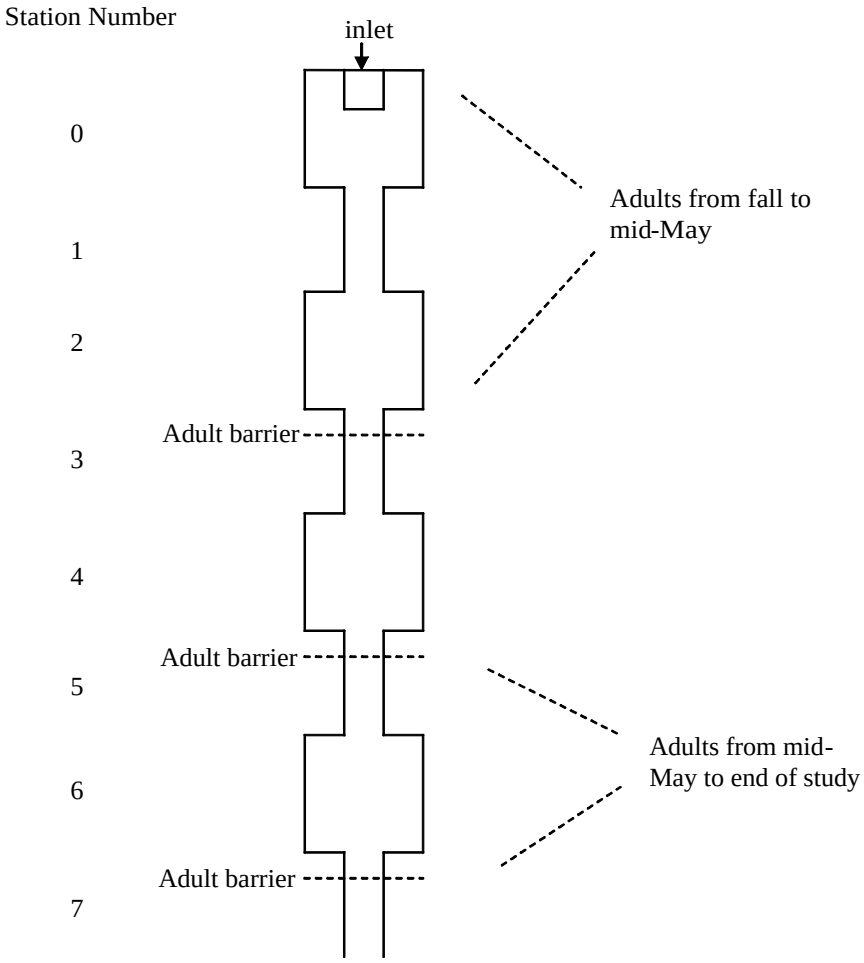


Table 2. Effects on Progeny - Study I[a]

Egg cup observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	% hatch mean ± SD	% survival to 4th day mean ± SD	% edema mean ± SD	% lordosis mean ± SD	% hemorr mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	93.3 ± 9.1	69.7 ± 13.9	0.1 ± 0.2	1.8 ± 2.6	0.1 ± 0.3
control	7	0.47	0.01	0.07	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	71.5 ± 22.5	28.8 ± 23.1	80 ± 1.0	11.6 ± 15.9	28.5 ± 40.6
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L	4	3.71	NA	3.71	15.46	15.46	60.3 ± 25.8	9.1 ± 12.9	50.3 ± 64.1	6.3 ± 1.8	26.8 ± 20.2

Nest observations											
treatment	stream	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[b]	Geomean ovary Se (mg/kg dw)	# active nests mean ± SD	# embryos Collected mean ± SD	% dead Embryos mean ± SD	# larvae Collected mean ± SD	% dead Larvae mean ± SD
		Early	Final	Geometric Mean							
control	5	NA	0.53	0.53	2.21	0.79	6.5 ± 2.1	1441 ± 205	0.9 ± 0.03	3947 ± 1888	3.0 ± 1.1
control	7	0.47	**NA**	**0.47**	0.29						
10 µg/L	3	4.29	2.53	3.29	13.73	17.71	5.0 ± 4.2	1282 ± 457	3.2 ± 2.9	1169 ± 1093	17.0 ± 21.3
10 µg/L	8	4.72	6.37	5.48	22.85						
30 µg/L [c]	4	3.71	NA	3.71	15.46	15.46	1.0 ± 1.4	361 ± 510	0.4	157 ± 222	12.1

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Hermanutz et al (1992).

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

[c] No active nests, embryos, or larvae found in one of the 30 µg/L streams. Therefore, N = 1 for % dead embryos and dead larvae in the 30 µg/L treatment

Table 3. Effects on Progeny - Study II[a]

Egg cup observations												
treatment	stream	No. of trials	% hatch	% survival to 3rd day	% edema	% lordosis	% hemorr	% healthy[b]	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
									Early	Final	Geometric Mean	
control	1	6	93.0	75.2	0	0	0	97.8	1.02	0.78	0.89	3.72
control	5	5	96.4	71.5	0	0	0	97.9	1.09	0.76	0.91	3.79
2.5 µg/L	2	0	NA	NA	NA	NA	NA	NA		1.82	1.82	7.58
2.5 µg/L	7	4	81.4	71.6	0	0	3.6	92.2	2.02	3.36	2.61	10.86
10 µg/L	3	3	83.3	57.7	100	11.1	49.3	0		8.1	8.10	33.75
10 µg/L	8	2	91.1	57.1	100	18.2	41.1	0	6.96	12.6	9.36	39.02
rec 30 µg/L	4	0	NA	NA	NA	NA	NA	NA				
rec 30 µg/L	6	6	92.9	73.0	17.4	0	11.5	70.7	5.87	13.2	8.80	36.68

Nest Observations														
Treatment	Stream	# active Nests	# embryos Collected	% dead embryos	# larvae collected	% dead larvae	#samples w larvae	% edema	% lordosis	% hemorr	ovary Se (mg/kg ww)			ovary Se (mg/kg dw)[c]
											Early	Final	Geometric Mean	
control	1	6	2458	0.94	3252	0.03	7	0	0	0	1.02	0.78	0.89	3.72
control	5	9	1329	0	3435	1.05	13	0	0	0	1.09	0.76	0.91	3.79
2.5 µg/L	2	1	0		2497	0.20	3	4.1	25	77.6		1.82	1.82	7.58
2.5 µg/L	7	5	1462	0	4717	0.08	8	0	0	52	2.02	3.36	2.61	10.86
10 µg/L	3	2	672	0	5376	0.50	9	81.4	5.0	55.5		8.1	8.10	33.75
10 µg/L	8	3	931	0.32	750	0.40	4	50	14.7	26.7	6.96	12.6	9.36	39.02
R 30 µg/L	4	0	NA	NA	NA	NA	NA	NA	NA	NA				
R 30 µg/L	6	8	646	0	6782	7.8	16	27.3	0	17.1	5.87	13.2	8.80	36.68

[a] Selenium concentrations in table were taken from Hermanutz et al. (1996); effect values were taken from Tao et al. (1999).

[b] Among live larvae that survived up to third day after first larvae hatched; assumes the observations of multiple abnormality types always co-occurred in the same organism. This may overestimate the actual % healthy when this assumption is violated.

[c] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Table 4. Effects on Progeny - Study III[a]

Egg cup observations									
treatment	**Stream**	**number of trials**	**% hatch**	**% survival to 3rd day**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	92	58.6	0	0	0	1.2	5.0
control	5	3	76.7	69.2	0	0.9	0.8	0.93	3.88
R 2.5 µg/L	2	3	87.3	66	0	0	0	1.84	7.67
R 2.5 µg/L	7	6	87.2	76.5	0	0	0	1.97	8.21
R 10 µg/L	3							6.25	26.04
R 10 µg/L	8	3	75.3	74.5	0	0	0	2.44	10.17
R 30 µg/L	4	5	92	78				3.82	15.92
R 30 µg/L	6								

Nest observations								
treatment	**stream**	**# active nests**	**# samples with larvae**	**% edema**	**% lordosis**	**% hemorr**	**ovary Se (mg/kg ww)**	**ovary Se (mg/kg dw)[b]**
control	1	2	5	0	0	0	1.2	5.0
control	5	2	3	0	0	0	0.93	3.88
R 2.5 µg/L	2	5	5	0	0	0	1.84	7.67
R 2.5 µg/L	7	5	2	0	0	0	1.97	8.21
R 10 µg/L	3	2	4	0	0	0	6.25	26.04
R 10 µg/L	8	4	4	0	0	0	2.44	10.17
R 30 µg/L	4	9	13	0	0	0	3.82	15.92
R 30 µg/L	6							

[a] The NOAEC for the study are from recovering 30 µg Se/L treatment.

[b] used 76% moisture for egg/ovary in bluegill (average of Gillespie and Bauman 1986 and Nakamoto and Hassler 1992) to convert egg/ovary ww to dw

R = recovering stream

Effects Data: Tables 2 through 4 include exposure and effects data for Study I, II, and III, respectively. Study I & II deformity and survival data reported in the tables above from the nest and egg in response to Se concentrations in parental ovaries (mg/kg dw) were compiled in Table 5 for TRAP analysis. Study I effects data were obtained from Hermanutz et al. (1992), and corresponding Study I ovary Se concentrations were obtained from Hermanutz et al. (1996). Study II and III exposure data were obtained from Hermanutz et al. (1996) and effects data from Tao et al. (1999).

In this study ovary concentrations were measured in an aliquot of females taken from each treatment. The exposure and effects data are thus not as directly linked as they would be in field studies of more recent design – where offspring health can be directly linked to measured tissue concentrations of their female parent.

In a change from the analyses published in drafts of this criterion document, ovary, muscle, and whole-body concentrations measured too early in the exposure period (that is, during the month of May, and labeled "early" in Tables 2 and 3) have not been used because they were not sufficiently co-occurrent with the effects measurements. On the other hand, the data for the Study II recovering stream and all Study III recovering streams *are* included in the analyses. For this analysis, the nest data continue not to be used, because they were less consistent than the egg-cup data.

EC_{10}s are based on the combined effects on survival and deformities: that is, reduction in the percentage of individuals surviving and normal. Table 5 shows the exposure and effects data used. Figures 2 and 3 show the ovary, and whole-body concentration-response curves and an explanation of how the EC_{10} values were derived. The same approach was used for the muscle data, that is, an interpolation using a nonlinear regression threshold sigmoid equation. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 11.2 mg/kg muscle and the average background survival/normal of 69.1% and the second point set to the LOEC of 21.0 mg/kg and a survival/normal of 5.8%. The resulting EC_{10} is 13.4 mg/kg muscle dw. The EC_{10} estimates for the three tissues (below) are slightly different than the EC_{10} values in the previous draft document. The reason for the difference is the use of the interpolation method in the current version rather than an inappropriate usage of a TRAP model in the previous document.

Chronic Value: This study's chronic values for bluegill based on percentage surviving and free of deformities are the following EC_{10} values:
Ovary: 14.7 mg Se/kg ovary dw
Muscle: 13.4 mg Se/kg muscle dw
Whole body: 10.6 mg Se/kg WB dw.

Table 5. Final Exposure Concentrations and Egg Cup Survival and Deformity Rates Used for TRAP Analysis (Studies I, II, & III). The percent deformity is the maximum percentage of the individual deformity types for each treatment.

		Tissue concentration at end of exposure (dw)			Effects data from Hermanutz et al. (1996) and Tao et al. (1999)		
Study	**Treatment (µg/L)**	**Se ovary (mg/kg)**	**Se muscle (mg/kg)**	**Se WB (mg/kg)**	**% Survival**	**% Deformity**	**%Normal +Surviving**
I	Control	2.21	2.05	1.546	69.7	1.8	68.4
I	10	16.73	21.03	18.131	28.8	80	5.76
I	30	>25[1]	No data	No data	9.1	50.3	4.52
II	Control	3.25	1.96	1.63	75.2	0	75.2
II	Control	3.17	2.61	1.47	71.5	0	71.5
II	2.5	7.58	6.73	5.40	No data	No data	No data
II	2.5	14	7.13	4.40	71.6	3.6	69
II	10	33.75	36.51	16.47	57.7	100	0
II	10	52.5	55.25	26.79	57.1	100	0
II	R-30	55	39.78	24.29	79	17.4	65.3
III	Control	5.0	3.37	1.27	62.9	0	62.9
III	Control	3.88	3.11	2.66	68	0	68
III	R-2.5	7.67	5.78	4.17	71.3	0	71.3
III	R-2.5	8.21	6.48	4.25	72.2	0	72.2
III	R-10	10.17	11.20	9.29	63.4	0	63.4
III	R-30	15.92	15.12	13.77	81.1	No data	No data

[1] No data were recorded for this treatment, but a value 50% higher than the 10 µg/L treatment was added for inclusion in the analysis.

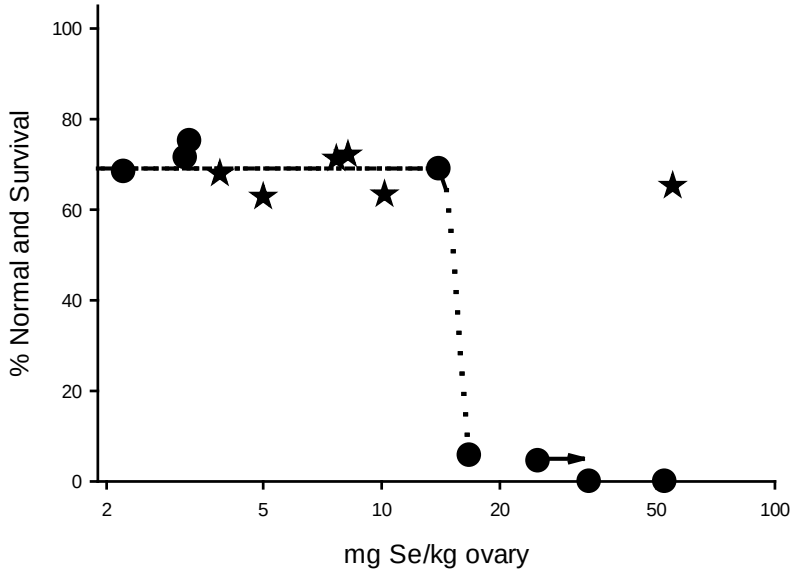


Figure 2. TRAP interpolation curve for the Table 5 ovary data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 14.0 mg/kg and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.7 mg/kg and a survival/normal of 5.76%. The resulting EC_{10} is 14.7 mg/kg ovary dw.

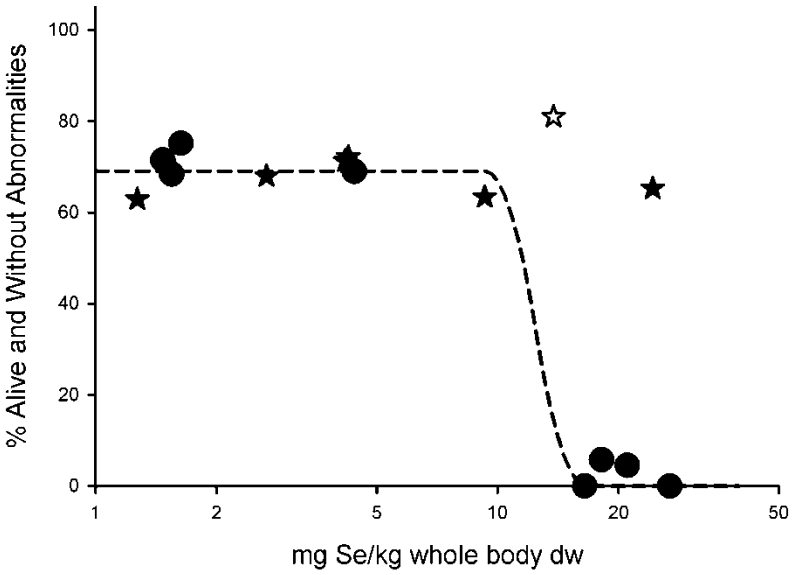


Figure 3. TRAP interpolation curve for the Table 5 whole body data. Circles denote active aqueous exposures and stars denote recovery periods. The interpolation is based on the threshold sigmoidal model, with the first interpolation point set to the HNOEC of 9.3 mg/kg whole body and the average background survival/normal of 69.1% and the second point set to the LOEC of 16.5 mg/kg and a survival/normal of 0%. The resulting EC_{10} is 10.6 mg Se/kg whole body dw.

Coyle, J.J., D.R. Buckler and C.G. Ingersoll. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). Environ. Toxicol. Chem. 12:551-565.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*; two-year old pond-reared adult fish and resultant fry)

Exposure Route: Dietary and waterborne
Dietary
Seleno-L-methionine added in an aqueous solution to Oregon moist pellets; moisture content of diet was 25 percent.
Waterborne
Flow through 10 µg Se/L nominal, 6:1 ratio of selenate:selenite, 98 percent purity, adjusted to pH 2 with HCl to prevent bacterial growth and change in oxidation states of Se(IV) and Se(VI).

Test Duration: 140 days

Study Design: The experiment consisted of a test control and food control (see Test Treatment table below) with fish (n=28 initially) in the four remaining treatments fed one of the four seleno-methionine diets in combination with 10 µg Se/L in water. Spawning frequency, fecundity, and percentage hatch were monitored during the last 80 days of the exposure period. Survival of resulting fry (n=20) was monitored for 30 days after hatch. Adults and fry were exposed in separate, modified proportional flow-through diluters. Fry were exposed to the same waterborne selenium concentrations as their parents. Adults were fed twice daily *ad libitum*. Whole-body selenium concentrations in adult fish were measured at days 0, 60, and were calculated from individually analyzed carcass and gonadal tissue (ovaries and testes) at day 140. Eggs not used in percentage of hatch determinations were frozen and analyzed for total selenium.

Measured Se in:	**Test Treatments**					
	1 (test control)	2 (food control)	3	4	5	6
water (µg Se/L)	0.56	8.4	10.5	10.5	10.1	11.0
diet (mg Se/kg dw)	0.76	0.76	4.63	8.45	16.8	33.3

Effects Data: There was no effect of the combination of highest dietary selenium concentration (33.3 mg/kg dw) in conjunction with exposure to a waterborne selenium concentration of 11.0 µg/L on adult growth (length and weight), condition factor, gonad weight, gonadal somatic index, or reproductive endpoints (i.e., spawning frequency, number of eggs per spawn, percentage hatch) during the 140-day exposure (Table 1). The mean corresponding whole-body selenium concentration in adults exposed to this waterborne and dietary selenium combination was 19 mg/kg dw. Survival of fry from the exposed adults was affected by 5 days post-hatch. Concentrations of whole-body selenium in adult tissue at day 60 were used to determine effects in the fry because eggs were taken for the larval tests beginning at day 60 of the adult exposure.

Table 1. Effects on Adults

Se in diet, mg/kg dw	Se in water, µg/L	whole-body Se (140 d), mg/kg dw	replicate	total no. spawns	eggs/spawn	hatchability, %
0.8	0.5	0.8	A	15	14,099	94.5
			B	10	5,961	90.5
0.8	7.9	1.0	A	12	9,267	89.5
			B	11	9,255	84.5
4.6	10.5	3.4	A	20	9,782	86.5
			B	12	13,032	96.5
8.4	10.5	6.0	A	2	10,614	96.5
			B	9	7,995	90
16.8	10.1	10	A	13	10,797	83
			B	13	9,147	91.5
33.3	10.1	19	A	14	8,850	80
			B	4	8,850	80

In the 30-d survival after hatch test, there was complete mortality after one week at the highest exposure and no significant differences in survival at lower concentrations. Table 2 provides the survival data at 5 days post hatch used in the analysis of the effect concentration. The day 5 data are given in Table 2 because this was the only day in which control survival was over 90%, with the control and all the treatments showing substantial and increasing toxicity over the next 4 days.

Because the survival in the fifth treatment was about 5% below the average of the lowest four and because the highest treatment still had some survivors, this

provided two partial effects for TRAP to fit a curve. However, the legitimacy of this depends on the lower survival in the fifth treatment actually being a significant Se effect, rather than reflecting random variation of background survival. Because there were multiple spawns with 200-500 total larvae tested for each survival value above, this might be expected to be a real effect, but there is insufficient data reported to test this. However, from day 6 through day 30, survival at the fifth treatment was above that in the first and third treatments, indicating this is not an effect level. These later data establish that the highest treatment is best considered an EC_{100} and the fifth treatment an EC_0. So an interpolation was done using 42 mg/kg as an EC_{100}, resulting in a slope of 7.6 and an EC_{10} of 26.3 mg/kg. The interpolation between the EC_0 and EC_{100} resulted in a slightly higher EC_{10} in the previous draft document (24.15 mg/kg) which used a TRAP model to estimate the EC_{10}. A figure is not provided here because this interpolation represents a synthesis of the data not tied to the data for a specific day.

As for the analysis with egg concentrations, the whole-body analysis recognizes the highest treatment as an EC_{100} (16 mg Se/kg dry wt whole body) and the second highest treatment as an EC_0 (7.2 mg Se/kg dry wt whole body). The interpolation method then results in an EC_{10} of 8.6 mg/kg. As for the egg concentration analysis, no plot is given because the EC_0 is not for a specific day or survival value.

Table 2. Survival of Larvae at Day 5 in the 30-day Post-hatch Test

Se in diet, mg/kg dw	Se in water, µg/L	egg, mg/kg dw	adult whole-body (60 d), mg/kg dw	mean survival, %
0.8	0.5	1.8	0.9	92
0.8	7.9	1.8	0.9	93
4.6	10.5	7.3	2.9	90
8.4	10.5	13	4.9	95
16.8	10.1	23	7.2	87
33.3	10.1	42	16	7

Chronic Value:

effect level	egg, mg Se/kg dw	whole body, mg Se/kg dw
EC_{10}	**26.3**	**8.6**

Cleveland, L. et al. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill sunfish (*Lepomis macrochirus*). Aquatic Toxicol. 27:265-280.

Test Organism: Bluegill sunfish (*Lepomis macrochirus*)

Life Stage: juvenile (5 months - waterborne exposure; 3 months - dietary exposure)

Exposure Route: waterborne (60-d) and dietary (90-d) - separate exposures
waterborne - 6:1 selenate:selenite at 0.17, 0.34, 0.68, 1.38, 2.73 mg/L; dietary - seleno-L-methionine in Oregon moist at 1.63, 3.25, 6.5, 13, 26 mg Se/kg dw)

Study Design: Fish were exposed using a flow-through diluter. Each test consisted of an exposure and a depuration phase. Whole body tissue measurements were made at 31 and 60 days of waterborne exposure and at 31, 59 and 90 days of dietary exposure. Mortality and condition factor, K (weight x 10^5/length^3), were reported at selected intervals.

Effects Data: The waterborne exposure (see table below) was determined to have an EC_{20} = 4.07 mg Se/kg dw (1.96-8.44 mg/kg 95% CL). However, because it was a water-only exposure, it was not considered in the derivation of the FCV. These data nevertheless provide evidence that exposure route influences the tissue concentration toxicity threshold, although the mechanistic explanation for this phenomenon is lacking.

A mortality effect level for the dietary exposure could not be calculated because the highest selenium whole body concentration (13.4 mg Se/kg dw) only had 17.5% mortality. The middle selenium concentration did have 22.5% mortality. Cleveland et al. reported a significant decrease in K between 4.7 and 7.7 mg/kg dw (see table below).

Waterborne Exposure Study

Measured selenium in water (:g/L)	**60-d measured selenium in whole body (mg/kg dw)**	**60-d mortality (%)**	**Condition factor (K)**
20 (control)	1.1	10	1.5
160	2.8	12.5	1.5
330	4	22.5	1.6
640	5.3	52.5	1.5
1120	9.8	70	1.6
2800	14.7*	97.5	NA

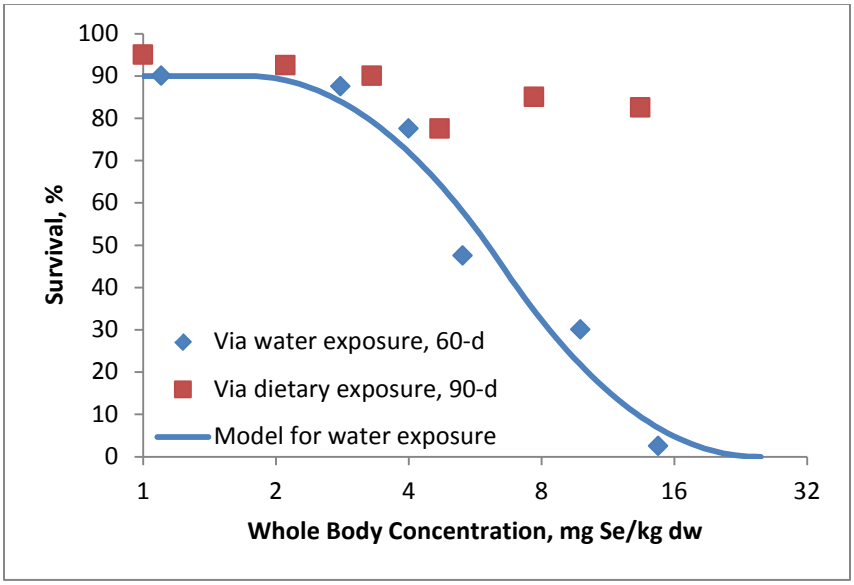
*[a] 30-d measurement because all fish were dead at 60 days in this concentration.

Dietary Exposure Study

Measured selenium in food (mg/kg ww)	90-d measured selenium in whole body (mg/kg dw)	90-d mortality (%)	Condition factor (K)
0.68 (control)	1	5	1.3
2.3	2.1	7.5	1.3
3.5	3.3	10	1.3
6.6	4.7	22.5	1.3
12.7	7.7	15	1.2
25	13.4	17.5	1.2

Discussion

The study demonstrates the influence of exposure route on the potency of a given tissue concentration, as shown in the figure. The TRAP threshold sigmoid concentration-response curve for the water-only exposure yields an EC50 of 6.5 mg Se/kg dw WB. In contrast, higher whole-body concentrations acquired via diet did not yield significant effects and cannot support a TRAP-fitted concentration-response curve or EC estimate. Examination of the graph indicates that the water-only concentration-response curve would need to be shifted to the right a minimum of 4-fold (or possibly more) to be able to fit the (lack of) effects observed in the dietary study. This supports the decision to derive the criteria only from studies relying on the environmentally relevant exposure route, diet.



Survival at 60-days (for water exposure) or 90-days (for dietary exposure) versus whole-body concentration.

Chronic Value: Given (a) the very slight reduction in K (1.3 to 1.2 between 4.7 and 7.7 mg Se/kg dw WB, with no further reduction at 13.4 mg Se/kg dw WB) and uncertain relevance of growth data, and (b) no apparent concentration-related effect on mortality between 4.7 and 13.4 mg Se/kg dw WB, the NOAEC is interpreted to be 13.4 mg Se/kg dw for this study; and the chronic value is >13.4 mg Se/kg dw whole body.

Lemly, A.D. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicol. 27:133-158.

Test Organism: Bluegill sunfish (*Lepomis macrochirus;* juvenile 50-70 mm)

Exposure Route: Waterborne and dietary
Water
1:1 selenite:selenate in stock at pH 2; metered in to reach 5 :g/L
Diet
seleno-L-methionine in TetraMin (5 mg/kg dw)

Test Duration: 180 days

Study Design: Fish were exposed (treatment and control) under intermittent flow-through conditions for 180 days. Tests were run at 4° and 20°C with biological (histological, hematological, metabolic and survival) and selenium measurements made at 0, 60, 120 and 180 days. Fish were fed at a rate of 3% body weight per day. All treatments were initiated at 20°C and then decreased in the cold treatment at a rate of 2°C per week for 8 weeks to reach 4°C and then maintained at that temperature for the remainder of the 180 days.

Effects Data : In the 20°C test, fish accumulated 6 mg/kg dw selenium (whole-body) with no significant effect on survival (4.3% and 7.4% mortality in control and treatment, respectively). In the 4°C test, fish exposed to selenium accumulated 7.9 mg/kg dw (whole-body) selenium and had significant mortality after 120 (33.6%) and 180 days (40.4%) relative to control (3.9%). Several hematological measurements were significantly different in both the warm and cold selenium exposures relative to controls. Both warm and cold selenium treatments also had greater O_2 consumption than controls. Fish lipid content in the cold Se treatment decreased more than the cold control; lipid content did not decrease in either the warm control or the warm Se treatment (see summary tables below). The results suggest significant mortality occurs in juvenile bluegill during winter months when tissue concentrations reach 7.91 mg/kg dw and lipid levels decrease to 6 percent.

Chronic Value: 20°C, >6 mg Se/kg whole-body; 4°C, <7.91 mg Se/kg dw whole body

Comments: See “Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies” in this appendix after presentation of the McIntyre et al. (2008) study.

Mean Concentration of Selenium in Tissues, Cumulative Survival*, Percent Lipid Content and Oxygen Consumption in Juvenile Bluegill

day	cold - Se control				cold + Se				warm - Se control				warm + Se			
	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]	Se[a]	Surv. %	lipid, %	O_2[b]
0	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98	1	100	13.2	98
60	1	97.1	12.5	58	5.8	92.9	10	63	1.2	95.7	13.3	98	5.8	100	13.3	103
120	1.1	97.1	11.5	57	7.9	66.4	6	81	1.1	95.7	13.4	100	6	96.7	13.4	120
180	1.4	97.1	10.5	57	7.9	59.6	6	78	1.2	95.7	13.6	100	6	92.6	13.5	120

[a] whole body Se tissue concentration, mg/kg dw
[b] oxygen consumption, mg/kg/hr

* Cumulative Survival: In this experiment, 240 juvenile bluegill were placed in three 400-L fiberglass tanks, 80 in each, and exposed to each control and treatment for a period of 180 days. Ten fish were removed at random from each treatment replicate on days 0, 60, 120, and 180 for selenium, histological, hematological, and metabolic measurements.

Replicate and Average Whole-body concentrations (mg/kg dry weight) of selenium in juvenile bluegill*

	day 0				day 60				day 120				day 180			
replicate	1	2	3	mean	1	2	3	mean	1	2	3	mean	1	2	3	mean
c+Se	0.87	1.21	0.95	1.01	6.30	5.49	5.76	5.85	8.36	7.31	7.85	7.84	7.53	8.01	8.19	7.91
w+Se	1.17	0.96	0.90	1.01	5.61	6.19	5.43	5.74	6.37	5.92	5.50	5.93	5.48	5.72	6.02	5.74
c-Se	0.89			0.89	0.97			0.97	1.01			1.01	1.10			1.10
w-Se	0.99			0.99	1.12			1.12	0.99			0.99	0.96			0.96

* Each value is for a composite sample made from 5 fish.

The Kaplan-Meier estimator was used to calculate survival at time *t*

$$\hat{S}(t) = \frac{\prod r(t_i) - d_i}{r(t_i)}$$

where $r(t_i)$ is the number of fish alive just before time t_i, i.e. the number at risk, and d_i is the number of deaths in the interval $I_i = [t_i, t_{i+1}]$. The 95% confidence interval for such estimate (Venables and Ripley 2002) was computed as

$$\exp\left\{-\hat{H}(t)\exp\left[\pm k_\alpha \frac{\text{s.e.}\left(\hat{H}(t)\right)}{\hat{H}(t)}\right]\right\}$$

where

$$\hat{H}(t) = \sum \frac{d_j}{r(t_j)} \qquad \text{and } j \# i$$

The following table lists the estimates of survival in the cold + Se treatment at 60, 120 and 180 days. The term n.event is the number of deaths at a given interval; n.risk is the number of organisms alive at the beginning of the interval; survival is computed by the Kaplan-Meier estimator.

Time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
60	210	15	0.929	0.0178	0.884	0.956
120	165	47	0.664	0.0350	0.590	0.728
180	88	9	0.596	0.0381	0.517	0.666

Hematological Measurements in Juvenile Bluegill Sunfish (*indicates significantly different from control)

Warm Exposure	day 0		day 60		day 120		day 180	
blood parameter	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se	warm-Se	warm+Se
total erythrocyte, 10^6/ml	2.95	2.92	2.96	2.93	2.99	2.95	2.96	2.89
% mature	85	86	86	93*	86	94*	85	94*
nuclear shadows, 10^4/ml	0.95	0.86	0.97	2.05*	0.83	2.38*	0.91	2.30*
total leucocytes, 10^4/ml	17.22	17.41	16.90	17.55	16.73	17.62	17.05	17.36
% lymphocytes	23	25	20	23	19	26	21	22
% neutrophils	15	13	14	15	17	19	17	16
hematocrit, %	37	36	37	29*	36	29*	38	28*
MCHC (mean corpuscular hemoglobin conc.)	23	25	25	19*	25	18*	25	17*
Cold Exposure	day 0		day 60		day 120		day 180	
blood parameter	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se	cold-Se	cold+Se
total erythrocyte, 10^6/ml	2.91	2.93	2.97	2.90	3.01	2.95	3.00	2.99

% mature	84	82	87	95*	85	96*	85	97*
nuclear shadows, 10^4/ml	0.86	0.84	0.83	2.30*	0.89	2.49*	0.90	2.36
total leucocytes, 10^4/ml	16.48	16.88	16.79	16.91	16.80	16.74	16.96	16.63
% lymphocytes	17	16	16	17	19	15	19	18
% neutrophils	13	12	15	11	15	12	12	14
hematocrit, %	39	37	40	30*	41	28*	39	27*
MCHC (mean corpuscular hemoglobin conc.)	26	25	25	18*	22	17*	23	17*
MCV (mean corpuscular volume)	182	171	188	146*	180	135*	185	130*

McIntyre et al. 2008. Effect of Selenium on Juvenile Bluegill Sunfish at Reduced Temperatures. US EPA, Health and Ecological Criteria Division. EPA-822-R-08-020

Test Organism: Bluegill sunfish (*Lepomis macrochirus*); juvenile; average length 47 mm, average weight 1 g

Exposure Route: Waterborne and dietary

Water

1:1 selenite:selenate; For exposure systems (ES) 1 and 3, fish were exposed to a control and a series of 6 nominal concentrations, 1.25, 2.5, 5, 10, 20 and 40 µg Se/L. For ES2, fish were exposed to a control and one nominal concentration, 5 µg Se/L.

Diet

For ES1 and ES3, fish were fed a series of six concentrations of selenium and a background control in *Lumbriculus variegatus*. The measured selenium concentrations in the *L. variegatus* treatments in ES1 were: 2.3 (control), 4.5, 5.3, 7.5, 14.2, 25.7 and 34.9 mg Se/kg dw; in ES3: 2.2 (control), 4.2, 5.0, 7.2, 15.2, 25.4 and 46.7 mg Se/kg dw. Fish were fed worms at a rate of 4% of the current biomass in each fish tank. Selenium was accumulated in *L. variegatus* by feeding the worms in separate tanks a series of six concentrations of selenized-yeast diluted with nutritional yeast: 1.7, 3.3, 6.7, 13.3, 26.7 and 53.5 mg Se/kg dw. Control worms were fed nutritional yeast only. Each tank was additionally exposed to the associated aqueous concentration selenium, e.g., the worms fed the 1.7 mg Se/kg dw selenized yeast were exposed to 1.25 :g Se/L, the worms fed the 3.3 mg Se/kg dw selenized yeast were exposed to 2.5 :g Se/L, and so on. For ES2, fish were fed TetraMin spiked with seleno-L-methionine at a nominal concentration of 5 mg/kg dw and at a rate of 3% of the current biomass in each tank.

Test Duration: 182 days

Study Design: Juvenile bluegill were exposed concurrently to selenium using three separate exposure systems, ES1, ES2 and ES3. In ES1 and ES3, 100 fish were exposed to each of 6 selenium treatments (low through high treatments are referred to as Treatments 1 through 6) and two controls in 200 L carboys under flow-through conditions. Each treatment consisted of an aqueous selenium concentration and an associated dietary selenium concentration, e.g., the fish in the lowest ES1 treatment were exposed to 1.25 :g Se/L and fed worms containing 4.5 mg Se/kg dw (see Exposure Route for other treatment concentrations). Temperature was controlled in each system through the immersion of the carboys in a temperature-controlled water bath and by controlling the temperature of the dilution water being added to the carboys. The temperature in ES1 was maintained at 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The only difference between ES1 and ES3 was temperature was decreased 2°C/week until it reached 9°C (test day 65) at which point temperature was maintained until test termination (test day 182).

The exposure of ES2 was similar to ES1 and ES3 in that 100 juvenile bluegill were exposed to treatment in 200 L carboys under flow-through conditions. The

ES2 selenium treatment consisted of two replicates of 5 µg Se/L waterborne and 5 mg Se/kg dw diet (Tetramin). Two controls were maintained with ES2. The temperature regime for ES2 was identical to ES1.

Observations on fish behavior and mortality were checked daily. Total selenium was measured in each fish tank weekly and selenium speciation was measured monthly in each fish tank. Whole body total selenium was measured in the worms from each tank (2 replicate 5 g samples) on test days 0, 30, 60, 112 and 182 and in the bluegill from each tank (3 replicates of 3-fish composites - total 9 fish) on test days 0, 7, 30, 60, 112 and 182. The standard length and weight of each fish was measured on each sample day. Lipid content was measured in fish at day 0 and from each treatment at test termination.

Effects Data:

Selenium increased in bluegill as the exposure concentrations increased (see following table). No meaningful mortality was observed in ES2. The number of fish that died in ES2 during the 182 day test was two fish in one treatment replicate and none in the other treatment replicate; no deaths were reported in ES2 controls. Significant mortality of juvenile bluegill was observed in ES1 and ES3. After 182 days, a total of 24 and 68 fish died in Treatments 5 and 6, respectively in ES1; and a total of 38 and 61 fish died in Treatments 5 and 6, respectively in ES3. See table below for mortalities in all treatments. Estimates of bluegill survival were adjusted for the removal of individuals from the test population. Individuals were removed from the experiments before test completion, for sampling tissue concentrations or because they suffered accidental deaths unrelated to selenium toxicity. For such data, it was necessary to account for the reduction in number of individuals at risk of death due to selenium over time. If $r(t_i)$ is the number of individuals at risk just before time t_i and d_i is the number of deaths in the interval, $I_i = [t_i, t_{i+1})$, then survival (S) at time t can be estimated as

$$\hat{S}(t) = \prod \frac{r(t_i) - d_i}{r(t_i)}$$

The product (P) was calculated for each period in which one or more deaths occur. The equation is the Kaplan-Meier estimator (Venables and Ripley 2002). This correction was applied to calculate the proportion of survival in treatments with ten or more deaths (10% mortality). The table below provides the adjusted proportion and surviving bluegill in each treatment along with the concentration of selenium in bluegill at test termination. The values in this table were used to calculate the EC_{20} and EC_{10} values using the TEAM software. Growth and lipid content of the bluegill was not negatively affected by the selenium exposures.

Measured total selenium concentrations in bluegill sunfish for all treatments and controls in Exposure System 1, 2 and 3.

Total Selenium in Whole Body Bluegill Tissue, mg/kg dw

ES1	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.43 (0.31)	2.48 (0.11)	2.43 (0.18)	2.64 (0.06)	2.72 (0.07)	3.27 (0.27)	4.27 (0.44)
30	2.10 (0.21)	2.85 (0.10)	3.10 (0.04)	2.94 (0.13)	4.24 (0.22)	6.62 (0.23)	10.21 (0.36)
60	2.11 (0.02)	2.70 (0.20)	3.07 (0.05)	3.69 (0.25)	5.21 (0.30)	8.62 (0.45)	12.66 (0.45)
112	1.98 (0.04)	3.16 (0.11)	3.41 (0.08)	3.99 (0.26)	6.42 (0.05)	11.60 (0.43)	
182	2.08 (0.10)	2.56 (0.21)	3.15 (0.25)	4.02 (0.21)	6.72 (0.09)	10.71 (0.55)	

ES3	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Test Day	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.50 (0.10)	2.60 (0.29)	2.38 (0.10)	2.82 (0.20)	3.19 (0.33)	4.29 (0.20)	6.13 (0.62)
30	2.24 (0.41)	2.44 (0.26)	2.70 (0.16)	3.13 (0.10)	3.95 (0.16)	6.06 (0.36)	11.07 (0.92)
60	2.70 (0.22)	2.88 (0.08)	3.04 (0.39)	3.79 (0.24)	5.54 (0.21)	9.50 (0.91)	15.14 (0.96)
112	2.16 (0.14)	2.49 (0.10)	3.10 (0.12)	3.64 (0.16)	6.54 (0.21)	11.50 (0.25)	17.24 (0.30)
182	1.67 (0.21)	3.20 (0.27)	3.83 (0.47)	5.48 (0.24)	9.38 (0.63)	16.01 (0.30)	

ES2	Control	5A	5B
Test Day	Average (SD)	Average (SD)	Average (SD)
0	1.93 (0.21)	1.93 (0.21)	1.93 (0.21)
7	2.19 (0.19)	3.55 (0.25)	3.08 (0.50)
30	2.49 (0.15)	7.05 (0.76)	7.51 (1.18)
60	1.53 (0.03)	8.23 (1.55)	8.09 (0.67)
112	1.57 (0.01)	8.97 (1.28)	9.45 (1.73)
182	1.38 (0.06)	9.41 (1.63)	10.61 (0.38)

Total number of deaths in ES1 and ES3 Treatments throughout the experiment's duration (182 days). Both ES1 and ES3 had two control tanks.

Treatment	ES1	ES3
Control (#1, #2)	0, 7	1, 1
1	5	0
2	1	1
3	0	0
4	3	3
5	24	38
6	68	61

The concentration of selenium in bluegill and the adjusted proportion of surviving fish at the end of the 182 day exposure.

	ES1		ES3	
Treatment	$[Se]_{tissue}$, mg/kg dw	surv	$[Se]_{tissue}$, mg/kg dw	surv
control	2.08	0.962	1.67	0.988
1	2.56	0.988	3.20	1.000
2	3.15	0.984	3.83	0.988
3	4.02	1.000	5.48	1.000
4	6.72	0.962	9.38	0.960
5	10.71	0.497	16.01	0.435
6	12.66	0.075	17.24	0.168

Chronic Value: The NOAEC for bluegill in ES2 was calculated as the geometric mean of the concentration of bluegill in the two replicates at the end of the exposure period, 9.992 mg Se/kg dw whole body. The chronic value for ES2 is therefore >9.992 mg Se/kg dw whole body. The EC_{20} and EC_{10} values for ES1 and ES3 are given in the following table.

	ES1 (4°C)	ES3 (9°C)
	Whole body	Whole body
EC_{20} mg Se/kg dw	9.78	14.64
EC_{10} mg Se/kg dw	9.27	14.00

Comparison of the Cold-Temperature Bluegill Juvenile-Survival Studies of Lemly (1993a) and McIntyre et al. (2008)

The Lemly (1993a) and McIntyre et al. (2008) cold-temperature juvenile bluegill studies are summarized on the previous pages. This discussion compares and contrasts these studies.

Both studies indicated that juvenile bluegill are more sensitive to selenium at lower temperature than at higher temperature. For a 4°C temperature regime, the EC_{10} of 9.27 mg Se/kg dw WB obtained with McIntyre's selenized yeast-worm-fish dietary bioaccumulation system is somewhat similar to the threshold of 5.85 mg Se/kg dw WB estimated from the time course of bioaccumulation and mortality in Lemly's single treatment with seleno-L-methionine in TetraMin. These chronic values differ by a factor of 1.58.

The difference in diet does not appear to explain the modest difference in results; however, since McIntyre's other 4°C experiment (Exposure System ES2), which used Lemly's seleno-L-methionine in TetraMin diet, experienced no significant toxicity, whereas Lemly's similarly exposed fish experienced 40 percent mortality by the end of the test. In addition to the difference in observed mortalities, Lemly's bluegill in the 4°C selenium exposure decreased in both lipid content and body condition over the 180 days whereas no decreases in these measurements were observed in the McIntyre et al. study, although the fish used in both studies were of comparable size and body condition at test initiation: 47 mm average standard length (range 44 to 54 mm) and a body condition index (100 x fish weight/standard length) of 3.2 in ES2 compared to 50 to 70 mm total length and a body condition factor of 3.9 in Lemly.

There are several possible reasons why such results could differ between studies. (1) ES2 maintained exposure at 20°C for the first 30 days of exposure before decreasing the temperature compared to 7 days in the Lemly study. (2) Lemly measured O_2 consumption by removing and reintroducing test fish to the test tanks, which was not done by McIntyre et al. (3) The two studies differed in photoperiod – Lemly "began with a 16:10 h light/dark photoperiod which was gradually reversed to 10:16" (sic) whereas McIntyre et al. used a fixed photoperiod of 16:8. (4) Some genetic differences between the tested batches of organisms may be expected, reflecting different origins, despite the similarities in their starting size and condition.

The modification to maintain 20°C for 30 days was to allow a longer period of time for the fish to accumulate selenium during a warmer condition prior to decreasing the temperature. This did result in shortening the exposure in ES2 at 4°C by 19 days (103 days at 4°C) compared to 122 days at 4°C in Lemly's study. However, as the majority of deaths in Lemly's study occurred between in the middle 60 days of the 180-day test, the slightly shorter cold period in the McIntyre study would not explain the differences in mortalities.

As stated above, Lemly removed fish (N = 15) from each treatment for oxygen consumption measurement and then returned these fish to the exposure tanks. There is the possibility that the fish removed from the cold plus selenium treatment were sufficiently stressed by the exposure conditions that the additional handling stress contributed to the mortality observed in this treatment. Between test days 60 and 180, 56 fish died Lemly's cold plus selenium treatment. Even if stress due to handling affected all the fish used in the oxygen consumption measurements (up to 30 fish), it does not explain all the mortality that was observed and therefore does not explain the difference between the two studies.

Both Lemly (1993) and McIntyre et al. (2008) showed reduced survival of juvenile bluegill exposed to elevated selenium under lab-simulated winter conditions, albeit at somewhat different concentrations. But only Lemly, not McIntyre et al., found the decreased survival to be accompanied by loss of lipid and body

condition. It was hypothesized that the decrease in EC_{10} observed by Lemly (1993) in the cold water treatment between 60-180 days was attributed to "winter stress syndrome" (WSS). WSS is hypothesized to occur in warmwater fish species because the presence of a stressor places additional metabolic costs on exposed organisms. These stresses can be better tolerated during periods of warm weather and active feeding. However, during the winter months, feeding and activity levels decrease but the metabolic costs of the stressor remain. As a result, fishes deplete their lipid stores, resulting in lower condition factors and increased susceptibility to mortality (Lemly 1996). Lemly noted three conditions that must be met simultaneously in order for WSS to occur: 1) a significant metabolic stressor must be present, 2) cold water temperatures must be present, and 3) fish must respond by reducing activity and feeding (Lemly 1996).

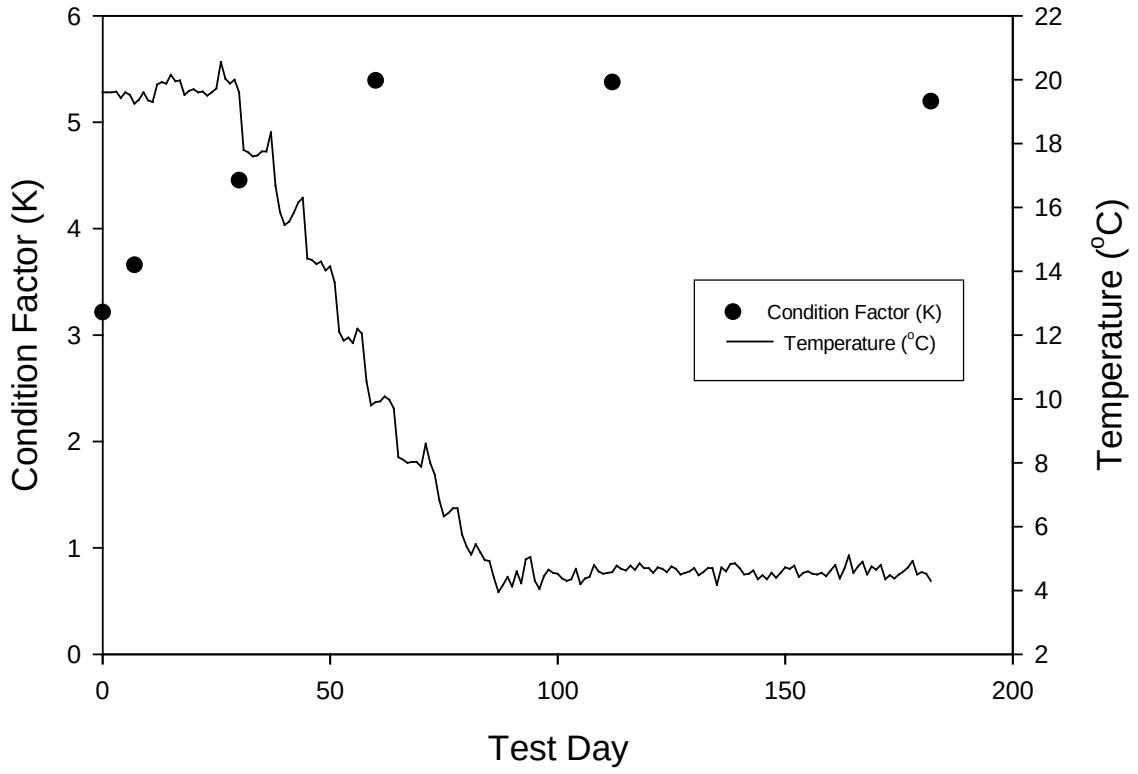
Several other studies have reported decreased feeding and activity levels for several fish species. McCollum et al. (2003) observed decreased overwinter feeding, and subsequent weight loss, of white crappie. Parrish et al. (2004) observed overwinter weight loss among mature, but not juvenile, salmon in a laboratory study in experimental raceways. Current speed, and by extension prey delivery rate, was the most important factor regulating overwinter feeding and growth. Eckmann (2004) observed overwinter reductions in feeding, weight, and lipid levels in yellow perch, but not in ruffe. Sogard and Olla (2000) observed walleye pollock could mitigate the effects of overwinter lipid depletion by moving to colder waters, where reduced metabolism allowed them to conserve energy. In all of these studies, fish continued to feed during the winter, but feeding rates decreased. The increase in weight among ruffe was attributed to its ability to feed on benthos in the dark during the winter months, suggesting that feeding reduction during winter may be more pronounced for species dependent on vision to feed. This was supported by Bennett and Janz (2007a), who observed that burbot, which rely primarily on smell while feeding on benthic invertebrates, experienced significant overwinter increases in weight and lipids in all sites, while northern pike, which rely primarily on vision while feeding on zooplankton, experienced slight but non-significant increases in weight and length.

WSS has not been definitively confirmed or refuted, although it has been investigated in the field. Bennett and Janz (2007a) observed no evidence of WSS for juvenile northern pike or burbot. Lengths, weights, and lipids increased for both species, particularly the olfactory feeding burbots, in the spring compared to the previous fall. Overall weights and lipids were higher in the low and high exposure lakes than the reference lake, possibly because of nitrogen limitation in the reference lake coupled with relatively low stressor concentrations in the exposed lakes. In a separate study, overwinter weights and lipids remained similar or increased in northern pike and burbot at both reference and exposure sites, while overwinter weights and lipids decreased at the exposure site for slimy sculpin (Bennett and Janz 2007b). However, this study neither supports nor refutes the WSS hypothesis, because stressor concentrations at the exposure site were not significantly different than at the reference sites, and the weight decrease in sculpin was attributed to higher turbidity at the exposure site, which inhibited food acquisition. In a final field test of WSS fathead minnow, creek chub, and white suckers were collected from reference and exposure sites (Driedger et al. 2009). Stressor levels at exposure sites were high, as whole body Se concentrations in fathead minnow ranged from 11-42 mg/kg dw. All three species either gained or maintained weight overwinter at all sites, indicating that active feeding occurred overwinter. Overall weights at exposure sites were higher, likely because of nutrient limitation at the reference sites, which confounded the ability to fully test the WSS hypothesis.

These results suggest that fish species responses to cold temperatures vary by species and environment. Many species lose weight, but this can be partially explained by the impact of low light levels on consumption levels, especially in northern latitudes where overwinter light limitation is pronounced. Field tests found no evidence of WSS, but were confounded by low stressor levels, nutrient limitation at reference sites, or both.

It may then be questioned whether the fixed photoperiod alone could account for the differences in the results of the two studies. More explicitly, did the longer light period in McIntyre et al. photoperiod allow the fish to feed more than the fish exposed to the shorter light period in the Lemly study, such that lipid and body condition in the McIntyre et al. fish were maintained and therefore not susceptible to "winter stress syndrome." The effects of photoperiod on fish and other ectotherms are well-documented. Temperature-independent seasonal changes in fish have been reported for growth and food conversion efficiency (Biswas and Takeuchi 2003; Jonassen et al. 2000; Simensen et al. 2000), feeding behavior (Volkoff and Peter 2006), metabolic rate (Evans 1984), and reproduction (Koger et al. 1999; Scott 1979). Some of these studies have found conflicting results on the effect of photoperiod on growth (Fuchs 1978; Jonassen et al. 2000; Simensen et al. 2000). Coupled with temperature being a dominant factor in controlling physiological functions in temperate-zone fish as indicated by a 3 to 4-fold fluctuation in metabolic activities over 10°C (Brett 1970; Fry 1971), it is difficult to use literature findings to explain the difference in the two bluegill studies. In field studies of fish at northern latitudes (Eckmann 2004), reduced light resulted in weight loss not though a bioenergetics interaction with cold temperatures, but by inhibiting feeding ability of visual, but not non-visual predators. If this mechanism applies to bluegill, then photoperiod is less likely to play a major role in the difference in results, as the overwinter light:dark cycle (8:16) should have been sufficiently long for the bluegill in Lemly (1993) to feed.

Observational recordings of the feeding behavior in McIntyre et al. noted that in both control replicates and in both treatment replicates the feeding of the juvenile bluegill went from active to not active on test day 78 when temperatures were decreased from 6.6 to 5.8°C. The feeding observations are reflected in a gradual slight decrease in the body condition factor (K) after test day 60 in the figure below. Although food intake was not quantified during the study, the lack of growth indicated in K suggests feeding markedly decreased as the temperature declined, as shown in the figure. Body condition decreased much more in the Lemly's cold plus selenium exposed fish after test day 60 (approximately 50%) but K in his cold-without-selenium exposure decreased only slightly, similar to McIntyre et al. Therefore it is not possible to determine if the greater decrease in K and in lipid content in Lemly's cold plus selenium treatment was due to decreased feeding because of a shorter photoperiod or because the bluegill fish population used in his study were more sensitive to selenium in cold conditions. McIntyre et al. obtained bluegill from Osage Catfisheries in Missouri whereas Lemly collected fish from ponds (assumed to be near Blacksburg, Virginia, not stated in paper). The fish obtained from Missouri, a location with colder winters than Virginia, may have been better adapted for withstanding colder winter temperatures than Lemly's fish and therefore were less sensitive to "winter stress syndrome" as induced by selenium exposure. Similarly, different populations of a species can have varying sensitivities to stressors. Furthermore, the relative difference in the Lemly and McIntyre et al. results is slightly **less** than Delos (2001) found to be typical when equivalent toxicity tests of the same species are compared. There should thus be no expectation that the two study results should agree more closely than they do.



Relationship between body condition factor (K) and temperature in juvenile bluegill fed a diet of Se-enriched TetraMin in the McIntyre et al. (2008) study.

Carolina Power & Light. 1997. Largemouth Bass Selenium Bioassay- Report. Carolina Power & Light Company, Environmental Services Section, 3932 New Hill, North Carolina. December 1997

Test Organism: Largemouth bass (*Micropterus salmoides*)

Exposure Route: Laboratory; dietary exposure only; DL-selenomethionine added to an artificial diet. Adult largemouth bass obtained from a commercial supplier were fed several months prior to spawning a series of selenium concentrations in the artificial diet.

Test duration: Embryo-larval monitoring through swim-up stage.

Study Design: Dietary exposure studies were conducted in 1995 and in 1996. In 1995, the measured dietary Se concentrations were 0.9 (control), 2.9, 7.5 and 11.2 mg Se/kg dw: in 1996, they were 26.7, 53.1 and 78.4 mg Se/kg dw. Parent fish were fed to satiation twice per day. Approximately 100 eggs from each spawn were transferred to each of 2 to 4 incubation cups. Eggs and larvae were monitored for mortality and deformities up to the larval swim-up stage. Selenium was measured in the liver, muscle and gonad tissues of the parent fish. All live deformed larvae at swim-up stage were considered as mortalities in the analyses.

Effects Data: Over the two year period, 56 successful spawns were obtained across all dietary treatments. Live larval fish with deformities (kyphosis, scoliosis, jaw gap, and lordosis) and edema at swim-up stage were considered mortalities for data analysis. The average concentration of selenium in ovaries ranged from 3.1 mg/kg dw in the control to 77.6 mg/kg dw in the high dietary treatment (Table 1). Larval survival generally decreased as the selenium concentration in the ovary increased (Table 1; Figure 1). A plot of the percent survival of larval largemouth bass as a function of the selenium concentration in the parental female ovary shows two groups of data; one at background survival with considerable variability (mean 90.3%, standard deviation 10.9%) and one with <10% survival, with most of the data being at 0% survival. Due to inadequate partial effects, a TRAP interpolation was used to estimate an EC_{10} value. Based on a risk management decision that the LOEC cannot be any higher than the lowest concentration with 0% survival (32.9 mg/kg) and that any ECx should be below this, this establishes the higher concentration point for the interpolation (an EC_{100} of 32.9 mg/kg) and requires that the highest 4 NOECs not be considered in setting the EC_0. The lower concentration point for the interpolation is therefore set here to 24.6, the next highest NOEC with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg (and a steep slope of 16).

An EC_{10} for the muscle tissue in Table 1 was not determined due to uncertainty in the values. The authors of this report also measured selenium in the ovaries and muscle tissues of largemouth bass collected from Mayo Reservoir (Table 2). There was a considerable difference in the proportion of selenium in the ovaries to the muscle tissues between the largemouth bass collected from the bioassay study and the field collected largemouth bass. The ratio of Se in ovaries to muscle in the laboratory fish was approximately 3.3 whereas it was 1.1 in the field collected fish. With the exception of mountain whitefish, the ovary to muscle ratio observed in the laboratory fish is also considerably higher than other

species (see Appendix B Table B-3). Based on this uncertainty in the muscle concentrations in the laboratory fish, an EC_{10} for this tissue was not calculated. The effect concentration based on the ovary selenium concentrations are not considered uncertain because these concentrations represent the direct exposure of selenium to the larvae from which the effect was observed.

Effect Concentration: **26.3** mg/kg dw in ovaries

Table 1. Selenium concentrations in the diet, ovary and muscle tissues and the percent mortality and deformities.

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
0.9 ± 0.1 (0.7 – 1.3)	6	1.62	5.38	3.1	75.5	95.3
	12	1.77	7.34		99.7	
	13	2.01	3.51		96.2	
	26	2.27	5.74		88.9	
	34	1.18	1.58		99.5	
	35	1.28	1.36		96.8	
	3	1.534	2.09		98.8	
	4	1.583	1.85		100	
	10 (2F)	1.15	2.11		97	
	13	1.181	1.86		97.1	
	14	1.341	1.40		98.4	
2.9 ± 0.5 (2.1 – 3.8)	9	2.075	9.59	8.8	84.9	94.8
	12	1.853	8.03		100	
	15	2.026	9.73		98.5	
	18	3.134	7.66		95.9	
7.5 ± 0.6 (6.3 – 8.4)	1	2.741	8.43	10.8	75	85.8
	2	3.737	25.15		63.9	
	5	5.709	15.31		90.6	
	7	3.468	1.20		79.1	
	8	2.545	6.78		95	
	16	7.302	8.25		96.8	
	19	4.776	10.20		100	
11.2 ± 1.4 (9.3 – 14.1)	6	4.521	35.44	25.0	91.5	88.7
	11	6.044	15.08		77.9	
	17	4.882	24.59		96.7	
26.7 ± 1.7 (23.6 – 29.5)	2	7.52	37.14	40.0	91.2	18.3
	5	12.42	44.67		0	
	11	9.73	34.26		75.9	
	16	10.1	35.58		0	
	17	5.74	33.48		9.9	
	19	11.74	48.24		0	
	36	10.21	35.81		6.3	
	37	14.12	37.88		0	
	51	11.68	32.95		0	

Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent **tissues**, mg/kg dw			Larval survival, %	
		Muscle	**Ovary**	Average	Individual	Average
	52	11.16	59.89		0	
53.1 ± 4.8 (45.5 – 61.9)	22	18.15	46.22	61.0	0	0
	25	21.07	70.45		0	
	30	25.02	81.62		0	
	31	16.63	54.99		0	
	32	14.3	53.96		0	
	41	17.73	51.48		0	
	48 (2F)	26.25	84.31		0	
	50 (2F)	11.66	32.87		0	
	55	18.36	73.33		0	
78.4 ± 4.3 (73.2 – 87.0)	4 (2F)	12.6	66.81	77.6	66	5.5
	7	17.24	56.98		0	
	8	20.36	86.49		0	
	10	19.59	65.99		0	
	18	22.52	72.35		0	
	21	18.58	71.89		0	
	24	22.08	62.44		0	
	28	29.15	99.02		0	
	38	58.2	52.37		0	
	44	17.7	102.82		0	
	47	24.14	88.15		0	
	49	18.94	105.29		0	

[a] ± standard error; range of concentrations in parentheses.

Table 2. Se concentrations in muscle and ovary of field-collected (Mayo Reservoir) female largemouth bass.

Date	Se Muscle (mg/kg dw)	Se Ovary (mg/kg dw)	Ovary to Muscle Ratio
05/10/95	8.48	14.79	1.74
05/10/95	8.48	14.79	1.74
05/09/95	7.29	8.35	1.15
04/21/94	15	19	1.27
04/20/94	15	15	1.00
04/22/94	12	14	1.17
04/22/94	10	18	1.80
04/25/94	18	15	0.83
04/25/94	18	15	0.83
04/27/94	11	12	1.09
04/27/94	11	9.4	0.85
04/27/94	13	10	0.77
05/04/94	11	11	1.00
			Median Ratio 1.09

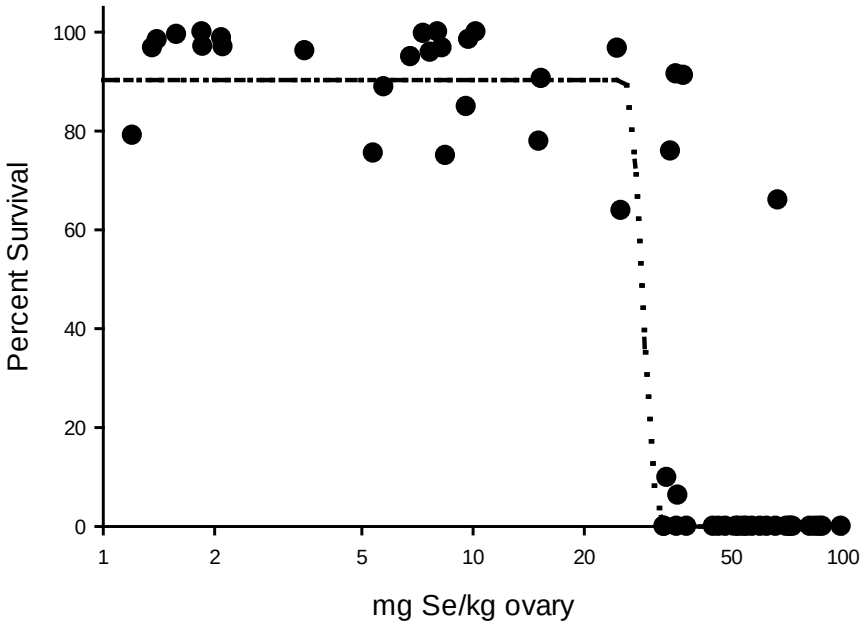


Figure 1. Largemouth bass larval survival relative to Se in ovary. TRAP interpolation was used to estimate the EC_{10} value. The higher concentration point for the interpolation was set at 32.9 mg/kg (EC_{100}) and the lower concentration point for the interpolation was set at 24.6 (NOEC) with greater than the average 90.3% background survival. This results in an EC_{10} of 26.3 mg/kg and a steep slope of 16.

APPENDIX D: SUMMARY STUDIES OF NON-REPRODUCTIVE EFFECTS

1.0 STUDIES OF NON-REPRODUCTIVE EFFECTS

1.1 Acipenseridae

1.1.1 Acipenser transmontanus (white sturgeon)

Juvenile white sturgeon were exposed for 8 weeks to a series of 5 concentrations of seleno-L-methionine added to an artificial diet (Tashjian et al. 2006). Survival was not affected by selenium treatment with a mean survival rate of 99% across all groups. Fish fed the highest three dietary treatments of selenium, 41.7, 89.8 and 191.1 mg Se/kg dw, exhibited significant declines in growth assessed by body weight measurements. The EC_{10} for reduction in body weight is 15.08 mg Se/kg dw in whole body or 27.76 mg Se/kg dw muscle; the EC_{20} is 17.82 mg Se/kg dw in whole body or 32.53 mg Se/kg dw muscle tissue. The criterion values derived in this document that are based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study.

1.2 Cyprinidae

1.2.1 Pogonichthys macrolepidotus (Sacramento splittail)

Teh et al. (2004) exposed juvenile Sacramento splittail (7 months-old) to 8 levels of dietary selenium, 0.4 (no added selenium), 0.7, 1.4, 2.7, 6.6, 12.6, 26.0, and 57.6 mg/kg. Selenium was added to the diet via selenized yeast which was diluted with Torula yeast (inactive) to attain the target levels. Mortality, growth, histopathology, deformities and selenium content in muscle and liver were observed or measured after 5 and 9 months of exposure. The appearance of deformities was the most sensitive endpoint. The authors determined the occurrence of deformities was higher in fish fed 6.6 and 12.6 mg Se/kg in their diet; however, such pathology was examined for only 15 of the 120 individuals per treatment, and a consistent concentration-response relationship did not occur (i.e., no deformities in the high concentration). The lack of a concentration-response relationship for the incidence of deformities has also been observed in another study. Crane et al. (1992) exposed a European species of perch, *Perca fluviatilis* to three aqueous and dietary selenium treatments in experimental ponds for 288 days up through spawning. Crane et al. (1992) found an increased occurrence of deformities in embryos and larvae in the lowest selenium treatment relative to the control, but a decrease in the middle treatment. No hatching occurred in the high treatment. Teh et al. (2004) proposed several physiological mechanisms to explain the lack of a dose-response relationship, but it appears that the underlying mechanism is not understood at this time. Toxicity tests with unusual dose-response relationships are typically not considered for criteria derivation, but since another assay (Crane et al. 1992) observed a similar relationship, the Teh et al. (2004) study with *P. macrolepidotus* is included. Using prevalence of deformities as the endpoint, the NOEC, LOEC and MATC (chronic value) in *muscle tissue* are 10.1, 15.1 and 12.34 mg Se/kg dw, respectively. The critieron value in muscle tissue, based on the reproductive EC_{10}, is 11.8 mg Se/kg dw. Appendix C provides further details on the study results and an approximate estimate of their relationship to egg-ovary and whole-body concentrations. Teh et al. (2004) is the only study in which deformities developed in fish that were not exposed to selenium from their mothers' ovaries. The selenium criterion values derived based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study, considering the non-reproductive muscle MATC of 12.3 mg Se/kg dw is greater than the reproductive muscle criterion of 11.8 mg Se/kg dw.

1.2.2 Pimephales promelas (fathead minnows)

Non-reproductive chronic values for fathead minnows were derived from two laboratory-based studies. These studies (Bennett et al. 1986 and Dobbs et al. 1996) involved exposing algae to selenium (either as

sodium selenite or sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fathead minnows. In the Bennett et al. (1986) study, larval fathead minnows were fed control rotifers (cultured in chambers without selenium containing algae) or selenium-contaminated rotifers (cultured in chambers with selenium containing algae previously exposed to sodium selenite in the water) in three separate experiments lasting 9 to 30 days. The different experiments were distinguished by 1) the day selenium-laden rotifers were first fed; 2) the day selenium-laden rotifers were last fed; and 3) the age of larvae at experiment termination. The results from the three experiments reported by Bennett et al. (1986) were conflicting. Larval growth was significantly reduced at larval whole-body selenium concentrations of 43.0 mg Se/kg dw in the first experiment and 51.7 mg Se/kg dw in the second experiment, but was slightly but not significantly reduced at 61.1 mg Se/kg dw in the third experiment (see Appendix C). Following the approach of Section 7.1.1, the geometric mean of these three values, 51.40 mg Se/kg dw, is the chronic value for this study.

Dobbs et al. (1996) used a test system similar to that of Bennett et al (1986) (described above). Larval fathead minnows were exposed to the same concentrations of sodium selenate in the water as their prey (rotifers), but also received additional selenium from the consumption of the selenium-contaminated rotifers. In this study, the fathead minnows did not grow well at concentrations exceeding 108.1 µg Se/L in water, and they survived only to 11 days at selenium concentrations equal to or greater than 393.0 µg/L in the water (75 mg Se/kg dw in the diet, i.e., rotifers). The LOEC for retarded growth (larval fish dry weight) in this study was <73 mg Se/kg dw tissue.

A third laboratory study, by Ogle and Knight (1989), examined the chronic effects of elevated foodborne selenium on growth and reproduction of fathead minnows. Juvenile fathead minnows were fed a purified diet mix spiked with inorganic and organic selenium in the following percentages: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine. The pre-spawning exposure lasted 105 days using progeny of adult fathead minnows originally obtained from the Columbia National Fishery Research Laboratory, as well as those obtained from a commercial fish supplier. After the 105 day exposure period, a single male and female pair from each of the respective treatment replicates were isolated and inspected for spawning activity for 30 days following the first spawning event of that pair. There was no effect from selenium on any of the reproductive parameters measured, including larval survival, at the dietary concentrations tested (5.2 to 29.5 mg Se/kg dw food). Sub-samples of larvae from each brood were maintained for 14 days post-hatch and exhibited >87.4 percent survival. The pre-spawning adult fish fed a mean dietary level of 20.3 mg Se/kg dw exhibited a significant reduction in growth compared to controls (16 percent reduction), whereas a nonsignificant reduction in growth (7 percent) occurred in the fish fed 15.2 mg Se/kg dw. The chronic value, as determined by the geometric mean of the NOEC and the LOEC measured at 98 days post-test initiation, was 17.57 mg Se/kg expressed as the above dietary concentrations, and 5.961 mg Se/kg dw as fathead minnow whole-body tissue. The concentration-response relationship, as indicated by the study data presented in Appendix E, was uniformly shallow; not resembling the sharp sigmoidal function characteristic of most selenium response curves.

Since Ogle and Knight reported that food in the higher selenium concentrations remained uneaten and fish were observed to reject the food containing the higher selenium concentrations, the authors suggested that the decreased growth was caused by a reduced palatability of the seleniferous food items, which contained unnatural percentages of inorganic selenium (Fan et al. 2002). This is a common observation also noted by Hilton and Hodson (1983) and Hilton et al. (1980) and apparent in Coughlan and Velte (1989). It is here interpreted to be an artifact of unrealistic spiking of the diet with inorganic selenium in this early experimental protocol. That is, in the real world it is not expected that avoidance of food items that were unpalatable because of excessive selenium would be either a mechanism by which selenium causes effects or a mechanism by which organisms can avoid exposure. (See Janz et al. (2010) for a more complete discussion of selenium's mechanism of toxicity.) Given the no observed effect on larval

survival and the apparent non-toxicological effect on growth in the Ogle and Knight study, a chronic value for this study is not included.

1.3 Catostomidae

1.3.1 Xyrauchen texanus (razorback sucker)

Two non-reproductive endpoint studies have been done with the endangered razorback sucker. In the first study, Beyers and Sodergren (2001a) exposed larval razorback suckers for 28 days to a range of aqueous selenate concentrations (6.12, 25.4, 50.6, 98.9, and 190.6 μg/L) and respectively fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw). Reflecting the lack of effects on survival and growth in any exposure, the chronic value for this study, based on selenium measured in the larvae at the end of the test, is >12.9 mg Se/kg dw.

In a second study, Beyers and Sodergren (2001b) exposed larval razorback suckers to a control water and three different site waters containing varying concentrations of selenium for 28 days. Two treatments were tested within each water type: fish fed rotifers cultured in the same water type (site diet) and fish fed rotifers cultured in control water. There were no reductions in survival or growth in fish exposed to both the site water and site diet compared to fish exposed to control water and control diet. There were, however, reductions in growth of fish exposed to site water/site food compared to the same site water and control food. The authors did not attribute the effect on larval growth by the diet to selenium and cited several lines of evidence, including: (1) there was not a dose-response relationship in the concentration of selenium in the food (rotifers) and growth, nor in the concentration of selenium in the fish larvae and growth across the three water types; and (2) water from the De Beque site promoted a significant reduction in the growth of fish exposed to site water/site food relative to site water/control food, but contained low levels of selenium in the water (<1 μg/L) and in food (2.10 mg/kg dw) typically lower than those that have been found to elicit effects. The chronic value for this study is >42 mg Se/kg dw based on the whole body concentration of selenium in the larval razorback suckers exposed to North Pond site water.

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the razorback sucker (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results, of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). Summaries of each of these two studies as well as a third study with razorback suckers (Hamilton et al. 2005d) are presented in Appendix E.

Due to the confounding results, lack of dose-response within and among related studies, and the uncertainty of the effect of other inorganic contaminants on larval response to the various dietary and waterborne treatments, the data from these three studies for razorback sucker (Hamilton et al. 2001a,b; Hamilton et al. 2005d) have not been included. A more detailed explanation of why these studies were not included is given in Appendix E. Because of the vastly different results between the Beyers and Sodergren studies and Hamilton et al. studies and the inability to resolve the differences, SMCV and GMCV were not calculated for the razorback sucker.

1.3.2 Catostomus latipinnis (flannelmouth sucker)

Beyers and Sodergren (2001a) exposed flannelmouth sucker larvae to a range of aqueous selenate concentrations (<1, 25.4, 50.6, 98.9, and 190.6 µg/L) and fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively). There were no survival or growth effects observed after the 28 day exposure. The chronic value based on the concentration of selenium measured in the larvae exposed to the highest test concentration was >10.2 mg Se/kg dw.

1.4 Salmonidae

1.4.1 Oncorhynchus tshawytscha (Chinook salmon)

Hamilton et al. (1990) conducted a 90-day growth and survival study with swim-up larvae fed one of two different diets. The first diet consisted of Oregon moist™ pellets where over half of the salmon meal was replaced with meal from selenium-laden mosquitofish (*Gambusia affinis*) collected from the San Luis Drain, CA (SLD diet). The second diet was prepared by replacing half the salmon meal in the Oregon moist™ pellets with meal from low-selenium mosquitofish (i.e., the same relatively uncontaminated mosquitofish that were used in the control diet) and spiked with seleno-DL-methionine (SeMe diet). Analysis of the trace element composition in the two different diets indicated that while selenium was the most toxic element in the SLD diet, concentrations of boron, chromium, iron and strontium in the high-selenium mosquitofish replacement diet (SLD diet type) were slightly elevated compared to the replacement diet. These trace elements were, however, only 1.2 (e.g., iron) to 2.0 times (e.g., chromium) higher in the SLD diet than the SeMe diet, which contained the following measured concentrations (dry weight basis) in the food: 10 mg boron/kg, 2.8 mg chromium/kg, 776 mg iron/kg, and 48.9 mg strontium/kg.

During the test, survival of control Chinook salmon larvae (consuming food at approximately 3 mg Se/kg dw) was 99 percent up to 60 days post-test initiation. Between 60 and 90 days of exposure, however, the control survival declined to 66.7% in the SLD test and to 72.5% in the test using the SeMe diet, indicating compromised health. Therefore, only data collected up to 60 days post-test initiation were considered for analysis. Nevertheless, there remains the possibility that even at 60 days, the control organisms were not healthy, although overt signs of stress did not appear until later.

For the SeMe diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 7.355 mg Se/kg dw and an EC_{20} of 10.47 mg Se/kg dw. For the SLD diet, regression analysis of the 60-day growth data yielded a whole-body EC_{10} of 11.14 mg Se/kg dw and an EC_{20} of 15.73 mg Se/kg dw. Note: The San Luis Drain mosquitofish (comprising the Chinook salmon's SLD diet) were not tested for contaminants other than certain key elements. Because the San Luis Drain receives irrigation drainage from the greater San Joaquin Valley, there is a possibility that the SLD diet might have contained elevated levels of pesticides, possibly a confounding factor, although the SLD diet was less toxic than the SeMe diet.

1.4.2 Oncorhynchus mykiss (rainbow trout)

Hilton and Hodson (1983) reared juvenile rainbow trout on either a high (25 percent) or low (11 percent) available carbohydrate diet supplemented with sodium selenite for 16 weeks. Body weights, feed: gain ratios, and total mortalities were followed throughout the exposure every 28 days. Tissues (livers and kidneys) were extracted for selenium analysis after 16 weeks. By the end of the exposure, fish fed diets (low carbohydrate and high carbohydrate) with the highest selenium concentrations (11.4 and 11.8 mg Se/kg dw food, respectively) exhibited a 45 to 48 percent reduction in body weight (expressed as kg per 100 fish) compared to control fish. The authors attributed such results to food avoidance. With only two dietary exposure concentrations and a control, these data were not amenable to regression analysis. The MATC for growth of juvenile rainbow trout relative to the concentrations of selenium in liver tissue of trout reared on the high carbohydrate seleniferous dietary type is the geometric mean (GM) of 21.00 mg Se/kg dw liver (NOEC) and 71.7 mg Se/kg dw liver (LOEC), or 38.80 mg Se/kg dw liver. The calculated MATC for the same group of experimental fish exposed to selenium in the low carbohydrate diet is 43.5 mg Se/kg dw liver tissue, which is the same MATC for trout exposed for an additional 4 weeks based on the occurrence of nephrocalcinosis in kidneys (see Hicks et al. 1984; Appendix C).

Hilton et al. (1980) employed a similar test design to that of Hilton and Hodson (1983) to examine the narrow window at which selenium changes from an essential nutrient to a toxicant affecting juvenile rainbow trout. The food consisted of a casein-Torula yeast diet supplemented with selenium as sodium selenite. As discussed previously for the Ogle and Knight (1989) study with fathead minnow, this represents an unrealistic fraction of inorganic selenium in the diet. The experiment lasted for 20 weeks. During this time, the trout were fed to satiation 3 to 4 times per day, 6 days per week, with one feeding on the seventh day. Organs (liver and kidney) and carcasses were analyzed for selenium from fish sacrificed at 4 and 16 weeks. No gross histopathological or physiological effects were detected in the fish, although trout raised on the highest dietary level of selenium (13.06 mg Se/kg dw food) had a significantly lower body weight (wet basis), a higher feed:gain ratio, and higher number of mortalities (10.7; expressed as number per 10,000 fish days). The MATC for growth and survival of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue is the geometric mean of the NOEC (40 mg Se/kg dw liver) and the LOEC (100 mg Se/kg dw liver), or 63.25 mg Se/kg dw, both of which hinge on accepting dietary spiking entirely with inorganic selenium as an acceptable experimental protocol.

The non-reproductive GMCV for *Oncorhynchus* (both rainbow trout and Chinook salmon) is 9.052 mg Se/kg dw whole body based on the EC_{10} value derived from the Hamilton et al. (1990) study with Chinook salmon. The NOEC values for the rainbow trout studies conducted by Hilton and Hodson (1983), Hilton et al. (1980), and Hicks et al. (1984) were not used in the GMCV calculation because of the large difference between the NOEC and the LOEC values. If adult fish contained whole-body selenium concentrations equal to 9.052 mg Se/kg dw, their egg-ovary concentrations would be estimated to be 21.5 mg Se/kg dw when translated using the factor 2.37. The criterion values derived based on reproductive endpoints are protective of the endpoint measured.

1.5 Moronidae

1.5.1 Morone saxitilis (striped bass)

A non-reproductive chronic value for selenium was determined from a laboratory dietary exposure conducted using yearling striped bass (Coughlan and Velte 1989). During the experiment, the bass were fed contaminated red shiners (38.6 mg Se/kg dw whole body) from Belews Lake, NC (treated fish) or golden shiners with low levels of selenium (1.3 mg/kg dw whole body) purchased from a commercial supplier (control fish). The test was conducted in soft well water and lasted up to 80 days. During the experiment, all fish were fed to satiation 3 times per day. Control fish grew well and behaved normally.

Treated fish behaved lethargically, grew poorly due to a significant reduction in appetite, and showed histological damage, all eventually leading to the death of animals. The final selenium concentration in muscle of treated striped bass averaged from 16.2 to 18.5 mg/kg dw tissue (assuming 78.4 percent moisture content), which was 3.4 to 3.6 times higher than the final selenium concentrations in control striped bass, which averaged 5.10 mg/kg dw tissue. The chronic value for this species was determined to be <16.2 mg Se/kg dw in muscle tissue.

1.6 Centrarchidae

1.6.1 Lepomis macrochirus (bluegill)

Bryson et al. (1985b) conducted juvenile survival toxicity tests using hatchery bluegill and various forms of selenium spiked to an artificial diet as well as a diet consisting of zooplankton collected from Hyco Reservoir. There was no effect on length or weight of the juvenile bluegill after 60 days of exposure. The highest concentration of selenium measured in whole body of the juveniles in these tests was in the seleno-DL-cysteine-2X treatment (3.74 mg Se/kg dw).

Cleveland et al. (1993) performed a 90-day diet-only laboratory exposure in which juvenile bluegill were fed a range of selenomethionine concentrations added to Oregon moist™ pellets. The authors observed no significant effects on survival, but did report a very small but apparently statistically significant decrease in the condition factor, K, from 1.3 at four concentrations between 1.0 and 4.7 mg Se/kg dw whole body, to 1.2 at the two concentrations 7.7 and 13.4 mg Se/kg dw whole body. The condition factor (weight x 10^5/length^3) is intended to reflect a fish's reserves. In contrast to the studies of Ogle and Knight (1989), Hilton and Hodson (1983), and Hilton et al. (1989), which appear to have involved an inorganic selenium food palatability problem, this study did not use inorganic selenium in the diet. Nevertheless, given that the reduction in K (1.3 to 1.2) is slight and shows no increasing effect between 7.7 and 13.4 mg Se/kg dw, thus not yielding a sigmoidal concentration-response curve to support an EC_{10} calculation, the chronic value for this study was estimated at >13.4 mg Se/kg dw in whole body tissue.

Data from Lemly (1993a) indicate that over-wintering fish may be more susceptible to the effects of waterborne and dietary selenium due to increased sensitivity at low temperature. The author exposed juvenile bluegill in the laboratory to a single elevated exposure level, waterborne (1:1 selenite:selenate; nominal 5 µg Se/L) and foodborne (seleno-L-methionine in TetraMin; nominal 5 mg Se/kg dw food) selenium for 180 days. Tests with a control and the treated fish were run at 4°C and 20°C with biological and selenium measurements made every 60 days. Survival and whole-body lipid content were unaffected at 20°C (whole-body selenium concentrations equal to 6 mg/kg dw, the sole treatment exposure) when compared to control fish. Thus, at 20°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium based on survival was >6 mg/kg dw in whole-body tissue. Fish exposed to the combination low-level waterborne and dietary selenium at 4°C exhibited significantly elevated mortality (40.4 percent) relative to controls (2.9 percent), and exhibited significantly greater oxygen consumption and reduced lipid content, which are indicative of stress. At 4°C the chronic value for juvenile bluegill exposed to waterborne and dietary selenium was <7.91 mg Se/kg dw in whole body based on mortality and tissue measurements at the end of the test (180 days), and 5.85 mg Se/kg dw in whole body based on mortality at 180 days and tissue measurements at 60 days. The increase in the concentration of whole-body selenium between Day 60 and 180 at 4°C was apparently due to reductions in body weight caused by loss of lipid (comparatively low in selenium) while body burden in other tissues remained relatively constant. If this concentration of selenium in tissues occurs in sensitive overwintering fish in nature, a concentration of 5.85 mg/kg dw (the selenium tissue concentration in the 4ºC exposure after 60 days) in fish collected during the summer or fall months could be considered a threshold concentration for the selenium-sensitive fish during the winter months. Therefore, this study's chronic value for the threshold concentration prior to winter stress is 5.85 mg Se/kg dw in whole body tissue.

McIntyre et al. (2008) also investigated the toxicity of selenium to juvenile bluegill under cold temperature conditions in the laboratory. Whereas relative to the control, Lemly (1993a) tested only one exposure level, 5 mg Se/kg in the diet and 5 µg Se/L and one low temperature regime, 4°C, McIntyre et al. (2008) evaluated a range of diet and water concentrations, two types of diet, and two low-temperature regimes. The goal of the study was to determine EC_{10} and EC_{20} values for selenium exposure to juvenile bluegill in 4°C and 9°C low-temperature regimes. Three separate exposure systems were run concurrently for 182 days. Two systems exposed juvenile bluegill to a series of six aqueous and dietary selenium treatments and a control; one exposure system (ES1) with a cold temperature regime (4°C), and one (ES3) with a cool temperature regime (9°C), both using a yeast-worm-fish food chain bioaccumulation system. That is, graded levels of selenized-yeast in ES1 and ES3 were fed to the oligochaete, *Lumbriculus variegatus*, which in turn was fed to bluegill. The third exposure system (ES2) used diet and exposure conditions similar to Lemly's 4°C treatment, i.e., nominal 5 µg Se/L in the water and nominal 5 mg Se/kg dw food (seleno-L-methionine in TetraMin). The cold temperature regime for ES1 and ES2 was 20°C for the first 30 days of exposure, and then decreased 2°C/week until it reached 4°C (test day 79) at which point temperature was maintained until test termination (test day 182). The cool temperature regime (ES3) was similar except when the temperature reached 9°C (test day 65), it was maintained until test termination (test day 182).

At the end of the 182 day exposure in the ES2 (with Lemly's diet and temperature), the bluegill accumulated an average (geometric mean) whole body concentration of 9.99 mg/kg dw with no meaningful mortality in the treatment or control. Significant mortality of juvenile bluegill was observed in the two highest treatments in the cold (ES1) and cool (ES3) *Lumbriculus*-fed tests. No effects on body weight or condition factor were observed. The EC_{10} and EC_{20} values for the cold treatment (ES1) are 9.27 and 9.78 mg Se/kg dw in whole body, respectively. The EC_{10} and EC_{20} values for the cool treatment (ES3) are slightly higher at 14.00 and 14.64 mg Se/kg dw in whole body, respectively.

The design and the results of the McIntyre et al. (2008) study have similarities and differences with Lemly (1993a), as presented in detail with comparisons and contrasts in Appendix C. Both studies found juvenile bluegill were more sensitive in a cold-temperature regime than in a cool (McIntyre et al.) or a warm regime (Lemly). The effect levels determined for the cold temperature regime differed by a factor of 1.58 (ES1 of McIntyre et al., 9.27 mg Se/kg; Lemly, 5.85 mg Se/kg), a difference rather typical of chronic studies conducted in different laboratories using different fish populations (Delos 2001) and similar to the 1.51 factor difference between two EC_{10}s of Hamilton et al. (1990) for chinook salmon.

The difference in the effect levels of the McIntyre ES2 exposure (>9.99 mg/kg) and the Lemly study (5.85 mg/kg) could have been due to the fitness of the fish entering the cold regime. The condition factor, K, in the ES2 selenium-exposed bluegill increased from 3.2 at the start of the exposure to 5.2 at day 60 (approximately 10°C at day 60) and decreased only slightly through over 100 days of 4°C exposure (see figure in bluegill summary in Appendix C). In contrast, K in the Lemly selenium-exposed fish decreased approximately 50% after 120 days of exposure. Shoup and Wahl (2011) conducted an overwinter exposure study with bluegill in which they fed and starved young of year bluegill (the larger size similar to the McIntyre and Lemly fish) under two temperature regimes, 4°C (harsh winter) and 9°C (mild winter) for 140 days and a 10 h light:14 h dark photoperiod. The juvenile bluegill in the Shoup and Wahl study ate in both temperature regimes. The 4°C exposed fish consumed 0.4-0.8% of their body weight/day and their K was not significantly different at the end of the test compared to the start. The Shoup and Wahl results only provide an indication that cold-exposed fish under a winter photoperiod feed and can maintain K.

The mortality observed in the Lemly laboratory study does not appear to be consistent with field observations. The occurrence of mortality in the field at the concentrations Lemly (1993a) reported to cause mortality in his lab was not observed in the Lemly (1993b) field study of centrarchid deformities in Belews Lake. In that field study, Lemly (1993b) found larval centrarchid deformities at concentrations ranging from 12-80 mg Se/kg dw WB. If juvenile mortality occurred at concentrations lower than those found to induce larval deformities and at concentrations as low as Lemly (1993a) reported in the lab (EC_{40} = 7.91 mg Se/kg WB), then centrarchids would likely not have been present in Belews Lake. The observations of Lemly (1993b) are evidence that larval deformity, not juvenile mortality, is the more sensitive endpoint.

The Crutchfield and Ferson (2000) predictions and field observations of recovery of bluegill at Hyco Reservoir likewise suggest that significant mortality was unlikely to be occurring at the concentrations Lemly (1993a) reported to cause substantial mortality. During a time period over which Crutchfield (2000) indicated dietary invertebrate concentrations exceeded 20 mg Se/kg dw, Crutchfield and Ferson (2000) indicated that bluegill population growth occurred at rates predicted to be natural for the unimpaired species. In contrast, if the Lemly (1993a) lab EC_{40} of 7.91 mg Se/kg dw whole-body were applicable to this field situation, the mortality associated with the resulting bluegill whole-body concentrations (25 mg Se/kg dw whole-body, assuming a trophic transfer factor of 1.27) would have prevented any recovery.

Selenium-induced cold temperature loss of lipid and body condition, a non-reproductive sublethal effect that Lemly (1993a) observed to accompany juvenile mortality in the laboratory (but which McIntyre et al. (2008) did not observe in a similar study) has also not generally been corroborated by field evidence (Janz 2008). Several studies have measured growth and energy storage indicators in juvenile fish just prior to and just after winter at reference sites and sites with elevated selenium in northern Canada (Bennett and Janz 2007a, b; Kelly and Janz 2008; Driedger et al 2009; Weber et al. 2008). The growth (length, weight, condition factor, muscle RNA:DNA ratio, muscle protein) and energy storage (whole body lipids, whole body triglycerides, liver triglycerides, liver glycogen) indicators for five fish species (northern pike, burbot, fathead minnow, creek chub, white sucker) measured just after winter were similar or greater than those measured just before winter at the selenium exposed sites. The slimy sculpin did show a decrease in whole body triglycerides, but the reduction was similar at exposed and reference sites.

Given the uncertainty in the occurrence of winter stress, the results of all four cold-temperature (4°C and 9°C) juvenile-survival lab studies were combined per the standard procedure described in the U.S.EPA Ambient Water Quality Criteria Guidelines, to determine the non-reproductive SMCV for bluegill. The SMCV for the combined 4°C and 9°C tests is 9.33 mg Se/kg dw whole body, based on the four chronic values: (a) the Lemly (1993a) concentration prior to winter stress (5.85 mg Se/kg dw whole body), (b) the McIntyre et al. (2008) ES1 EC_{10} (9.27 mg Se/kg dw whole body), (c) the McIntyre et al. (2008) ES2 NOEC (>9.992 mg Se/kg dw whole body), and the McIntyre et al. (2008) ES3 EC_{10} of 14.00 mg Se/kg dw whole body. This value is not less than the reproductive endpoint-based whole-body criterion concentration of 8.5 mg Se/kg dw. The studies of Bryson et al (1985b) and Cleveland et al. (1993) were not conducted at cold temperatures and were thus not used for these SMCV calculations.

Table D-1. Freshwater Chronic Values from Acceptable Tests - Non-Reproductive Endpoints (Parental Females Not Exposed).
(Same as Table 6.2 in the main document).

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

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab) 60 days	mosquitofish spiked with seleno-DL-methionine	EC_{10} for juvenile growth	7.355 WB	EC_{10} 9.052 WB EC_{20} 12.83 WB	EC_{10} 9.052 WB
				EC_{20} for juvenile growth	10.47 WB		
			mosquitofish spiked with SLD diet	EC_{10} for juvenile growth	11.14 WB		
				EC_{20} for juvenile growth	15.73 WB		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab) 16 weeks	sodium selenite in food preparation	juvenile growth NOEC	21 Liver	NOAEC 28.98 L LOAEC 84.68 L MATC 49.52 L	
				LOEC	71.7 Liver		
				MATC	38.80 Liver		
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juvenile survival and growth NOEC	40 Liver		
				LOEC	100 Liver		
				MATC	63.25 Liver		
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab) 80 days	Se-laden shiners from Belews Lake, NC	LOEC for survival of yearling bass	<16.2 M[c]	<16.2 M	<16.2 M
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab) 180 days 20 to 4°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOEC for juvenile mortality at 4oC	<7.91 WB	4°C EC_{10}-NOAEC 8.15 WB 4°C EC_{20}-LOAEC 8.80 WB 9°C EC_{10} 14.0 WB 9°C EC_{20} 14.6 WB	4°C & 9°C 9.33 WB
				Threshold prior to "winter stress"	5.85 WB		
		dietary and waterborne (lab) 180 days 20°C	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC for juvenile mortality at 20oC	>6.0 WB		
Lepomis macrochirus bluegill	McIntyre et al. 2008	dietary and waterborne (lab) 182 days 20 to 4°C (ES1)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES1	9.27 WB		
				EC_{20} juv. survival ES1	9.78 WB		
		dietary and waterborne (lab) 182 days 20 to 9°C (ES3)	diet: Lumbriculus fed selenized-yeast water: 1:1 selenate:selenite	EC_{10} juv. survival ES3	14.00 WB		
				EC_{20} juv. survival ES3	14.64 WB		

Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
		dietary and waterborne (lab) 182 days 20 to 4°C (ES2)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOEC juv. surv. ES2	>9.992 WB		
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary (lab) 60 days	seleno-DL-cysteine	NOEC for juvenile growth	>3.74 WBb		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary (lab) 90 days	seleno-L-methionine	NOEC for juvenile survival	>13.4 WBb		

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

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

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

APPENDIX E: OTHER DATA

1.0 SELENITE

Additional data on the lethal and sublethal effects of selenium on aquatic species are presented in Table E-1. Bringmann and Kuhn (1959a,b, 1976, 1977a, 1979, 1980b, 1981), Jakubczak et al. (1981), and Patrick et al. (1975) reported the concentrations of selenite that caused incipient inhibition (defined variously, such as the concentration resulting in a 3% reduction in growth) for algae, bacteria, and protozoans (Table E-1). Although incipient inhibition might be statistically significant, its ecological importance is unknown. Albertano and Pinto (1986) found the growth of three red algal species was inhibited at selenite concentrations that ranged from 790 to 3,958 μg/L.

2.0 SELENATE

Dunbar et al. (1983) exposed fed *D. magna* to selenate for seven days and obtained an LC_{50} of 1,870 μg/L. This value is in the range of the 48-hr EC_{50}s in Table E-1.

Watenpaugh and Beitinger (1985a) found that fathead minnows did not avoid 11,200 μg/L selenate during 30-minute exposures (Table E-1). These authors also reported (1985b) a 24-hr LC_{50} of 82,000 μg/L for the same species and they found (1985c) that the thermal tolerance of the species was reduced by 22,200 μg/L. Westerman and Birge (1978) exposed channel catfish embryos and newly hatched fry for 8.5 to 9 days to an unspecified concentration of selenate. Albinism was observed in 12.1 to 36.9% of the fry during the five years of such exposures. Pyron and Beitinger (1989) also investigated fathead minnows, and after a 24-hr exposure, no effect on reproductive behavior was found at 36,000 μg/L, but when adults were exposed to 20,000 μg/L selenate for 24-hr, edema was observed for their larvae.

The respiratory rate of the eastern oyster, *Crassostrea virginica*, was unaffected by exposure to selenate at 400 μg/L for 14 days (Fowler et al. 1981). Embryos of the striped bass were quite tolerant to selenate in dilute salt water (Klauda 1985a, b). There was a 93% successful hatch of embryos at 200,000 μg/L, but 50% of 72-day-old juveniles died after four days at 87,000 μg/L. Exposure of juvenile fish for up to 65 days to concentrations of selenate between 39 and 1,360 μg/L caused developmental anomalies and pathological lesions.

Table E-1. Other Data on Effects of Selenium on Aquatic Organisms

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	96 hr	Incipient inhibition (river water)	2,500	Bringmann and Kuhn 1959a,b
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	Decreased dry weight and chlorophyll a	75	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 12-21[b]	10-100	Foe and Knight, Manuscript
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	72 hr	BCF = 11,164[c]	150	Foe and Knight, Manuscript
Alga, *Chrysochromulina breviturrita*	Selenious acid	-	30 days	Increased growth	320	Wehr and Brown 1985
Red alga, *Cyanidium caldarium*	Selenious acid	-	20 days	Inhibited growth	3,958	Albertano and Pinto 1986
Red alga, *Cyanidioschyzon merolae*	Seleniousacid	-	20 days	Inhibited growth	3,140	Albertano and Pinto 1986
Red alga, *Galdieria sulphuraria*	Seleniousacid	-	20 days	Inhibited growth	790	Albertano and Pinto 1986
Algae (diatoms), Mixed population	Sodium selenite	-	18 days	Inhibited growth	11,000	Patrick et al. 1975
Bacterium, *Escherichia coli*	Sodium selenite	-	-	Incipient inhibition	90,000	Bringmann and Kuhn 1959a
Bacterium, *Pseudomonus putida*	Sodium selenite	-	16 hr	Incipient inhibition	11,400 (11,200)	Bringmann and Kuhn 1976; 1977a; 1979; 1980b
Protozoan, *Entosiphon sulcatum*	Sodium selenite	-	72 hr	Incipient inhibition	1.8 (1.9)	Bringmann 1978; Bringmann and Kuhn 1979; 1980b; 1981
Protozoan, *Microreqma heterostoma*	Sodium selenite	-	28 hr	Incipient inhibition	183,000	Bringmann and Kuhn 1959b
Protozoan, *Chilomonas paramecium*	Sodium selenite	-	48 hr	Incipient inhibition	62	Bringmann and Kuhn 1981; Bringmann et al. 1980

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Protozoan, *Uronema parduezi*	Sodium selenite	-	20 hr	Incipient inhibition	118	Bringmann and Kuhn 1980a; 1981
Snail, *Lymnaea stagnalis*	Sodium selenite	-	7.5 days	LT50	3,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenite	-	48 hr	EC50 (river water)	2,500	Bringmann and Kuhn 1959a,b
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	LC50	16,000	Bringmann and Kuhn 1977a
Cladoceran, *Daphnia magna*	Sodium selenite	214	24 hr	EC50 (swimming)	9.9	Bringmann and Kuhn 1977b
Cladoceran, *Daphnia magna*	Sodium selenite	329	48 hr 96 hr 14 days	EC50 (fed)	710 430 430	Halter et al. 1980
Cladoceran (<24 hr), *Daphnia magna*	Sodium selenite	-	48 hr 21 days	EC50 (fed)	685 160	Adams and Heidolph 1985
Cladoceran (5th instar), *Daphnia magna*	Sodium selenite	-	48 hr	LC50 (fed)	680	Johnston 1987
Cladoceran, *Daphnia magna*	Selenious acid	220[d]	48 hr	LC50 (fed)	1,200	Kimball, Manuscript
Cladoceran (preadult), *Daphnia pulex*	Sodium selenite	42	24 hr	Did not reduce oxygen consumption or filtering rate	>498	Reading and Buikema 1980
Ostracod, *Cyclocypris* sp.	Sodium selenite	100.8	48 hr	LC50	130,000	Owsley 1984
Amphipod, *Hyalella azteca*	Sodium selenite	329	14 days	LC50 (fed)	70	Halter et al. 1980
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	48 hr	LC50	623	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodiumselenite	133	10 days	LC50 (fed)	312	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenite	133	24 days	LOEC reproduction (static-renewal)	200	Brasher and Ogle 1993
Midge (first instar), *Chironomus riparius*	Sodium selenite	134	48 h	LC50	7,950	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenite	40-48	48 h	LC50	14,600	Ingersoll et al. 1990
Coho salmon (fry), *Oncorhynchus kisutch*	Sodium selenite	325	43 days	LC50	160	Adams 1976
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	LC50	460	Adams 1976

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout (fry), *Oncorhynchus mykiss*	Sodium selenite	334	21 days	Reduced growth	250	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	330	5 days	LC50	2,700 2,750	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	48 days	LC50	500	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	325	96 days	LC50	280	Adams 1976
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	200	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	MATC survival	4.7 µg/g dw (whole-body)	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	4 wk	BCF = 23	100	Gissel-Nielsen and Gissel-Nielsen 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC growth (dietary only exposure)	>9.96 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Sodium selenite	-	42 wk	MATC survival (dietary only exposure)	5.34 µg Se/g dw (food)	Goettl and Davies 1978
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	9 days	LC50	7,020	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	7,200 5,410	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	96 hr 9 days	LC50 (fed)	8,200 6,920	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	41 days	LOAEC (Reduced hatch of eyed embryos)	26	Hodson et al. 1980
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	135	50 wk	Decreased iron in blood and red cell volume	53	Hodson et al. 1980
Rainbow trout (fertilized egg), *Oncorhynchus mykiss*	Sodium selenite	135	44 wk	BCF = 33.2 BCF = 21.1	53	Hodson et al. 1980
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	120 hr	Did not reduce survival or time to hatch	10,000	Klaverkamp et al. 1983b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Rainbow trout, *Oncorhynchus mykiss*	Sodium selenite	-	90 days	Chronic value for survival	14	Mayer et al. 1986
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	LC50	55.2[e]	Hunn et al. 1987
Rainbow trout (sac fry), *Oncorhynchus mykiss*	Sodium selenite	272	90 days	MATC survival	31.48	Hunn et al. 1987
Rainbow trout (egg), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 17.5 BCF = 3.5	0.4 45.6	Hodson et al. 1986
Rainbow trout (embryo), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 3.1 BCF = 3.0	0.4 45.6	Hodson et al. 1986
Rainbow trout (sac-fry), *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 13.1 BCF = 1.6	0.4 45.6	Hodson et al. 1986
Rainbow trout (swim-up fry) *Oncorhynchus mykiss*	Sodium selenite	-	96 hr	BCF = 80.3 BCF = 20.2	0.4 45.6	Hodson et al. 1986
Northern pike, *Esox lucius*	Sodium selenite	10.2	76 hr	LC50	11,100	Klaverkamp et al. 1983a
Goldfish, *Carassius auratus*	Selenium dioxide	157	14 days	LC50	6,300	Cardwell et al. 1976a,b
Goldfish, *Carassius auratus*	Sodium selenite	-	10 days	Mortality	5,000	Ellis 1937; Ellis et al. 1937
Goldfish, *Carassius auratus*	Sodium selenite	-	46 days	Gradual anorexia and mortality	2,000	Ellis et al. 1937
Goldfish, *Carassius auratus*	Selenium dioxide	-	7 days	LC50	12,000	Weir and Hine 1970
Goldfish, *Carassius auratus*	Selenium dioxide	-	48 hr	Conditional avoidance	250	Weir and Hine 1970
Fathead minnow, *Pimephales promelas*	Selenium dioxide	157	9 days	LC50	2,100	Cardwell et al. 1976a,b
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	96 hr	LC50 (fed)	1,000	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Sodium selenite	329	14 days	LC50 (fed)	600	Halter et al. 1980
Fathead minnow, *Pimephales promelas*	Selenious acid	220[d]	8 days	LC50 (fed)	420	Kimball, Manuscript
Creek chub, *Semotilus atromaculatus*	Selenium dioxide	-	48 hr	Mortality	∃12,000	Kim et al. 1977
Bluegill, *Lepomis macrochirus*	Sodium selenite	318	48 days	LC50	400	Adams 1976
Bluegill, *Lepomis macrochirus*	Selenium dioxide	157	14 days	LC50	12,500	Cardwell et al. 1976a,b

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	16	323 days	MATC larval survival (dietary only exposure)	19.75 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Sodium selenite	25 and 200	120 days	No mortality	>10	Lemly 1982
Largemouth bass (juvenile), *Micropterus salmoides*	Sodium selenite	25 and 200	120 days	No mortality	10	Lemly 1982
Yellow perch, *Perca flavescens*	Sodium selenite	10.2	10 days	LC50	4,800	Klaverkamp et al. 1983a,b
African clawed frog, *Xenopus laevis*	Sodium selenite	-	7 days	LC50	1,520	Browne and Dumont 1980
African clawed frog, *Xenopus laevis*	Sodium selenite	-	1-7 days	Cellular damage	2,000	Browne and Dumont 1980
Selenium (VI)						
Alga, *Chrysochromulina breviturrita*	-	-	30 days	Increased growth	50	Wehr and Brown 1985
Rotifer, *Brachionus calyciflorus*	Sodium selenate	120	96 hr	EC20 Growth (dry weight)	42.36 (µg/g dw)	Dobbs et al. 1996
Snail, *Lymnaea stagnalis*	Sodium selenate	-	6 days	LT50	15,000	Van Puymbroeck et al. 1982
Cladoceran, *Daphnia magna*	Sodium selenate	129.5	7 days	LC50 (fed)	1,870	Dunbar et al. 1983
Cladoceran (juvenile), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	550	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	48 hr	LC50 (fed)	750	Johnston 1987
Cladoceran (5th instar), *Daphnia magna*	Sodium selenate	-	90 hr	42% of organisms had visible changes in gut morphology	250	Johnston 1989
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	48 hr	LC50	2378	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	10 days	LC50 (fed)	627	Brasher and Ogle 1993
Amphipod (2 mm length), *Hyalella azteca*	Sodium selenate	133	24 days	LOEC reproduction (static renewal)	>700	Brasher and Ogle 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	18 (SO_4=3.4)	10 days	LC50 (fed)	43	Borgmann et al. 2005
Amphipod (1-11 days old), *Hyalella azteca*	Sodium selenate	124 (SO_4=32)	10 days	LC50 (fed)	371	Borgmann et al. 2005
Midge (first instar), *Chironomus riparius*	Sodium selenate	134	48 h	LC50	16,200	Ingersoll et al. 1990
Midge (first instar), *Chironomus riparius*	Sodium selenate	40-48	48 h	LC50	10,500	Ingersoll et al. 1990
Rainbow trout (embryo, larva), *Oncorhynchus mykiss*	Sodium selenate	104 (92-110)	28 days	EC50 (death and deformity)	5,000 (4,180) (5,170)	Birge 1978; Birge and Black 1977; Birge et al. 1980
Goldfish (embryo, larva), *Carrassius auratus*	Sodium selenate	195	7 days	EC50 (death and deformity)	8,780	Birge 1978
Goldfish, *Carassius auratus*	Sodium selenate	-	24 hr	BCF = 1.42 BCF = 1.15 BCF = 1.47 BCF = 0.88 BCF = 1.54	0.45 0.9 1.35 2.25 4.5	Sharma and Davis 1980
Fathead minnow, *Pimephales promelas*	Sodium selenate	337.9	48 days	LC50	2,000	Adams 1976
Fathead minnow, *Pimephales promelas*	Sodium selenate	338	48 days	LC50	1,100	Adams 1976
Fathead minnow, *Pimephales promelas*	-	51	30 min	No avoidance	11,200	Watenpaugh and Beitinger 1985a
Fathead minnow, *Pimephales promelas*	-	-	24 hr	LC50	82,000	Watenpaugh and Beitinger 1985b
Fathead minnow, *Pimephales promelas*	-	-	24 hr	Reduced thermal tolerance	22,200	Watenpaugh and Beitinger 1985c
Fathead minnow, *Pimephales promelas*	Sodium selenate	44-49	7 days	Chronic value - growth Chronic value-growth Chronic value-survival	1,739 561 2,000	Norberg-King 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	No effect on reproductive behavior	36,000	Pyron and Beitinger 1989
Fathead minnow, *Pimephales promelas*	Sodium selenate	160-180	24 hr	Edema in larvae produced from adults exposed to Selenium VI	20,000	Pyron and Beitinger 1989

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Channel catfish (embryo, fry), *Ictalurus punctatus*	Sodium selenate	90	8.5-9 days	Induced albinism	-	Westerman and Birge 1978
Narrow-mouthed toad (embryo, larva), *Gastrophryne carolinensis*	Sodium selenate	195	7 days	EC50 (death and deformity)	90	Birge 1978; Birge and Black 1977; Birge et al. 1979a
Organo-selenium						
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	16	323 days	MATC larval survival (dietary only exposure)	20.83 µg Se/g dw (food)	Woock et al. 1987
Bluegill (juvenile), *Lepomis macrochirus*	Seleno-L-methionine	283	90 days	EC20 survival (dietary only exposure)	>13.4 µg/g dw (food)	Cleveland et al. 1993
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	53.83 µg Se/g dw (liver)	Reash et al. 1999
Bluegill (2 yr and adult), *Lepomis macrochirus*	Selenium	-	field	NOEC deformities	23.38 µg Se/g dw (ovaries)	Reash et al. 1999
Redear sunfish (adult), *Lepomis microlophus*	Selenium	-	field	LOEC Adverse histopathological alterations	<38.15 µg Se/g dw	Sorensen 1988
Selenium Mixtures						
Phytoplankton, Mixed population	Selenium	-	field	Reduced growth rates	18	Riedel et al. 1991
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC growth	115.2 µg Se/L	Ingersoll et al. 1990
Cladoceran (<24 hr), *Daphnia magna*	Selenite-Selenate mixture	138	21 days	MATC productivity	21.59 µg/g dw (whole-body)	Ingersoll et al. 1990
Midge (<24-hr), *Chironomus riparius*	Selenite-Selenate mixture	138	30 days	MATC emergence	503.6	Ingersoll et al. 1990
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	NOEC survival	340	Cleveland et al. 1993
Bluegill (juvenile), *Lepomis macrochirus*	Selenite-Selenate mixture	283	60 days	EC20 survival	4.07 µg/g dw (whole body)	Cleveland et al. 1993

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Anaerobic bacterium, *Methanococcus vannielli*	Sodium selenite	-	110 hr	Stimulated growth	79.01	Jones and Stadtman 1977
Bacterium, *Vibrio fisheri*	Sodium selenite	-	5 min	50% decrease in light output (Microtox7)	68,420	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenite	32	14 days	5-12% increase in growth	10-10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenite	32	14 days	23% increase in growth	100-10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenite	32	20 days	Increased growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b
Diatom, *Skeletonema costatum*	Selenium dioxide	-	5 days	BCF = 18,000 BCF = 16,000 BCF = 10,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Chaetoceros muelleri*	Selenium dioxide	-	6 days	BCF = 337,000 BCF = 65,000 BCF = 5,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Phaeodactylum tricornutum*	Selenium dioxide	-	8 days	BCF = 109,000 BCF = 27,000 BCF = 7,000	0.06 0.79 3.6	Zhang et al. 1990
Diatom, *Thallassiosira aestivalis*	Selenium oxide	29-30	72 hr	No effect on cell morphology	78.96	Thomas et al. 1980a
Brown alga, *Fucus spiralis*	Sodium selenite	-	60 days	1355% increase in growth of thalli	2.605	Fries 1982
Red alga, *Porphyridium cruentum*	Sodium selenite	32	27 days	Increase growth; induced glutathione peroxidase	4,600	Gennity et al. 1985a,b

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Selenium (VI)						
Bacterium, *Vibrio fisheri*	Sodium selenate	-	15 min	50% decrease in light output (Microtox7)	3,129,288	Yu et al. 1997
Green alga, *Chlorella* sp.	Sodium selenate	32	14 days	No effect on rate of cell	10-1,000	Wheeler et al. 1982
Green alga, *Chlorella* sp.	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10-100	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	14 days	71% reduction in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Dunaliella primolecta*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	No effect on rate of cell population growth	10	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	16% decrease in rate of cell population growth	100	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	14 days	50% decrease in rate of cell population growth	1,000	Wheeler et al. 1982
Green alga, *Platymonas subcordiformis*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982
Brown alga, *Fucus spiralis*	Sodium selenate	-	60 days	160% increase in growth rate of thalli	2.605	Fries 1982
Red alga, *Porphridium cruentum*	Sodium selenate	32	14 days	23-35% reduction in rate of cell population growth	10-1,000	Wheeler et al. 1982
Red alga, *Porphyridium cruentum*	Sodium selenate	32	4-5 days	100% mortality	10,000	Wheeler et al. 1982

Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (µg/L)[a]	Reference
Eastern oyster (adult), *Crassostrea virginica*	Sodium selenate	34	14 days	No significant effect on respiration rate of gill tissue	400	Fowler et al. 1981
Striped bass (embryo), *Morone saxatilis*	Sodium selenate	7.2-7.5	4 days	93% successful hatch and survive	200,000	Klauda 1985a,b
Striped bass (larva), *Morone saxatilis*	Sodium selenate	4.0-5.0	4 days	LC50 (control survival= 77%)	13,020	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	9-65 days	Significant incidence of development anomalies of lower jaw	39-1,360	Klauda 1985a,b
Striped bass (juvenile), *Morone saxatilis*	Sodium selenate	3.5-5.5	45 days	Significant incidence of severe blood cytopathology	1,290	Klauda 1985a,b

[a] Concentration of selenium, not the chemical. Units are µg selenium/L of water unless noted otherwise.
[b] Converted from dry weight to wet weight basis (see Guidelines).
[c] Growth of algae was inhibited.
[d] From Smith et al. (1976).
[e] Calculated from the published data using probit analysis and allowing for 8.9% spontaneous mortality.

3.0 OTHER DATA - ENDANGERED SPECIES

Two similar studies were conducted in 1996 and 1997 to determine effects of site water and site food, both contaminated with selenium, on the endangered species, razorback sucker, *Xyrauchen texanus* (Hamilton et al. 2001a,b; published later in a peer-reviewed journal in 2005, see Hamilton et al. 2005 a,b,c). Both studies show marked effects of selenium on survival of razorback sucker larvae exposed to contaminated food and to a lesser extent, contaminated water. Although the data convincingly demonstrate effects to larval survival from exposure to contaminated food, interpretation of the results in the context of chronic criterion derivation is complex because of inconsistencies between: 1) levels of selenium in the food and larvae relative to larval survival; 2) the time to larval mortality relative to selenium in the diet and selenium in the larvae; and 3) levels of other inorganic contaminants in food and water (possible organic contaminants were not measured). A summary of each of these two studies is presented below.

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1996 (Hamilton et al. 2001a; also Hamilton et al. 2005 a,b,c)

This study was initiated with 5-day old razorback sucker larvae spawned from adults (first time spawners) which were previously held (9 months) in three different locations along the Colorado River that contained varying levels of selenium: Horsethief (the designated reference site which receives water pumped directly from the Colorado River near Fruita, CO, and where dissolved selenium concentrations in water ranged from <1.6 to 3.9 µg/L during the period of exposure), Adobe Creek (low level selenium contamination - dissolved selenium concentrations in water ranged from 1.5 to 11.6 µg/L; avg. = 3.8 µg/L), and North Pond (high level selenium contamination - dissolved selenium concentrations in water ranged from 3.8 to 19.6 µg/L; avg. = 9.5 µg/L). The selenium content in eggs from three Horsethief females ranged from 5.8 to 6.6 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 3.4 to 5.0 mg Se/kg dw. The selenium content in the eggs from three Adobe Creek females ranged from 38.0 to 54.5 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 11.5 to 12.9 mg Se/kg dw. The selenium content in the eggs from three North Pond females ranged from 34.3 to 37.2 mg Se/kg dw, and the selenium content in adult muscle plugs at spawning was from 14.1 to 17.3 mg Se/kg dw. The selenium content in eggs from one of three hatchery brood stock females was 7.1 mg Se/kg dw, and the selenium content in muscle plugs of two of three hatchery brood stock females at spawning ranged from 2.6 to 13.8 mg Se/kg dw. The razorback sucker larvae spawned from fish hatchery brood stock (older, previously spawned females) and held in Colorado River (Horsethief) water were used as an additional reference group of test fish.

The experimental groups were subdivided into those receiving reference water (hatchery water; 24-Road Fish Hatchery) or site water (Table E-2). They were further subdivided into those receiving a daily ration of reference food (brine shrimp) or zooplankton (predominantly cladocerans and copepods) collected from each site where their parents were exposed for the previous 9 months. A total of 60 larvae from each of the four adult sources (Horsethief, Adobe Creek, North Pond, Brood Stock held in different ponds at Horsethief) were exposed to each treatment (2 replicates x 3 spawns x 10 fish/beaker). The larvae were held in beakers containing 800 ml of test water. Fifty percent of the test water was renewed daily.

Table E-2. Treatment conditions during the 30-day larval study.

Source of Larvae	Treatments	Se in food (mg/kg dw)	Dissolved Se in water (µg/L)
Horsethief Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9
Adobe Creek Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	5.5
	Site food: Reference water	20	< 1.6
	Site food: Site water	20	5.5
North Pond Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	10.7
	Site food: Reference water	39	<1.6
	Site food: Site water	39	10.7
Hatchery raised Adults	Reference food: Reference water	2.7	< 1.6
	Reference food: Site water	2.7	0.9
	Site food: Reference water	5.6	< 1.6
	Site food: Site water	5.6	0.9

Growth, survival and development were evaluated amongst treatment groups for up to 30 days in the treatment conditions. Each treatment group was fed once daily after renewal. Test waters were collected every day from each site as grab samples for the renewal. A small portion of this water was retained at 3- and 7-day intervals for an analysis of total and dissolved selenium concentrations. At approximately 2-day intervals, aquatic invertebrates and brine shrimp not used for feeding were sieved from the media for selenium analysis. The number of live fish was recorded daily. After the 30-day exposure period, the surviving fish were sacrificed and measured for total length. At this same time,

approximately four fish from each treatment, when available, were collected as a composite sample and analyzed for total selenium.

After 30 days of exposure in the reference food-reference water treatment, survival of razorback sucker larvae from brood stock and Horsethief adults (89 and 87 percent, respectively) was slightly higher than those from Adobe Creek adults (84 percent) and North Pond adults (75 percent). Corresponding selenium concentrations in larval whole-body tissue were 3.6, 3.3, 7.7 and 9.7 mg Se/kg dw, respectively. Survival was similar or slightly reduced in larvae from all four sources after 30 days of exposure in the reference food-site water treatments; corresponding selenium concentrations in larval whole-body tissue were 5.2, 5.1, 12.7 and 15.2 mg Se/kg dw, respectively. In contrast, none of the larvae spawned from parents from Horsethief, Adobe Creek, or North Pond survived to 30 days when fed zooplankton collected from the three sites, irrespective of the water type they were exposed to (i.e., reference or site). Only the larvae from brood stock adults, which were fed zooplankton from the Horsethief site for this treatment, survived, and even these larvae suffered substantial mortality (40 and 60 percent respectively). The mean selenium concentrations in whole-body tissue of larvae from brood stock adults after the 30-day exposures were 5.4 mg Se/kg dw (site food-reference water treatment) and 6.9 mg Se/kg dw (site food-site water treatment).

Several inconsistencies were observed that indicate selenium may not be solely responsible for the effect on larval survival. Larval survival in the Adobe Creek treatment group exposed to reference water (<1.6 µg/L) and reference food (2.7 mg Se/kg dw) was 84 percent, similar to survival of larvae from brood stock (89 percent). The selenium concentration in the larvae from this Adobe Creek treatment group after 30 days was higher (7.7 mg/kg dw) than that of the brood stock fish (5.4 mg Se/kg dw) in the reference water (<1.6 µg/L) and site food (5.6 mg Se/kg dw) treatment, which had a 30-day survival of 62 percent. Also, the time to 50 percent mortality between the site food treatments, where most mortality occurred, was not related to selenium concentration in the diet or in the larvae.

Although the larvae from brood stock held at Horsethief and the larvae from the first-time spawning adults held at Horsethief that were used for the 9 month exposure received the same site food, no larvae from the latter group survived the 30 day exposure. Concentrations of selenium in the larvae of these two treatment groups were essentially the same between days 6 and 12 of the exposure (8.1 to 8.9 mg Se/kg dw). During this same general time frame (6 to 7 days of exposure), larvae from Adobe Creek and North Pond adults apparently tolerated up to 32 and 39 mg Se/kg dw in tissue, respectively, without any increase in mortality when exposed to reference food and reference water. Larvae grown out under hatchery conditions from adults in the Horsethief and Adobe Creek treatments also did not differ in total deformities compared to larvae from brood stock. There was also no difference between treatments (brood stock, Horsethief, Adobe Creek, and North pond) in percent egg viability, percent hatchability,

percent embryos with deformities, and percent mortality of deformed embryos and larvae from a separate test initiated with eggs in the same study (Hamilton et al. 2005b).

Evaluation of Contaminant Impacts on Razorback Sucker held in Flooded Bottomland Sites Near Grand Junction , Colorado - 1997 (Hamilton et al. 2001b)

In a similar 30-day larval study conducted by the authors in the following year (1997), razorback sucker larvae from a single hatchery brood stock female (11 mg Se/kg dw muscle) were subjected to the sixteen different combined water and dietary exposure conditions described in the earlier (1996) study. The female parent was held at Horsethief Canyon State Wildlife Area before spawning. The larvae were held in beakers containing 800 ml of test water as before; fifty percent of the test water was renewed daily. Specific treatment conditions for the 1997 30-day larval study are listed in Table E-3.

Table E-3. Treatment conditions during the 30-day larval study.

Water Treatments	Se in food (mg/kg dw)	Se in water (µg/L)
Reference food (brine shrimp): Reference water (24-Road Hatchery)	3.2	< 1
Reference food: Site water (Horsethief)	6.0	1.6
Reference food: Site water (Adobe Creek)	32.4	3.4
Reference food: Site water (North Pond)	52.5	13.3
Horsethief food: Reference water	3.2	< 1
Horsethief food: Site water (Horsethief)	6.0	1.6
Horsethief food: Site water (Adobe Creek)	32.4	3.4
Horsethief food: Site water (North Pond)	52.5	13.3
Adobe Creek food: Reference water	3.2	< 1
Adobe Creek food: Site water (Horsethief)	6.0	1.6
Adobe Creek food: Site water (Adobe Creek)	32.4	3.4
Adobe Creek food: Site water (North Pond)	52.5	13.3
North Pond food: Reference water	3.2	< 1
North Pond food: Site water (Horsethief)	6.0	1.6
North Pond food: Site water (Adobe Creek)	32.4	3.4
North Pond food: Site water (North Pond)	52.5	13.3

After 30 days of exposure in this study, there was also good survival of razorback sucker larvae fed reference food (brine shrimp) and held in reference water or water from Horsethief (83 and 81 percent, respectively). The survival of these larvae was significantly greater than survival of larvae fed brine shrimp and held in water from North Pond (52 percent). Corresponding selenium concentrations in larval whole-body tissue after 10 days were 6.3, 6.7, and 11 mg Se/kg dw, respectively. The average concentrations of selenium in the water for the three treatments were <1, 1.6, and 13.3 µg Se/L. After 30

days the mean selenium concentrations in these larvae were 5.2, 5.2, and 16 mg Se/kg dw, respectively. Survival was markedly reduced (0 to 30 percent survival) in the remaining treatments where larvae were fed zooplankton from the various sites. Complete mortality was experienced by larvae exposed to Horsethief food and reference water treatment after 30 days.

Similar to the previous study, several inconsistencies in results suggested that selenium may not have been solely responsible for the effect on larval survival. The most notable inconsistency was that the greatest effect on larval survival (percent survival or time to 50 percent mortality) was from exposure to Horsethief food, the food with the lowest selenium contamination.

The authors of the above two studies (Hamilton et al. 2001a,b) make a strong argument that some of the inconsistency in response observed in their studies between larvae fed reference and site diets may be related to the difference in arsenic concentration between the two diets. The arsenic concentration measured in the brine shrimp used in the reference diet was 24 mg total As/kg dw (measured in the second larval study) versus between 6 and 7.5 mg total As/kg dw measured in the zooplankton from the various sites. In their publication (Hamilton et al. 2005c), the authors cite several studies reporting an ameliorating effect of arsenic against the toxicity of a variety of forms of selenium in various animals (Dubois et al. 1940, Hoffman et al. 1992, Klug et al. 1949, Levander 1977, Moxon 1938, Thapar et al. 1969). In terms of the survival of larvae from Horsethief, Adobe Creek and North Pond adults when fed the reference diet, the authors propose that the arsenic concentrations in the brine shrimp diet may have resulted in an antagonistic interaction with selenium and reduced adverse effects in larvae. Such hypothesis is questionable, because their studies included diets spiked with inorganic arsenic salts, whereas the arsenic in brine shrimp (and other natural diets), is most likely predominantly organic arsenic (US EPA 2003). Additionally, in a separate but related study by the same authors (Hamilton et al. 2005d), larval razorback sucker spawned from one female at the Ouray Native Fish Facility were fed zooplankton from six sites (S1, S3, S4, S5, SR, and NR) adjacent to the Green River, Utah at four different initial ages (5, 10, 24, and 28 day old larvae) for 20 to 25 days. The selenium concentrations in zooplankton from the S1 reference site ranged from 2.3 to 3.5 mg Se/kg dw (dissolved Se in water <0.6 to <1.1 µg/L). The concentrations in zooplankton from sites S3 and S4 were slightly higher (range 2.4 to 6.7 mg Se/kg dw; water, 0.3-0.8 µg/L), substantially elevated at S5 (12- 26 mg Se/kg dw; water, 0.6-3.1 µg/L), and highest at SR and NR (44-94 mg Se/kg dw; water, 14-107 µg/L). All larvae in the test initiated when they were 5 days old (study 1) died after 25 days of exposure. Median time to death was shortest in fish fed zooplankton from the reference site (S1) and longest for SR and NR. Interestingly, the concentration of arsenic measured in zooplankton collected from S1 was 12 mg As/kg dw, half that of the brine shrimp used in the above study (Hamilton et al. 2001b), which did not appear to antagonize the toxicity of the

selenium in the diet in this test. In this and the previous two studies, additional inorganic contaminants such as vanadium and strontium were elevated in the zooplankton fed to the larval razorback sucker.

De Riu, D., L. Jang-Won, Huang, S., Monielloa, G., and Hung, S. 2014. Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon. Aquat. Toxicol. 148:65-73.

Test Organisms: Green sturgeon (*Acipenser medirostris*)
White sturgeon (*Acipenser transmontanus*)

Exposure Route: Dietary only
Three different concentrations of L-selenomethionine were added to an artificial diet mixture: nominal concentrations of 0 (control), 50, 100, and 200 mg SeMet/kg (measured: 2.2 mg/kg Se in control diet (no added Se) and 19.7, 40.1 and 77.7 mg/kg Se in the three treatment diets).

Test Duration: 8 weeks

Study Design: Daily rations of the treatment diets (3% BW/d for first 4 weeks and 2% BW/d for second 4 weeks) were fed to the juvenile sturgeon (approximately 30 g). Each of the four dietary treatment consisted of 3 replicate 90 L tanks with 25 juveniles in each tank. Several endpoints were monitored over the 8 week exposure period including survival, percent body weight increase (% BWI), and hepatosomatic index (HSI).

Effects Data: White sturgeon had no mortalities through the highest dietary treatment. Green sturgeon juveniles had 0%, 7.7% and 23.1% mortality with the three dietary treatments (see table below). %BWI had a greater response to selenium concentration in juvenile tissues than HSI (see table below). Of note is the relatively high concentration of Se in the whole body and muscle tissues of the juvenile sturgeon in the control treatment (both species). The reason for the relatively high Se control concentrations was not due to accumulation of Se from the artificial diet because the concentration of Se remained relatively constant over the 8 week exposure.

Chronic Value: TRAP analysis (threshold sigmoid nonlinear regression) of the green sturgeon survival data resulted in a whole body EC_{10} value of 28.93 mg/kg dw. EC_{10} values were lower for % BWI and HSI using TRAP. For % BWI, the whole body EC_{10} value for green sturgeon was 16.36 mg/kg dw, and for white sturgeon, 23.94 mg/kg dw. For HSI, the whole body EC_{10} value for green sturgeon was 10.86 mg/kg dw (with a very wide 95% confidence interval, 1.842-64.08 mg/kg dw), and for white sturgeon there were no discernible effects.

Selenium in Juvenile Sturgeon Tissues and Endpoints Measured at end of Eight Week Exposure

Green Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	7.1	8.4	100	6.6	2
19.7	22.8	31.1	100	2.6	1.3
40.1	27.8	37	92.3	0.8	0.8
77.7	34.3	36.8	76.9	-1	0.9

White Sturgeon

Dietary [Se] mg/kg dw	whole body [Se] mg/kg dw	muscle [Se] mg/kg dw	survival %	%BWI	HIS
2.2 (control)	5.6	9.2	100	4.2	2.6
19.7	20.1	27	100	4.2	3.6
40.1	31.8	41.3	100	2.8	3
77.7	47.1	57.9	100	1	2.2

4.0 OTHER DATA – CHRONIC STUDIES WITH FISH SPECIES

Some chronic studies met the requirements of an acceptable chronic test but were excluded from being included in the data set used for criterion derivation for a variety of reasons. Summaries of these studies are provided below.

Vidal, D., S.M. Bay and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol.49:71-75.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*)

Exposure Route: Dietary only
Selenomethionine was added to dry fish food; the measured dietary concentrations were 4.6, 12 and 18 µg Se/g dw. The measured selenium in the control diet was 0.23 µg Se/g dw.

Test Duration: 90 days

Study Design: Each of the three dietary treatments and control had 5 replicates, each replicate contained 12 to 16 larval rainbow trout that were 27 days old at initiation. Each fish was fed an average of 10 mg/d for 30 days; 25 mg/d on days 30-60; and 40 mg/d thereafter. Fish were sampled on days 30, 60 and 90 for length, weight, selenium, hepatic GSH and thiobarbituric acid-reactive substances (TBARS) measurements.

Effects Data: The authors reported significant decreases in weight and length after the 90-day exposure (Table E-4). There were no significant differences in the hepatic lipid peroxidation and hepatic GSH to GSSH ratios among the treatments. The authors found significant differences in weight and length in the 4.6 and 12 µg Se/g dw dietary treatments, but not the 18 µg Se/g dw treatment. Based on larval trout body burden, the authors reported an LOEC of 1.20 µg/g ww, the concentration of Se in fish fed the 12 µg Se/g dw dietary treatment. The Se concentration in larval rainbow trout associated with the lowest dietary treatment that showed significant decreases in larval weight and length was 0.58 µg Se/g ww or 2.06 µg Se/g dw based on 71.8% moisture in whole body rainbow trout (NCBP).

Chronic Value: The data from this study was not used to calculate a chronic value for selenium due to several inconsistencies. The significant decreases in length and weight observed in the two lowest concentrations were not observed in the highest dietary treatment. The Se concentrations in the larval rainbow trout were irregular with the 60-day concentrations being considerably higher than the 90-day concentrations. The authors explain this observation to rapid growth in the fish causing dilution of the Se body burden. However, the increase in fish weight from 30 to 60 days was similar to the 60 to 90 day increase and the 60 day Se concentrations increased from day 30. Also, the Se concentration in the control fish went from below detection on day 0 to 0.46 µg/g ww on day 30; to 1.24 µg/g ww on day 60; and to 0.31 µg/g ww on day 90. The 60-day measured Se in the control fish (1.24 µg/g ww) was more than twice the concentration of Se in the fish with lowest concentration showing effects (0.58 µg/g ww).

Table E-4. Mean (SD) rainbow trout growth after four SeMet dietary treatments.

test day	Treatment, µg/g dw	weight, g	fork length, cm	[Se] whole body, µg/g ww	[Se] whole body, µg/g dw**
0	control	0.37 (0.30)	3.14 (0.41)	ND	ND
30	control	1.33 (0.92)	4.66 (0.41)	0.46 (0.20)	1.63
	4.6	1.25 (0.21)	4.84 (0.29)	1.05 (0.77)	3.72
	12	1.33 (0.30)	5.09 (0.46)	1.81 (1.04)	6.42
	18	1.31 (0.37)	4.97 (0.50)	1.60 (0.93)	5.67
60	control	2.96 (0.92)	6.91 (0.56)	1.24 (0.54)	4.40
	4.6	2.33 (0.63)	6.69 (0.67)	1.70 (0.72)	6.03
	12	2.52 (0.38)	6.88 (0.35)	1.83 (0.94)	6.49
	18	2.59 (0.24)	6.92 (0.24)	2.62 (1.22)	9.29
90	control	5.17 (1.09)	7.70 (0.33)	0.31 (0.20)	1.09
	4.6	3.45 (0.35)*	6.93 (0.19)*	0.58 (0.21)	2.06
	12	3.45 (0.35)*	6.84 (0.68)*	1.20 (0.21)*	4.25
	18	3.82 (0.62)	7.37 (0.62)	1.41 (0.27)*	5.00

* Significantly different than the control.

** ww converted to dw using 71.8% moisture for whole body rainbow trout (NCBP).

Pilgrim, N. 2009. Multigenerational Effects of Selenium in Rainbow Trout, Brook Trout, and Cutthroat Trout. Master's Thesis. University of Lethbridge.

Test Organisms: Rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*) and brook trout (*Salvelinus fontinalis*)

Exposure Route: Dietary only
Selenomethionine added to trout chow and gelatin. Two dietary treatment levels, nominal Se concentrations, 15 (low) and 40 (high) mg/kg.

Test Duration: Rainbow trout were fed the experimental diets from August - December 2009, brook trout July - November 2010, and cutthroat trout December 2010 - April 2011.

Study Design: Fish were obtained from a fish hatchery brood stock. Mature females and were fed the experimental diets in 710 L tanks. Spawning was stimulated by injecting Ovaprim® into the females. Eggs were fertilized and incubated at the fish hatchery until the eye spots were visible. A portion of the eyed stage larvae from each treatment was shipped to the University of Lethbridge Aquatic Research Facility for the swim-up stage of the experiment conducted in gravel bed flumes. Endpoints measured included percent survival in the first (spawned eggs to eyed eggs) and second (eyed eggs to yolk-absorbed fry) stages of development, swim-up success, and malformations (spinal, craniofacial and finfold deformities and edema).

Effects Data: Selenium affected larval survival, swim-up success and the percent of malformations in larvae in one or more of the three species tested (see table below). Visual inspection of plots of the replicate data in Pilgrim (2009) showed considerable variation between the endpoints and selenium in eggs. The distribution of selenium among the tissues was markedly inconsistent with other studies that have used these species. For example, the amount of selenium in the eggs was 8 and 18 times greater than the concentration in the respective muscle tissues in cutthroat and rainbow trout. Median ratios (egg Se:muscle Se) calculated for rainbow trout (Casey and Siwik 2000; Holm et al. 2005) and cutthroat trout (Golder 2005; Kennedy et al. 2000; Rudolph et al. 2007) were 1.9 and 1.8, respectively. Due to the considerable variation in the concentration response of the replicate data and anomalous selenium distribution, these data were not included in the data set to derive the criterion.

Table E-5. Mean selenium concentrations in the diet and selected tissues and selected endpoints measured in rainbow trout (RN), brook trout (BK) and cutthroat trout (CT).
Adapted from Table 3.1 in Pilgrim (2009).

		Tissue, mg/kg ww			Survival, %			
Species	**Diet ww**	**Muscle**	**Liver**	**Egg**	**Stage 1**	**Stage 2**	**Swim-up success**	**Total malformations, %**
	1.47	0.21	3.77	1.17	82.36	61.56	57.18	10
	12.7	0.51	6.53	4.30	77.86	48.64	73.83	9.86
RBT	35.2	0.74	17.21	13.0	54.72	30.33	27.45	29.63
	1.47	0.23	0.72	0.81	86.3	82.68	84	21.3
	12.7	1.14	7.23	5.01	71.37	88.72	83.42	23.93
BK	35.2	3.41	20.4	8.15	71.37	44.63	50.11	24.23
	1.47	0.31	1.00	2.02	61.41	61.87	55.3	6.13
	12.7	0.93	6.00	9.80	30.65	14.75	21.71	48.06
CT	35.2	2.05	14.4	18.0	21.99	0	0.08	NA

Formation Environmental. 2012. Appendix E – Yellowstone Cutthroat Trout Adult Laboratory Reproduction Studies. Technical Support Document: Proposed Site-Specific Selenium Criterion, Sage and Crow Creeks, Idaho. Prepared for J.R. Simplot Company. January 2012.

Test Organism: Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*)

Exposure Route: Field collected. Adult female and male Yellowstone cutthroat trout were collected at five field sites from four streams near the Smokey Canyon mine. In addition Yellowstone cutthroat trout eggs were obtained from a hatchery as method controls.

Test Duration: Test duration was from hatch through 15 days post swim up, and averaged 55-56 days for larvae hatched from field collected fish and 64 days for larvae hatched from laboratory collected fish.

Study Design: Eggs were collected from 15 ripe females at five sites from four streams upstream and downstream of the Smokey Canyon mine. This included one selenium impacted stream downstream of the mine, Sage Creek (LSV), one site along Crow Creek upstream of Sage Creek (CC-150) and one site along Crow Creek downstream of Sage Creek (CC-350), and in sites within the reference streams Deer Creek (DC), and South Fork Tincup Creek (SFTC). Eggs were fertilized in the field with milt collected from males collected at the same site as females. Fertilized eggs were water hardened at the site using stream water, then placed in oxygenated plastic bags and stored on ice in the dark (cooler) for transportation to laboratory. In addition, eggs were collected from 16 ripe females obtained from Henry's Lake hatchery (HL) to serve as method controls. Hatchery females were stripped of eggs and fertilized by milt from males obtained from the same hatchery. For field and hatchery fish, Se was measured in adult fish (whole body) and in eggs of field collected females.

A target of approximately 600 fertilized eggs from each female (were placed in egg cups for hatching and monitoring. After swim up, remaining fry were thinned to a target of 100 fry/treatment and monitored for an additional 15 day post swim up feeding trial.

Endpoints measured in the laboratory were hatch, survival (hatch to swim up, and hatch through 15 days post swim up), and deformities. Deformities were combined as assessed as having at least one deformity, or being fully free of deformities (i.e., normal).

Effects Data: Eggs failed to hatch for one of the field treatments (SFTC-1), and six of the hatchery treatments, resulting in a final dataset of eggs fertilized from 14 field collected fish and 10 hatchery fish. Se concentrations in eggs obtained from field collected females ranged from 11.4 mg/kg in Deer Creek through 47.6 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in eggs obtained from Henry's Lake hatchery fish ranged from 0.83 mg/kg – 3.23 mg/kg (Table E-6). Se concentrations in whole body tissue samples obtained from field collected females ranged from 8.17 mg/kg in Deer Creek through 25.7 mg/kg in Crow Creek downstream of Little Sage Creek (Table E-6). Se concentrations in whole body tissue samples obtained from Henry's Lake hatchery fish ranged from 0.23-0.91 mg/kg (Table E-6).

Table E-6. Yellowstone cutthroat trout selenium concentrations, survival, and deformity data from hatch to test end.

Sample ID[a]	Egg Se mg/kg	WB[b] Se mg/kg	# Free From Deformities	# Assessed For Deformities	# Died	# Survived	# Assessed + # Died
CC-150/001	17.6	16.3	22	182	33	182	215
CC-350/001	27.9	20.7	14	138	120	138	258
CC-350/002	29.7	19.4	143	602	83	602	685
CC-350/003	22.3	17.0	73	330	36	330	366
CC-350/004	14.6	16.7	149	480	19	480	499
CC-350/005	47.6	25.7	91	392	71	392	463
DC/001	22	8.17	95	275	30	275	305
DC/002	15.4	9.07	133	465	26	465	491
DC/003	11.4	8.63	59	380	39	380	419
DC/004	12.7	16.6	7	38	23	38	61
HL/002	2.03	0.45	5	39	10	39	49
HL/003	2.48	0.44	121	302	19	302	321
HL/004	1.36	0.36	154	416	20	416	436
HL/006	0.83	0.36	21	244	103	244	347
HL/007	2.26	0.44	120	404	18	404	422
HL/008	1.87	0.28	147	412	37	412	449
HL/011	3.23	0.31	69	296	22	296	318
HL/012	1.58	0.23	112	454	27	454	481
HL/013	1.93	0.72	148	483	24	483	507
HL/015	2.06	0.91	0	36	6	36	42
LSV2C/001	40.1	19.4	2	200	536	0	536[c]
LSV2C/002	30.0	21.0	40	319	105	319	424
LSV2C/003	35.6	18.6	92	487	138	487	625
LSV2C/004	30.5	22.5	107	476	75	476	551

a – CC – Crow Creek; DC – Deer Creek; LSV2C – Sage Creek; HL – Henry's Lake (Hatchery)
b – whole body
c – does not include the 200 fish assessed that were dead prior to assessment, as all fish for that treatment died during the swim up stage in this sample.

Figure E-1 is a plot of % free from deformities versus egg concentration. The previous draft used TRAP to estimate an effect level for these data but after further review it was concluded these data just do not demonstrate any clear effect of Se and therefore inappropriate for analysis by TRAP. There is no obvious trend, especially one that is substantial relative to the data variability. The correlation coefficient for these data is not significant and a t-test of the two data clusters is likewise not significant. The survival data also do not show a useful trend, especially one suitable for EC10 estimation. Although no effect concentration was determined for this test, the data do not contradict the other cutthroat trout datasets in that there are no effects up to 30 mg/kg and of the three points in excess of 30 mg/kg, one did show 100% mortality. The data are consistent with *Oncorhynchus* not being one of the four most sensitive genera.

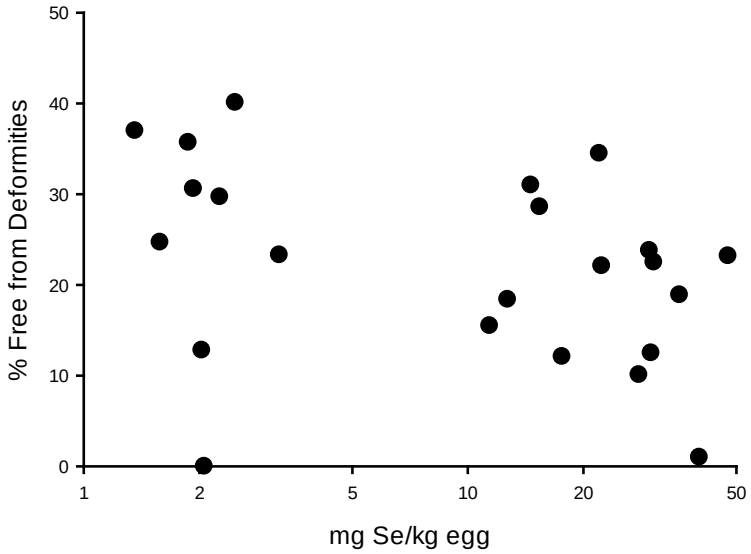


Figure E-1. Plot of percent free from deformities relative to the concentration of selenium in cutthroat trout eggs.

Effect Concentration: NA

Deng, X. 2005. Early life stages of Sacramento splittail (*Pogonichthys macrolepidotus*) and selenium toxicity to splittail embryos, juveniles and adults. Doctoral dissertation, University of California, Davis.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*)

Exposure Route: Dietary only
Four concentrations of selenium in the fish diet (0.6, 17.3, 33.0, and 70.1 mg/g) were created by mixing different proportions of selenized and Torula yeast. A different batch of selenized yeast was used in the adult exposure.

Test duration: 24 weeks

Study Design: Fourteen adult fishes were placed in each circular tank (92 cm diameter, 33 cm height) and fed one of the four diets. Each diet was provided to fishes in three tanks. The twelve tanks were arranged in three rows. Each row had all four treatment concentrations with randomly assigned positions. Thus, the experiment had a randomized block design. Adult splittail fishes were obtained from the Tracy Pump Station (U.S. Bureau of Reclamation, Tracy, CA). After 12 and 24 weeks of exposure, blood samples were collected, the liver, gonad, kidney and white muscle were dissected, and liver and gonad were weighed to calculate hepatosomatic and gonadosomatic indices. Stages of ovarian and testicular development were determined from histological studies.

Effects Data: No mortality occurred throughout the experiment. Fish in control, 17.3, and 33.0 mg/g treatments exhibited normal behavior. Fish exposed to 70.1 mg/g in did not consume as much food as fishes exposed to lower selenium concentrations, and displayed abnormal behaviors. Splittail adults were less sensitive to dietary selenium than juveniles. Relative to control, no changes in body weight, total length, GSI, and condition factor were observed in fishes exposed to selenium concentrations in food up to 33 mg/g. In general, tissue concentrations in fishes exposed to selenium were higher than in the control, but differences in selenium concentrations among them were often small and not significant (Table E-7). Percentages of ovaries with atretic follicles increased with higher concentrations of selenium in their diet: 30% in control, 45.5% in the 17.3 mg Se/g, and 100% in the 33.0, and 70.1 mg/g treatments. The average concentration of selenium in ovaries of fish exposed to 17.3 mg/g in their diet was 6.5 mg/g. This low effect level, though, is disputable because of the very low number of ovaries analyzed, the occurrence of atresia in 30% of ovaries in control, and the lack of significant differences in concentrations of selenium in ovaries among treatments exposed to elevated levels of this element.

Table E-7. Mean concentration of selenium in ovaries (SE).[‡]

	Diet Concentration (mg Se/g)			
	0.6	17.3	33.0	70.1
[Se] in ovary (mg/g dw)	4.4 (0.57)	6.5 (1.0)	8.3 (0.14)	8.9 (0.46)

[‡] Values estimated from Figure 4 in Deng (2005) (pg. 111)

de Rosemond, K. Liber and A. Rosaasen. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker. Bull. Environ. Contam. Toxicol. 74: 1134-1142.

Test Organism: White Sucker (*Catostomus commersoni*)

Exposure Route: Field collected.
In June, 2002, eggs were collected from 4 females from Island Lake (exposed site); milt was obtained from 2 males. Island Lake is downstream from Cluff Lake uranium mine located in northern Saskatchewan. Selenium concentrations in Island lake range from 1 to 11 µg/L and in recent years have been typically 4-5 µg/L. No fish/eggs were collected from a reference site.

Test duration: Through the end of yolk absorption by the larvae; 33 days post-fertilization.

Study Design: Individual batches of eggs were fertilized in the field with milt and water-hardened. Eggs were air transported to the laboratory in Saskatoon for testing. 200 eggs were randomly selected from each clutch and then separated into groups of 100 which were placed into individual test chambers (n = 8).

On test day 30 (3 days prior to test termination), all fish larvae that exhibited macroscopic deformities (e.g., kyphosis, lordosis, scoliosis and edema) were removed, photographed and preserved. At test termination, (day 33), 40 larvae from each female whites sucker were evaluated for deformities using a microscope.

Effects Data: Although all four females were collected from the exposed site, selenium concentrations in eggs were grouped into two low (Fish 2 and 3 in Table E-8) and two high (Fish 1 and 4 in Table E-8). Larval mortality and developmental deformities were not related to selenium concentrations in eggs (Table E-8). The data suggest that embryo/larval effects are not observed at concentrations in eggs reaching 40.3 mg/kg dw (geometric mean of the two high selenium concentrations in eggs). However, because a reference condition with low selenium exposure was not established, it is not appropriate to estimate an effect concentration for this study. Note: the average percent moisture for the four clutches of eggs was 92.6%.

Effect Concentration: NA

Table E-8. Embryo/larval endpoints for eggs from four female white sucker collected from Island Lake in June 2002.

Measurement	Fish 1	Fish 2	Fish 3	Fish 4
Successfully hatched larvae[a]	161	140	176	141
Deformed larvae[b]	21	25	16	13
Dead larvae[c]	6	14	6	4
Macroscopic deformities , %				
Embryological[d]	6.8	6.4	5.7	1.4
Developmental[e]	6.2	11.4	3.4	7.8
Microscopic deformities, %				
Developmental[f]	7.5	5	2.5	7.5
Total developmental deformities, %[g]	13.7	16.4	5.9	15.3
[Se] eggs mg/kg ww[h]	2.7	0.7	0.6	3.2
[Se] eggs mg/kg dw[h]	33.6	9.4	8.4	48.3

[a] Initial number was 200 per fish

[b] Total number of deformed larvae throughout study; includes embryological and macroscopic deformities

[c] Total number of larvae that died throughout study.

[d] Percent of curled deformities that appeared in embryonic fish; deformities were evident immediately after embryos hatched.

[e] Percent of deformities that were designated developmental; deformities became evident as larvae grew and absorbed yolk sac (after experimental day 15).

[f] Percent of microscopic developmental deformities that were evident in the 40 fish examined per female white sucker.

[g] The estimated percentage of offspring that had microscopic and macroscopic developmental deformities combined.

[h] Selenium concentration measured in a subsample of embryos collected on test day 0.

Ogle, R.S. and A.W. Knight. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). Arch. Environ. Contam. Toxicol. 18:795-803.

Test Organism: Fathead minnows (*Pimephales promelas*; juvenile, 59 to 61 d old)

Exposure Route: Dietary only
Purified diet mix spiked with inorganic and organic selenium: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine, homogenized in dextrin.

Test Treatments: Completely randomized block design (2 blocks); 4 replicates per block (n = 8 replicates total per treatment). Actual mean total selenium levels in each exposure treatment were: 0.4 (control), 5.2, 10.2, 15.2, 20.3, and 29.5 mg/kg dw. Fish used in the first randomized block (F_2 generation fish) were progeny from F_1 generation originally obtained from the Columbia National Fishery Research Laboratory, some of which were used in an initial range-finding experiment. Fish obtained from a commercial supplier were used in the second randomized block. The prepared diet was extruded into 1.5 mm pellets which were air-blown dried to 5 percent moisture content and crushed and sieved so that only particles retained by an 11.8 mesh/cm sieve were used in the study. The amount of selenium in water that leached from the food during the experiment averaged only 0.8 μg/L.

Test Duration: 105 days, F_2 generation (block one) and commercial fish (block two);
14 days F_3 generation

Study Design: Ten fish were randomly placed in each cell per block (n = 8x10, or 80 fish total per treatment). Fish were fed twice daily at 6 percent body weight per day, with wastes and uneaten food removed 30 min. after each feeding. Test tanks were flushed with two tank volumes of fresh test water after each feeding (solution renewal). Growth (as wet weight) was determined every two weeks by bulk weighing, and one fish from two of the cells per treatment in a given block (n = 4 total per treatment) was removed for selenium (whole-body) analysis. After 105 days of exposure, a single male and female fish from each treatment replicate (n = 4 breeding pairs per treatment in a given block, or 8 breeding pairs per treatment total) were placed in 250 ml beakers and inspected for spawning activity for 30 days following the first spawning event for that pair (each pair being one replicate). Gonads and muscle tissue were dissected for selenium analysis from these fish at the end of the 30 days spawning period. The spawning substrates were inspected daily for eggs to determine fertility and viability. Samples of not more than 50 eggs from each spawn were incubated in flowing, aerated water and inspected for percent hatch determination. Ten larvae from each incubated brood were transferred to separate glass test chambers and maintained (48 h renewal; fed brine shrimp twice daily) for 14 days to determine percent larval survival.

Effects Data: There was no effect of selenium on any of the reproductive parameters measured at the dietary concentrations tested. Percent hatch and percent larval survival were very high (>87.4 percent) and essentially equal for all of the treatments. Growth of pre-spawning adults was affected by the selenium exposure (Table E-9).

Table E-9. Effects on fathead minnow growth after 98 days of exposure to dietary selenium.

Measured mean selenium in diet, mg/kg dw	Whole-body selenium, mg/kg dw	Mean fish weight, g ww
0.4	1.76	1.30
5.2	2.78	1.24
10.2	3.42	1.20
15.2	5.40	1.21
20.3	6.58	1.09
29.5	7.46	0.94

Chronic Value: An EC value could not be calculated for these data because the data did not meet the minimum requirements for analysis.

GEI Consultants. 2008. Maternal Transfer of Selenium in Fathead Minnows, with Modeling of Ovary Tissue to Whole Body Concentrations.

Test Organism: Fathead Minnow (*Pimephales promelas*)

Exposure Route: Field collected.
Gravid adult fathead minnows were collected from creeks with a wide range of surface water selenium concentrations near the city of Denver, CO during the 2006 summer breeding season.

Sites
Low selenium exposure:

- Sand Creek at Colfax. In 2002, aqueous selenium averaged 0.9 µg/L.

Moderate to high selenium exposure:

- Sand Creek downstream of refinery
- East Tollgate Creek
- Mainstem Tollgate Creek

Control fish – no field exposure

- Laboratory-reared fish from Aquatic BioSystems

Test duration: Embryo-larval test was 48 hours post hatch.

Study Design: Field collected adult fish were either field dissected for selenium measurement in paired tissues or transported live back to the laboratory in coolers with site water. Fish were transported to the laboratory where mating pairs were bred in individual chambers containing spawning substrates. Eggs were removed from the spawning substrate and reared in a standard Falcon dish with lab water. Eggs were screened under a dissecting microscope for viability. Dead eggs were removed and numbers recorded on a datasheet. Three separate breeding experiments were conducted.

Upon hatching, larvae were moved to standard bioassay cups containing lab water and maintained in the laboratory incubator at 25ºC. Larvae were maintained via static conditions in exposure cups for 48 hours post-hatch without food to ensure full absorption of the yolk sac before they were fixed in formalin. Deformity assessment was performed on fixed embryos using a dissection microscope. Test endpoints consisted of egg production, fertilization success, mortality, and deformities (includes edema and skeletal, craniofacial and finfold malformations). The authors used a graduated severity index (GSI) for deformities in which larvae were scored 0 (normal), 1 (slight), 2 (moderate), and 3 (severe) based on the level of defect.

Effects Data: All fish successfully spawned except those collected from Sand Creek downstream from the refinery. These fish had visible parasites and were only used in the ovary-to-whole body selenium analysis. A suite of metal and metalloids were measured in fish samples from each location. Fish collected from East Tollgate Creek had higher concentrations of 9 of the 15 metals that were

measured in fish from at least one site. Aluminum and iron showed the highest difference with an approximate 10-fold increase in the East Tollgate Creek fish.

Only the first brood of each mating pair was used for the analysis because effects appeared to be muted in subsequent broods. The lower response in the second brood was thought to be due to clearing of selenium in the oocytes. There was poor correlation between egg fertilization ($R^2 = 0.13$) and embryo mortality ($R^2 = 0.18$) data with whole body selenium concentrations in the adult fish (see Table E-10 for summary data; see Table E-11 for individual brood data). Neither the fraction of embryos surviving nor fertilization rate as a function of the concentration of selenium in maternal fathead minnows was suitable for estimating EC values. Although there were low survival and fertilization rates at some higher selenium concentrations, these responses were quite varied and did not follow a defined concentration-response relationship (Figure E-2).

Of the 9 broods from fish collected at the three exposed sites only one brood (from East Tollgate Creek) had deformities greater than 10%. The fathead minnow females that produced the brood with the greatest number of deformities and highest GSI also had the second highest concentration of whole body selenium, 46.4 mg/kg dw (Table E-12; Figures E-3 and E-4). Approximately half of the larvae from this brood exhibited some sort of malformation. Similar to the embryo parameters, EC values were not able to be estimated for any of the 4 malformation parameters.

The authors used probit analysis and TRAP to determine effect levels for each of the embryonic and larval endpoints (Table E-13). Although there is an indication of effect due to selenium exposure in both the embryonic and larval endpoints, there is too much variation in the responses observed with the embryos and insufficient response observed with the larvae to derive a reasonable estimate of effect levels. Therefore, no effect level was determined for this study.

Effect Concentration: Unable to determine due to high variability or insufficient response.

Table E-10. Mean fathead minnow first brood embryo and larval parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); CON = control, SCC = Sand Creek at Colfax Avenue bridge, TGC = Tollgate Creek, and ETC = East Tollgate Creek.

Parameter	Site			
	Con	SCC	TGC	ETC
n (number of breeding pairs)	10	3	3	4
WB Se concentration (mg/kg dw)	2.86 ± 0.18	9.17 ± 0.46	35.87 ± 3.73	44.53 ± 2.41
Egg fertilization (%)	84.75 ± 3.32	23.99 ± 22.45	63.42 ± 31.82	59.6 ± 22.26
Embryo mortality (%)	22.03 ± 3.34	89.04 ± 9.70	46.40 ± 26.86	50.76 ± 23.63
Mean spawn size (# of eggs per spawn)	129 ± 23	318 ± 63	162 ± 61	317 ± 158
Total larva evaluated (total # of broods)	957	89	281	254
Mean brood GSI score	4.85 ± 1.22	8.88 ± 8.88	14.88 ± 4.63	21.75 ± 9.53
Larval craniofacial defects (%)	2.64 ± 0.90	4.65 ± 4.65	6.26 ± 3.63	18.48 ± 13.84
Larval skeletal defects (%)	4.74 ± 0.89	9.30 ± 9.30	6.21 ± 1.48	19.62 ± 12.11
Larval finfold defects (%)	2.19 ± 0.78	4.07 ± 4.07	5.71 ± 3.08	17.23 ± 14.48
Larval edema (%)	3.89 ± 1.01	5.23 ± 5.23	6.26 ± 3.63	20.32 ± 12.93
Larval length (mm)	4.90 ± 0.05	4.97 ± 0.12	4.83 ± 0.14	4.90 ± 0.07

Table E-11. Fathead minnow first brood embryo parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); for site acronyms see Table E-9.

Brood	Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-1a-1	CON		2.90	19	0.79	0.96
T-1f-1	CON		3.24	238	0.77	0.88
T-1f-1	CON		1.94	19	0.63	0.73
T-2a-1	CON		2.25	135	0.98	0.98
T-3a-1	CON		2.71	154	0.68	0.72
T-3b-1	CON		2.64	90	0.90	0.95
T-3d-1	CON		3.67	76	0.70	0.71
T-4d-1	CON		3.43	199	0.85	0.91
T-5d-1	CON		3.33	149	0.73	0.87
T-6d-1	CON		2.52	183	0.76	0.78
T-2b-1	SCC		9.92	395	0.00	0.00
T-4a-1	SCC		8.35	193	0.03	0.03
T-6a-1	SCC		9.25	340	0.30	0.69
T-2a-1	TGC		32.29	132	0.83	0.91
T-3a-1	TGC		43.33	79	0.00	0.00
T-4a-1	TGC		31.99	262	0.77	1.00
T-1f-1	ETC		39.76	141	0.52	0.70

Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total eggs (total dead+total hatch+not hatched)	Survival fraction (total dead/total eggs)	Fert. Rate ((Initial Egg Count - 1st day mortalities)/Initial Egg Count)
T-3b-1	ETC	47.47	208	0.88	0.92
T-5a-1	ETC	46.37	634	0.07	0.17

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

Brood Code	Treatment	Maternal WB Se Conc dw (mg/kg)	Total Larvae	Spinal Incidence	%larvae w/o spinal deformity	%larvae w/o craniofacial deformity	%larvae w/o finfold deformity	%larvae w/o edema	Total GSI Score
T-1f-1	CON	1.94	11	9	91	100	100	100	1
T-2a-1	CON	2.25	141	3	97	99	98	96	24
T-6d-1	CON	2.52	117	2	98	99	99	97	16
T-3b-1	CON	2.64	81	4	96	98	99	98	12
T-3a-1	CON	2.71	96	1	99	100	100	100	1
T-1a-1	CON	2.90	14	7	93	93	93	93	10
T-1f-1	CON	3.24	189	8	92	98	98	94	53
T-5d-1	CON	3.33	95	4	96	97	99	98	20
T-4d-1	CON	3.43	164	3	97	98	99	96	28
T-3d-1	CON	3.67	49	6	94	92	94	90	29
T-4a-1	SCC	8.35	3	0	100	100	100	100	0
T-6a-1	SCC	9.25	86	19	81	91	92	90	71
T-4a-1	TGC	31.99	190	5	95	97	97	97	41
T-2a-1	TGC	32.29	91	8	92	90	91	90	78
T-1f-1	ETC	39.76	65	5	95	95	98	94	20
T-5a-1	ETC	46.37	39	44	56	54	54	54	152
T-3b-1	ETC	47.47	150	11	89	95	96	91	89

Table E-13. Authors calculation and comparison of fathead minnow larval deformity EC_{10} estimates using probit analysis and TRAP.

Effect	Endpoint	Probit Results WB [Se] mg/kg, dw (±SE)	TRAP Results WB [Se] mg/kg, dw (95% CL)	Probit Results Ovary [Se] mg/kg, dw (±SE)	TRAP Results Ovary [Se] mg/kg, dw (95% CL)
Edema	EC_{10}	39.48 ± 16.21	45.78 (40.95 - 51.20)	52.99 ± 19.99	61.43 (55.04 – 68.55)
Finfold	EC_{10}	68.55 ± 27.26	48.31 (39.41 - 59.21)	87.95 ± 32.16	64.81 (53.01 – 79.24)
Skeletal	EC_{10}	27.80 ± 9.53	46.08 (41.94 - 50.62)	38.67 ± 12.32	61.82 (56.36 – 67.80)
Craniofacial	EC_{10}	53.86 ± 18.77	47.41 (38.92 - 57.76)	70.83 ± 22.84	63.56 (52.37 – 77.16)
All abnormalities	EC_{10}	16.98 ± 5.38	45.50 (41.10 - 50.37)	24.23 ± 7.06	61.06 (55.26 – 67.48)
All abnormalities except edema	EC_{10}	21.35 ± 6.45	45.69 (41.10 - 50.79)	30.32 ± 8.51	61.27 (55.23 – 67.97)

Figure E-2. The fraction total survival of embryos (top left), fraction of embryos successfully fertilized (right), survival adjusted for fertilization (bottom) versus maternal whole body selenium concentration. Bottom figure EC10=35.2 mg/kg Se dw WB.

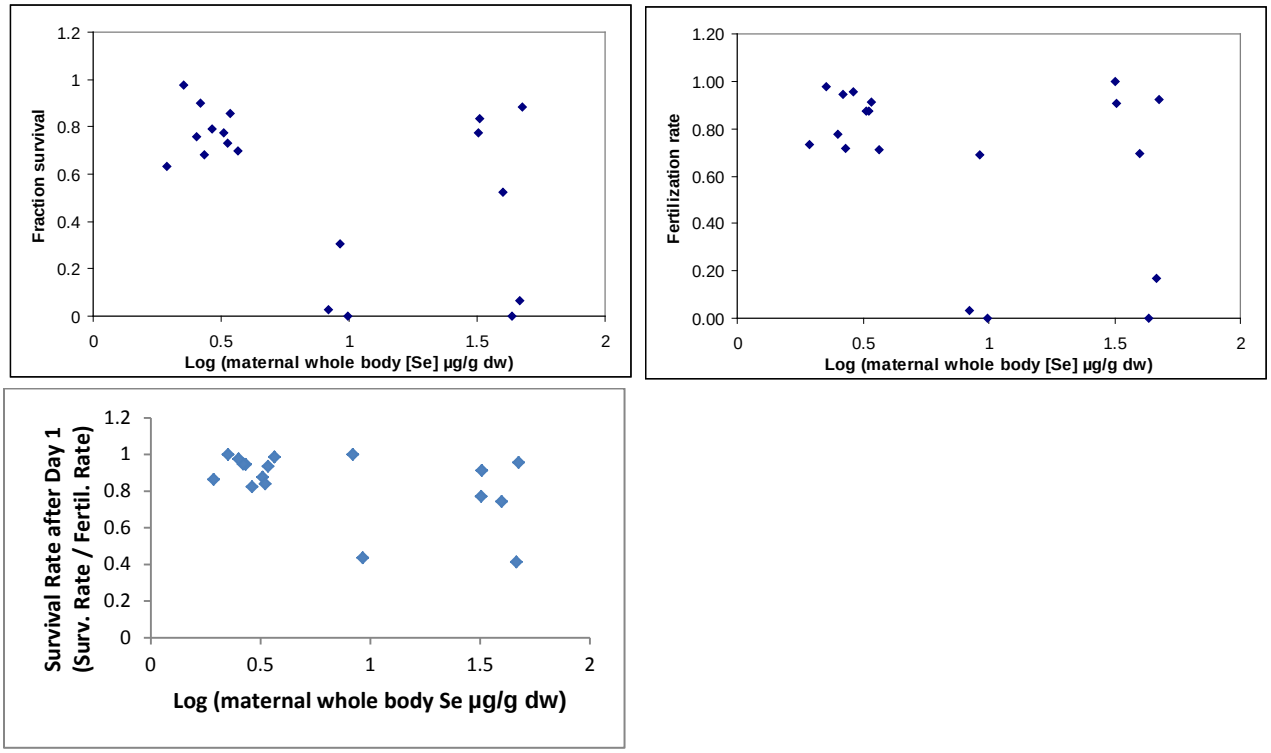


Figure E-3. Percent 2-day post-hatch larvae without edema (A), finfold deformity (B), craniofacial deformity (C), and spinal deformity (D) relative to maternal whole body selenium concentration. EC10s: 61.4 – 64.8 mg/kg dw WB.

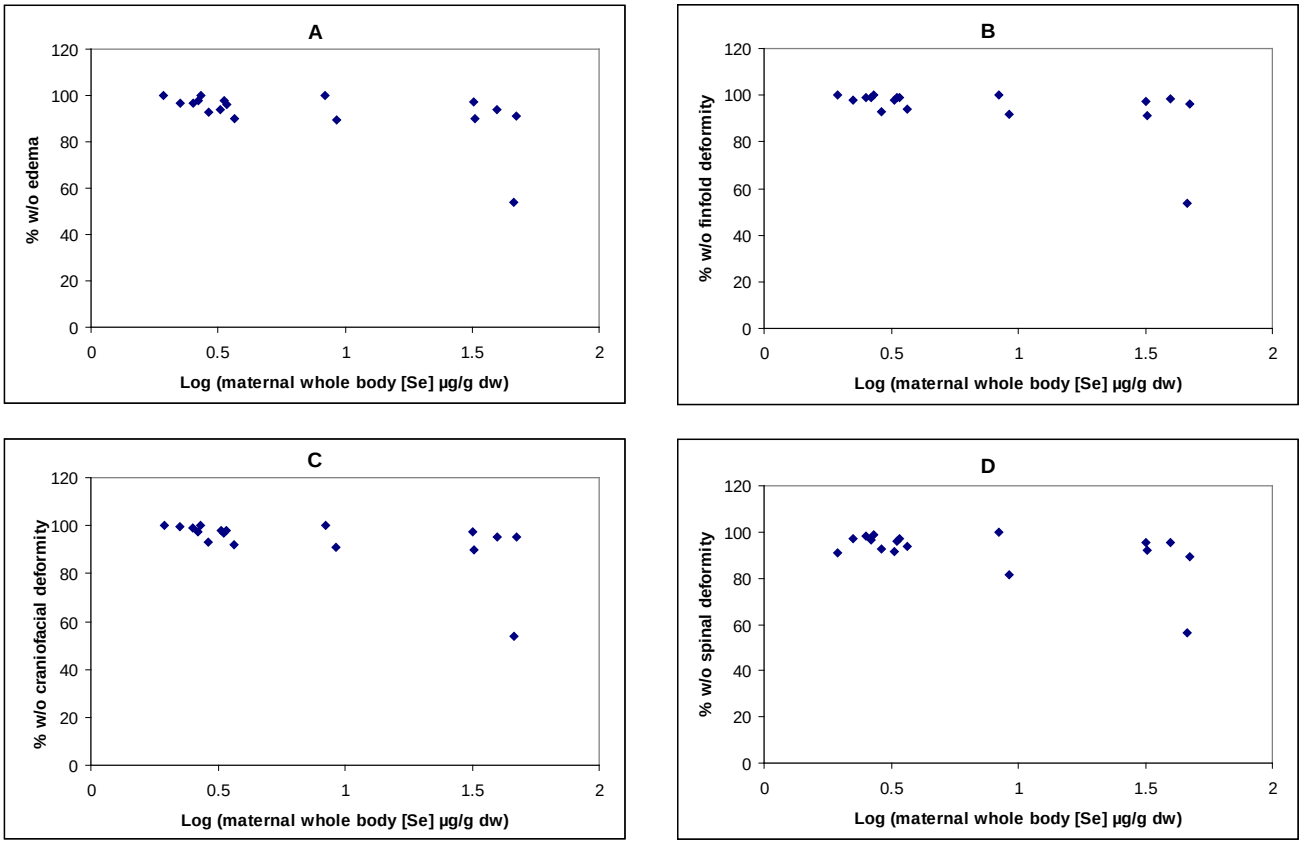
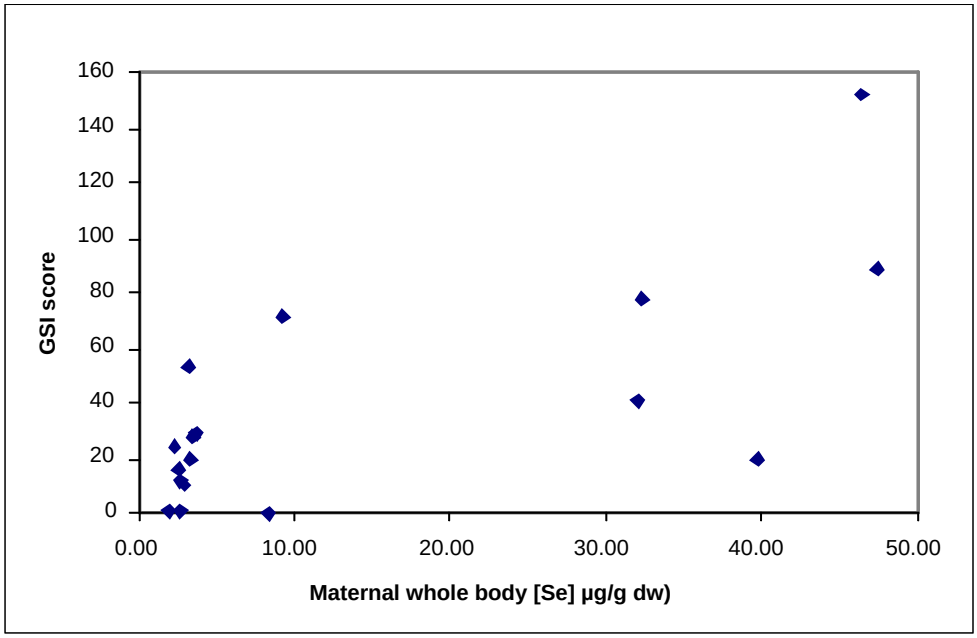


Figure E-4. Percent 2-day post-hatch larvae Graduated Severity Index (GSI) relative to maternal whole body selenium concentration



4.1 Evaluation of zebrafish (Danio rerio) and native cyprinid sensitivity to selenium

Overview:

Two new studies on zebrafish (*Danio rerio*), Thomas and Janz (2014), Thomas (2014), and Penglase et al. (2014), were made available to EPA by David Janz, one of the external peer reviewers. Thomas (2014) and Thomas and Janz (2014) were the original dissertation and peer reviewed paper, respectively, of the same body of work. The apparent sensitivity of the zebrafish to selenium relative to other species in the EPA selenium criteria document was the subject of several public commenters, as well as Dr. Janz in the comments received by EPA.

EPA calculated an EC10 of 7.004 mg Se/kg egg dw, or approximately 3.5 mg/kg whole body) from the Thomas (2014) and Thomas and Janz (2014) study. EPA was not able to calculate an EC10 from Pengalese et al. (2014). The Thomas (2014) and Thomas and Janz (2014) study is summarized in the following section (Part I). Penglase et al. (2014) is summarized in section 7.1.5 of the main document.

EPA noted that the concentration-response curves for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species. The shallow slope indicates partial effects across the range of test doses, with some individuals being very sensitive, and others being less sensitive than other test species. A typical test signature of the nutritionally essential element selenium is that above a particular concentration there is a precipitous increase in adverse effects, with most test organisms affected within a narrow dose range. Additional issues discovered during the analysis of available information in the literature and supplied by the investigator raised questions of test quality that introduced uncertainty in the results reported. This uncertainty, and the fact that zebrafish may not represent the sensitivity range for cyprinids native to the US (discussed in Part II), led to the decision to include this study qualitatively in the effects characterization.

The paucity and relative insensitivity of the available data for cyprinids (fathead minnow EC10 = < 23.9 mg/kg dw; based on LOEC in ovary) relative to other fish families like centrarchids (sunfish), and salmonids (trout and salmon) caused additional concern. This led EPA to investigate the field significance of the zebrafish EC10 (7.004 mg/kg egg) compared to what we know about cyprinid occurrence in selenium impacted waters. The available studies with native cyprinids indicate that a variety of native cyprinid genera (e.g. chubs, shiners, dace) have stable, diverse populations and are reproducing successfully (based on length frequency data) in selenium impacted waters at whole body concentrations far exceeding our proposed whole body criterion element of 8.0 mg/kg dw. Taken together, the available studies (Hamilton et al. (1998), NAMC (2008), Presser (2013), USGS (2012)), indicate that native cyprinids as a family are not expected to be overly sensitive to selenium when compared with other families of freshwater fish. This is important because zebrafish are non-native, and have only been recently discovered in U.S. waters due to accidental introduction.

EPA believes there is significant uncertainty regarding the actual sensitivity to zebrafish, and therefore proposes inclusion of the zebrafish studies in the effects characterization section, as well as inclusion of a comprehensive analysis of the studies as well as the studies on sensitivity of selenium to native cyprinids (below) in its own technical appendix, and issuing an FRN soliciting additional studies or information on zebrafish, as well as native cyprinids.

4.1.1 Part I. Chronic summary of Thomas (2014) and Thomas and Janz (2014)

Thomas, J.K. 2014. Effects of Dietary and in ovo Selenomethionine Exposure in Zebrafish (*Danio rerio*). Dissertation. University of Saskatchewan, Saskatoon, Canada.

Thomas and Janz, D.M. 2014. *In ovo* exposure to selenomethionine via maternal transfer increases developmental toxicities and impairs swim performance in F1 generation zebrafish (*Danio rerio*). Aquatic Toxicol. 152:20-29.

Test Organism: Zebrafish (*Danio rerio*)

Exposure Route: Dietary only
Selenomethionine spiked into Nutrafin® basic flake food

Test Treatments: Control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw).

Test Duration: 90 days

Study Design: Adult zebrafish were fed a control diet (1.3 mg/kg Se dw) and three selenium-spiked diets (3.7, 9.6, and 26.6 mg/kg Se dw) for 60 days, followed by an additional 30-40 days with equal rations (2.5%) of control or SeMet-spiked diets and clean chironomids. After 90 days of feeding exposure, adult fish from each exposure group were bred 3-4 times and embryos were collected and used to assess a number of different effects including larval survival and deformities. Eggs from each treatment were pooled from which replicate samples were collected for selenium measurement, larval survival and deformity assessment

Effects Data: The authors presented mortality and deformities in the F1 generation graphically for days up to 6 days post fertilization (dpf). The bar graphics were initially converted to numeric values using a length measuring tool in GIMP (GNU Image Manipulation Program). EC10 values for both mortality and deformities were very low with deformities being slightly lower. Upon request, the authors provided a table of the number of deformities in observed in 2-6 days post fertilization (dpf) fish larvae for each replicate pool of eggs (Table E-14) (David Janz, pers. comm.). TRAP analysis of these data produced a very low EC10 of 7.0 mg/kg egg Se dw. The concentration-response curve in Figure E-5 is extremely shallow compared to similar tests on other species, such that the apparent sensitivity of zebrafish relative to other species depends on what level of effect is considered. A comparison of egg-ovary zebrafish concentration-response curves for survival and deformities with well-founded concentration-response curves for other species is presented in Figure E-6. The shallow survival and deformity slopes for the zebrafish stand out as atypical for a selenium response. Note the EC50 values for the zebrafish are very similar to the EC50 values for the majority of other fish species and the zebrafish EC90 is similar to the EC90 of the least sensitive fish, Dolly Varden.

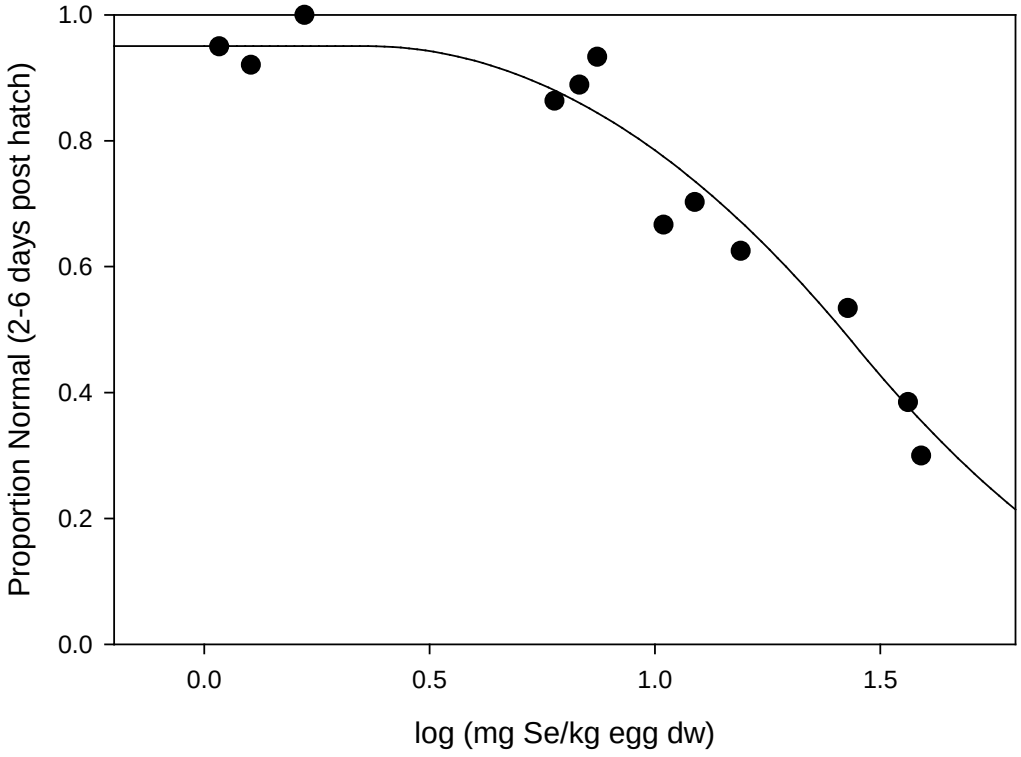
A GMCV based on this test has not been included in the Sensitivity Distribution for several reasons. Although the deformity and survival EC50s are within the range observed for a number of other species, the concentration-response curves

for both deformities and survival are anomalously shallow, yielding EC10s far below that of any other sensitive species (Figure E-6). Furthermore, if the concentration-response curves are log-symmetrical, as generally has been assumed in estimating EC10s, the projected EC90s for zebrafish would place it among the least sensitive known species**,** indicating greater variability among individuals within this one species than among individuals across the entire class of other fishes represented in the figure. The implication of such a shallow concentration-response curve is that this species has exceptional genetic diversity with respect to selenium tolerance, such that populations could adapt to very high or very low selenium concentrations. The field significance of its exceptionally low EC10 is thus uncertain. The low EC10 might or might not have some relationship to the selenium deficiency reported by Hook (2008) in substantial portions of its home range in the Ganges and Brahmaputra basins in India and Bangladesh.

An assessment of the relative sensitivity of cyprinids using both field and laboratory data is provided in the following section (Part II).

Table E-14. Selenium concentrations in zebrafish eggs and deformities in 2-6 dpf larvae.

Se in eggs, mg.kg dw	Total	Deformed	% Deformity
1.67	35	0	0.00
1.27	63	5	7.94
1.08	40	2	5.00
5.99	44	6	13.64
7.45	45	3	6.67
6.80	36	4	11.11
12.26	37	11	29.73
10.46	39	13	33.33
15.51	48	18	37.50
38.98	30	21	70.00
36.44	65	40	61.54
26.81	88	41	46.59



Parameter Summary:					
Parameter	**Initial**	**Final**	**Std. Error**	**95%LCL**	**95%UCL**
LogX50	1.45	1.4421	0.0408	1.3632	1.5247
Standard Deviation	0.44	0.4421	0.0586	0.3514	0.5964
Y0	0.95	0.9503	0.0184	0.9	0.9799

Effect Concentration Summary:			
%Effect	**ECx**	**95%LCL**	**95%UCL**
90	65.15	45.28	93.73
50	27.79	23.08	33.47
20	11.12	8.647	14.29
10	7.004	4.884	10.04
5	5.053	3.208	7.958

Figure E-5. Tolerance distribution model (triangular distribution model shape) of the proportion of normal zebrafish larvae (1-fraction with deformities) vs. the logarithm of concentration of selenium in zebrafish eggs.

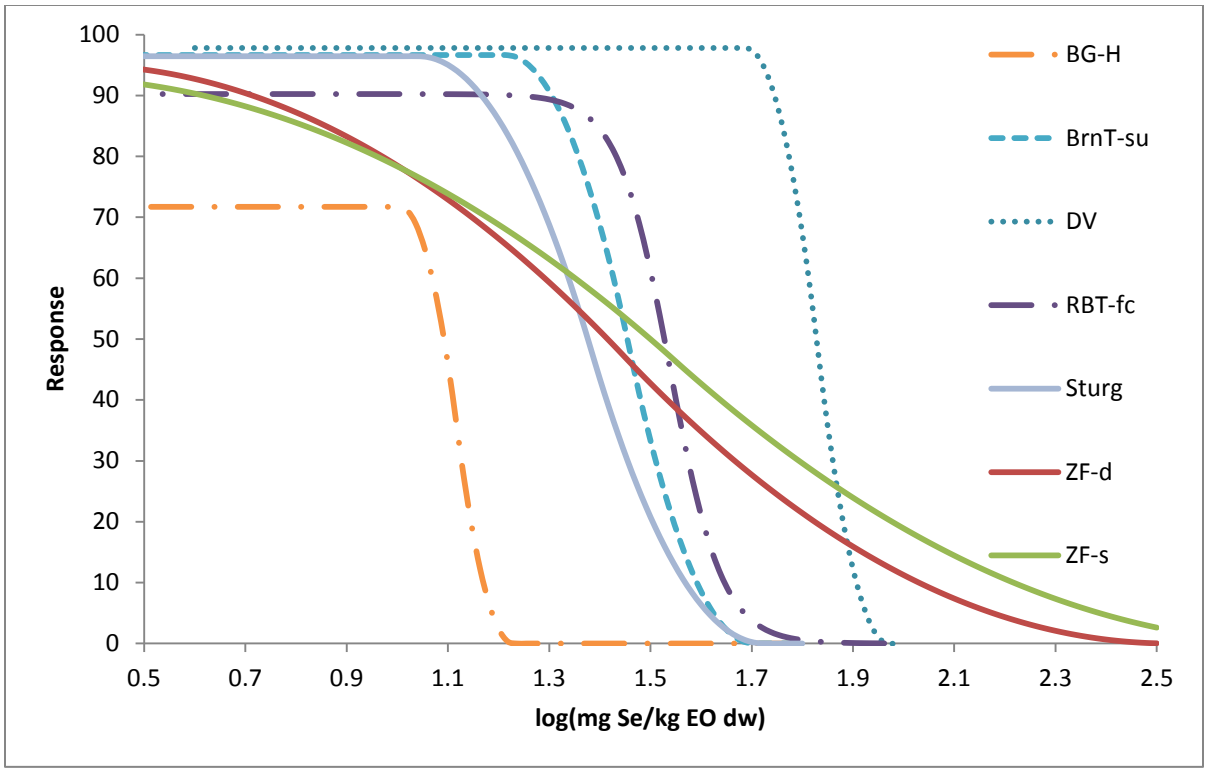


Figure E-6. Thomas and Janz (2014) zebrafish concentration-response curves for deformities and survival, ZF-d and ZF-s, compared with representative concentration-response curves for other species spanning the full range of EC10s.
BG-H: bluegill, Hermanutz et al. (1992, 1996); BrnT-su: brown trout survival to swim-up (Formation 2011); DV: Dolly Varden, Golder (2009; RBT-fc: rainbow trout facial-cranial deformities, Holm (2002) and Holm et al. (2003, 2005); Sturg: sturgeon deformities, Linville (2006).

4.1.2 Part II - Evaluating Sensitivity of Cyprinids (Cyprinidae) to Selenium from Field and Laboratory Data

Background:

The draft selenium criteria document is based on reproductive effects (mortality deformities) to larval fish following maternal exposure. These chronic tests are based primarily on species from the families salmonidae and centrarchidae. There is a paucity of data for a number of fish families used for development of selenium criteria. This limitation in data is particularly notable for the family cyprinidae ("minnows"), because it is comprised of approximately 180 general and is one of the most diverse families in North America. A recent toxicity test with zebrafish (*Danio rerio*), discussed above in Part 1, indicated that some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. This study was very different than all previous studies

examining larval effects in that the slope was very shallow, whereas the slopes for all other species were steep (see Figure E-6).

This analysis considers the results of the zebrafish laboratory survival study and several field collection studies, which evaluated cyprinid abundance and diversity in watersheds impacted by selenium, to compare the sensitivity of the zebrafish evaluated by Thomas (2014) and Thomas and Janz (2014) to native cyprinid populations. Available water and whole body tissue selenium concentrations (> 8.0 mg/kg dw), were compared to the translated egg-ovary to whole body zebrafish EC10 values (~ 3.5 mg/kg dw) to evaluate the relative sensitivity of native cyprinids to the non-native zebrafish test outcome.

Executive Summary:

The occurrence and effect of selenium on native cyprinids were evaluated based on the results of field studies conducted in four aquatic systems (CO, NC, UT, and WV) having elevated selenium concentrations. The objective of this evaluation was to compare the sensitivity of native cyprinid populations with the results of a recent toxicity test with zebrafish (*Danio rerio*) (Thomas (2014), Thomas and Janz (2014)) that suggests some cyprinids may be markedly more sensitive to the effects of selenium than other fish families for which toxicity data are available. The following set of analyses evaluated studies of widely-distributed native cyprinid species occurring in waters impacted by selenium from various sources and the relationships between whole body tissue levels, (and water concentrations where available) and impacts from selenium via toxicity or population metrics.

Cyprinid genera representing many species native to the US were found to be present in waters with selenium concentrations exceeding the current national criteria value (5µg/L). Cyprinid species present in the four studies examined represent 169 of the approximately 180 species present (at the genus level) in the United States. Abundance and diversity at sites impacted by selenium (water concentrations > 5.0 µg/L) were found to be no different than at sites in the Arkansas River, Colorado with low selenium concentrations (3.0-3.5 µg/L) watershed, with the exception of one location where extremely high selenium concentrations (Wildhorse Creek, CO; approximately 413 µg Se/L) were detected.
Whole body tissue concentrations within several widely distributed cyprinid genera exceeded the proposed whole body tissue element of 8.0 mg/kg dw and had sustainable reproducing populations, as indicated by length frequency analysis and occurrence data for the four studies. When evaluated by itself, the influence of selenium whole-body concentration in reducing family Cyprinidae densities was not statistically significant ($R^2 = 0.02$; $p = 0.51$). Rather, substrate characteristics of the waterbodies sampled had the strongest influence. In contrast, when evaluated by itself, the influence of selenium whole-body concentration in reducing family Centrarchidae densities was significant ($R^2 = 0.53$; $p = 0.02$).

In spite of the potential for confounding factors, GEI (2008) obtained parallel results at a different location, Dixon Creek and Canadian River in Texas, affected by refiner effluent selenium. Again, selenium whole-body selenium had no relationship to cyprinid density ($R^2 = 0.00$) but was a significant negative factor for centrarchid density ($R^2 = 0.41$, $p = 0.003$). And in the Sand Creek Drainage, CO, GEI found no negative association between fathead minnow densities and selenium concentrations of 3-26 mg Se/kg whole-body dw and 8-45 mg Se/kg ovary dw.

These findings suggest that native cyprinids are less sensitive than centrarchids, and are thus likely to be protected by a national criterion based heavily on centrarchid and salmonid sensitivity. Based on these available data, native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species, and much greater than indicated by the non-native zebrafish test outcome. It is therefore expected that the proposed selenium criterion will be protective of native cyprinids occurring throughout the United States.

Laboratory Exposures:

1. Chronic Toxicity and Hazard Assessment of an Inorganic Mixture Simulating Irrigation Drainwater to Razorback Sucker and Bonytail. Hamilton et al. (2000). USGS CERC Laboratory

Toxic effects from inorganics associated with irrigation activities, and possibly contributing to the decline of endangered fish in the middle Green River, Utah were investigated. Two 90-day chronic toxicity studies were conducted with two endangered fish, razorback sucker (*Xyrauchen texanus*) and bonytail chub (*Gila elegans*). Swim-up larvae were exposed in a reconstituted water simulating the middle Green River. The inorganic mixtures were tested at 1X, 2X, 4X, 8X, and 16X the measured environmental concentrations of the evaluated inorganic constituents (2 ug/L arsenic, 630 ug/L boron, 10 ug/L copper, 5 ug/L molybdenum, 51 ug/L selenate, 8 ug/L selenite, 33 mg/L uranium, 2 ug/L vanadium, and 20 ug/L zinc).

Bonytail chub survival was 95% or greater at 30, 60, and 90 days except for the 16X treatment (1232 ug/L Se), whereas growth was reduced after 30, 60, and 90 days at the 8X treatment (532 ug/L Se). Swimming performance of bonytail chub was reduced after 90 days of exposure at the 8X treatment. Whole-body residues of copper, selenium, and zinc increased in a concentration-response manner, but did not increase at 90 days of exposure at the 8X treatment for most species tested, and at lower treatment concentrations for the bonytail chub. Mean whole body selenium residues at the 8X treatment were 23.3, 16.7, and 9.4 mg/kg Se dw at 30, 60 and 90 days respectively. Hamilton et al. (2000) concluded that adverse effects in bonytail chub were associated with whole-body concentrations of 9.4 to 10.8 mg/kg Se dw in this study. One key uncertainty is the effect that the combination of toxic elements, in contrast to selenium alone, had on outcomes measured in this study. However, basing the selenium toxicity

evaluation on exposure to multiple contaminants is expected to provide a more conservative estimate of effect on the bonytail chub (*Gila elegans*) than if selenium is tested alone.

Field Collection Studies

2. Selenium Tissue Thresholds: Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field. Part III: Field Application of Tissue Thresholds: Potential to Predict Population or Community Effects in the Field. NAMC Report (2008).

Field studies were conducted by GEI in the Arkansas River, CO mainstem and selected tributaries between 2005 and 2006 to examine the relationship between selenium concentrations as well as habitat characteristics in surface waters and cyprinid abundance and diversity in the Arkansas River. The data collected for the study included:

1) Seasonal fish and macroinvertebrate (not shown) sampling to determine species composition and the relative abundance of aquatic organisms);
2) Whole-body fish tissue, composite macroinvertebrate tissue (not shown), and water and sediment (not presented) sample collection for the evaluation of Se concentrations in these tissues and the evaluation of bioaccumulation pathways; and
3) Physical habitat measurements (not presented), to determine relationships between the occurrence of biota and their physical environment. Data were collected from fall 2004 to fall 2006 from the Arkansas River, Fountain and Wildhorse Creeks, and the St. Charles River.

Total selenium (dissolved) was measured at 4 sites mainstem and 6 sites on three tributaries of the Arkansas River watershed near Pueblo Colorado (Table E-15). Multiple site visits (6 to 17) to collect water for selenium determination were conducted at the 10 sampling stations between 2005 and 2006.

Table E-15. Selenium water column data: Total Selenium (µg/L, dissolved).

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
AR (Arkansas River)				
AR1 (ARM) Mainstem, in Pueblo below Whitlock WWTP	8 months	15	7.05	3.69
AR2 (ARE) Mainstem below Pueblo WW Reclamation Center and Fountain Creek	12 months	9	10.6	4.06
AR3 (ARB) Mainstem, downstream of Pueblo	10 months	7	8.72	4.0
AR4 (ARN) Mainstem, downstream of St. Charles River	10 months	8	8.81	2.85

Site	Sampling Duration 2005-06	Sample Size	Mean [Se] (µg/L)	Standard Deviation
Arkansas River Tributaries				
WHC (Wildhorse Creek)	6 months	17	418	115
FC (Fountain Creek)				
FCP (Upstream)	12 months	9	3.43 (4.9)*	1.05
FC4 (Downstream)	6 months	12	12.1	4.34
SC (St. Charles River)				
SC1 (Upstream)	6 months	6	3.09 (4.8)*	1.37
SC2 (Mid-Point)	6 months	11	11.7	6.22
SC5 (Downstream)	8 months	13	20.3	13

* Maximum [Se] in FCP and SC1 < 5.0 ug/L, current selenium criterion

Summary of Selenium Concentrations in Water:

1) Total selenium concentrations exceeded the EPA chronic selenium standard of 5 µg/L in surface water samples collected from most locations, with only the upper reaches of the St. Charles River and Fountain Creek having mean selenium concentrations below the EPA chronic selenium standard.
2) Selenium concentrations in water samples from Wildhorse Creek were more than 20X greater than in water samples collected from all other sample locations, with a mean selenium concentration of 418 ± 115 µg/L.
3) The minimum concentration measured in water samples from Wildhorse Creek (315 µg/L) was approximately 7X greater than the maximum selenium concentration measured at other study sites (43.6 µg/L at St. Charles River, SC5).

Selenium in Fish Tissue:

Selenium concentrations in fish tissue (whole body) were measured for three representative cyprinid species (central stoneroller, sand shiner, red shiner), one catostomid (white sucker), and three centrarchids (green sunfish, smallmouth bass, and largemouth bass) (Table E-16).

Table E-16. Mean fish tissue concentrations.
[Average whole body mg/kg dw estimated by eye from graphs in NAMC (2008)].

Sample Site	ARM	ARN	ARE	ARB	WHC	FCP	FC4	SC1	SC2	SC5
Mean water [Se] ug/L	7.0	8.8	10.6	8.7	418	3.43	12.1	3.1	11.7	20.3
Cyprinids										
Sand Shiner	10	10-21	25			10-17	15-21			
Red Shiner		23	42				25			30
Central Stoneroller	8	10-20			18-47	12	14	5	45	33
Centrarchids										
Green Sunfish								12	30	
Largemouth Bass	11-15	14-36	22	26						40
Smallmouth Bass	7		20	20						
Catostomids										
White sucker	8-11	10-24	16-18	14-21	32-33	6-10	24	6-14		47

Summary of selenium in fish tissue:

1) The mean concentrations in all cyprinids across all sites was 21.06 mg/kg dwt; SE = 1.38).
2) For comparison, the mean concentration in all centrarchids across all sites was 19.73 mg/kg dw; SE = 1.32; and the mean concentration in white sucker (catostomids) across all sites was 17.52 mg/kg dw; SE = 1.52.
3) Most mean whole-body Se concentrations were well above the U.S. EPA (2014) proposed chronic tissue criterion element for whole body of 8.13 mg/kg dry weight.

Comparison to national draft fish tissue criteria:

Given that these are waters known to be impacted by selenium there were only a few fish samples (Tables E-17, E-18) that were at or below the proposed whole body criteria element of 8.1:

1) The Arkansas River mainstem (mean water [Se] = 7.05 ug/L), had samples from three species that met the criteria in 2006, central stoneroller, smallmouth bass and white sucker.
2) In the tributaries to the Arkansas River that were sampled, white sucker in both Fountain Creek (mean water [Se] = 3.43 ug/L) and St. Charles River met the whole body criteria in 2004 and 2005, whereas the only cyprinid to meet the proposed whole body criterion was the central stoneroller in 2005.

Cyprinid Abundance and Diversity:

Table E-17. Cyprinid Diversity (native spp. present– excludes carp): NAMC 2008 Study.

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	1/6	3/6
ARB	8.72	6/6	5/6
ARN	8.81	5/6	3/6
ARE	10.6	5/6	4/6
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	5/6	4/6
FC4	12.1	4/6	6/6
Whitehorse Creek (WHC)	413	1/6	1/6
St. Charles River			
SC1	3.09	5/6	5/6
SC2[1]	11.7	4/6	NS
SC5	20.3	6/6	5/6

[1]SC2 only sampled in 2005

Table E-18. Cyprinid Abundance (native spp. present– excludes carp): NAMC 2008 Study

Site	[Se] in water ug/L	2005	2006
Arkansas River Mainstem			
ARM	7.05	8	460
ARE	8.72	643	950
ARB	8.81	697	521
ARN	10.6	446	116
Arkansas River Tributaries			
Fountain Creek			
FCP	3.43	746	2352
FC4	12.1	1978	1825
Whitehorse Creek (WHC)[1]	413	926	81
St. Charles River			
SC1	3.09	2920	14583
SC2[2]	11.7	2757	NS
SC5	20.3	3102	2568

[1]Whitehorse Creek comprised 1 species, central stoneroller
[2] SC2 not sampled in 2006

Summary of cyprinid abundance and diversity:

1) Diversity as well as abundance of cyprinids in the tributaries vs the Arkansas River mainstem more likely a function of habitat and/or predator density rather than influence of selenium.
2) Several sites on Wildhorse Creek, Fountain Creek, and the St. Charles River, had substantial changes in the populations of some fish species between sample years 2005 and 2006, with fish that were present in one year in high numbers and with a variety of age classes, either

absent or present in low numbers the other year. These changes are likely to be linked to higher stream flows present in 2006 and significant habitat changes due to beaver activity at some sites. Variable population compositions and numbers of cyprinids are not uncommon in plains streams with highly variable flow regimes and habitat conditions (Schlosser 1987).

3) Based on an evaluation of age class distribution (indicated by length-frequency distribution data), it was concluded that the following sites had viable and reproducing cyprinid populations (NAMC 2008:

> Arkansas River mainstem: The length-frequency data collected for the fish species at these sites indicates multiple age groups present for most of the species at the sites.
> Fountain Creek - Length-frequency analysis of the flathead chubs indicated that the populations are reproducing, with juvenile and older adult fish present in relatively high numbers at both sites and years.
> St. Charles River - Length-frequency analysis of the fish populations indicated that sites had reproducing populations of central stonerollers, fathead minnows, and sand shiners, with juvenile and adult fish collected during both years (GEI 2007a).
> Wildhorse Creek - the age class distribution of central stonerollers was similar between years, indicating a reproducing population that includes both juvenile and adult fish in both years, despite the extremely high [Se] in water.

Relevance/Surrogacy of Arkansas River Cyprinids to all Cyprinid Species in US

Cyprinids captured from the Arkansas River are representative of cyprinid species occurring throughout the US. This conclusion is based on the following lines of evidence:

- Six of the seven cyprinid species (central stoneroller, fathead minnow, flathead chub, longnose dace, red shiner, and sand shiner) captured from the Arkansas River during this investigation are native to the United States;
- Four of the six cyprinid species found in the Arkansas River basin (central stoneroller, fathead minnow, sand shiner and red shiner) are widely distributed throughout the United States (see species specific distribution maps Attachment 1); and,
- Six of the native species present in the Arkansas River Basin are direct surrogates at the genus level for the 142 native cyprinids in North America (Table E-19).

Table E-19. Cyprinid species surrogacy and occurrence in water for native species inhabiting the Arkansas River and select tributaries.

Species	Cyprinid group	# of species represented by genus	[Se] in waterbodies where species occurred	Average tissue concentration or range
Campostoma anomalum Central stoneroller	stonerollers	5 species	3.1-418 ug/L	5-47 mg/kg dw
Pimephales promelas Fathead minnow	Blunthead minnows	4 species	3.1 - 20.3 ug/L	No tissue
Platygabio gracilis Flathead chub	Flathead chub	1 species	3.1 - 20.3 ug/L	No tissue
Rhynichthys cataractae Longnoise dace	dace	9 species	3.1 - 20.3 ug/L	No tissue
Cyprinella lutrensis Red shiner	Satinfin shiners	32 species	3.1 - 20.3 ug/L	23-42 mg/kg dw
Notropis stramineus Sand shiner	Eastern shiners	91 species	3.1 - 20.3 ug/L	10-25 mg/kg dw

Summary cyprinid surrogacy:

Cyprinid species collected from the Arkansas River watershed are representative (at the genus level) of the 142 cyprinid species native to North America. With the exception of one sample location (Whitehorse Creek), the abundance and diversity of cyprinid species present and the occurrence of multiple age classes indicates that cyprinids are successfully surviving and reproducing in the Arkansas River watershed, even with selenium concentrations exceeding 5ug/L in water and 8 mg/kg bw in whole body fish tissue. North American species not represented at the genera level comprise 54 species (mostly chubs – 40 species), many of which are geographically isolated.

3. Observations of cyprinids in NC Reservoirs (Hyco Reservoir and Belews Lake) – (located at end of NAMC 2008 report).

Crutchfield et al. (2000) evaluated long-term water quality data, selenium chemical concentration data collected for sediment, invertebrate and fish tissues, and invertebrate and fish population data collected from the Hyco Reservoir to document the recovery of the aquatic community following the 1990 installation of a dry fly ash pollution abatement system. Since 1973, data have been collected from six locations in the Hyco Reservoir, with varying fly ash exposure. Gamefish including bluegill sunfish and largemouth bass were reproductively extirpated due to high selenium concentrations prior to installation of the pollution abatement system. The fish community was dominated by green sunfish (*Lepomis cyanellus*), eastern mosquitofish (*Gambusia holbrooki*), gizzard shad (*Dorosoma cepedianum*), and satinfin shiner (*Cyprinella analostana*). Their main observation was that satinfin shiner was a dominant cyprinid in the Se limited fish community prior to selenium reduction.

Barwick and Harrell (1997) evaluated fish population monitoring and tissue selenium concentration data to document the recovery of fish populations in Belews Lake for the ten years following installation of a dry fly ash pollution abatement system. Fish diversity and biomass data were collected from 1977 to 1994 (with the exception of 1978-1979 and 1982-1983) at two sites on the lake. In 1980 and 1981, fathead minnows (*Pimephales promelas*) dominated the fish community, representing 62 percent and 81 percent of the biomass, respectively (Barwick and Harrell 1997). By 1984, red shiner (*Cyprinella lutrensis*), common carp (*Cyprinius carpio*), and fathead minnows (*Pimephales promelas*) were the dominant cyprinids in the selenium limited fish community prior to selenium reduction. The authors noted that cyprinid abundance started to decrease as green sunfish, a more Se- tolerant sunfish recovered in 1989-1990, followed by further decreases in 1990-1994, as channel catfish, bluegill, and largemouth bass populations increased (Barwick and Harrell 1997).

Young et al. (2010), reviewing the studies of Belews Lake, NC, note that during the period of maximal selenium inputs, egg and ovary concentrations reached 40-159 mg Se/kg dw. Out of as many as 29 resident species prior to contamination, only catfish and the cyprinids common carp and fathead minnows remained during the period of maximum impact.

4. Presser, T.S., 2013, Selenium in ecosystems within the mountaintop coal mining and valley-fill region of southern West Virginia—assessment and ecosystem-scale modeling: U.S. Geological Survey Professional Paper 1803, 86 p. http://dx.doi.org/10.3133/pp1803.

USGS sampled southern West Virginia ecosystems affected by drainage from mountaintop coal mines and valleys filled with waste rock (valley fills) in the Coal, Gauley, and Lower Guyandotte watersheds during 2010 and 2011. Sampling data from earlier studies in these watersheds (for example, Upper Mud River Reservoir) and other mining-affected watersheds in WV are also are included to assess additional hydrologic settings and food webs for comparison.

1) Site-specific fish abundance and richness data documented the occurrence of various species of chub, shiner, dace, minnow, and central stoneroller (*Campostoma anomalum*) in the sampled watersheds.
2) Model species for streams were limited to creek chub (*Semotilus atromaculatus*) and central stoneroller. Creek chub was present at all sites during USGS sampling in 2010-2011. However, both of these species are considered to have high tolerance for environmental stressors based on results of traditional comparative fish community assessments. Concentrations of Se in water and whole body tissues of creek chub, blacknose dace, and stoneroller are shown in Table E-20.
3) The order of abundance for species with greater than 28 individuals was: creek chub, striped shiner, mottled sculpin, green sunfish, central stoneroller, blacknose dace, bluntnose minnow, and northern hog sucker. Shiners and darters were prevalent, but bluegill sunfish were absent during the 2010 survey.

Table E-20. Se in fish whole body tissue samples: Upper Mud River Basin and Tributaries.
(Compilations of data from different sources presented in (Presser et al. 2013).

Stream Segment	Year	[Se] in water Mean (Range) in ug/L	Creek Chub Mean (Range) in mg/kg dw	Blacknose Dace Mean (Range) in mg/kg dw	Stoneroller Mean (Range) in mg/kg dw
Upper Mud River	2011	10.5, 18.2	9.0 (6.4–11)	Not Sampled	Not Sampled
Upper Mud River 1	2010	Not Sampled	10.3 (9.4–10.9)	Not Sampled	Not Sampled
Lower Mud River	2008	7.9	10.3 (9.4-15.4)	Not Sampled	Not Sampled
	2011	5.2, 7	9 (6.4-11)	Not Sampled	Not Sampled
Upper Mud River 2 (above Upper Mud River 1)	2005	9.8 (4–22)[1]	2.9 (<1-8.7)	Not Sampled	Not Sampled
	2006	Not Sampled	5.6 (2.2-10)	Not Sampled	Not Sampled
	2007	Not Sampled	7.7 (3.7-10)	Not Sampled	Not Sampled
Berry Branch	2009-2010	8.3 (1.7–18)[2]	4.0 (3.3–5.0)	9.6 (7.8–13)	Not Sampled
Stanley Fork	2009-2010	6.0 (3.0–7.4)[3]	10.3 (7.2–13)	Not Sampled	Not Sampled
Lower Kanawha River Watershed					
Little Scary Creek	2006	20	Not Sampled	55	Not Sampled
	2009	31.4 (23-42)	28 (3-80)	Not Sampled	Not Sampled
Connor Run	2009	47.8 (4-90)	(21-36)	Not Sampled	Not Sampled
Upper Kanawha River Watershed					
Jack's Branch Mining Complex					
Bull push fork w/pond	2010	9.0-10.0	Not Sampled	66 (19–113)	Not Sampled
Bull push fork downstream	2010	9.1–10	8.6 (6.2–13)	10.7 (5.5–14)	6.9 (3.1–17)
Hughes Fork	2005 - 2007	5.3 (2–10)	7.8 (4.1–10.9) 2005 7.9 (2.7–12.9) 2007	Not Sampled	12.4 (0.5–34.5) 2005
Hughes Creek	2010-2011	2.1-13	9.9 (3.7–17)	16.9 (6.8–25)	9.0 (3.6–14)
Big Coal River Watershed					
Beech Creek	2005-2007	Not Sampled	(3-18)	Not Sampled	Not Sampled
Seng Creek	2005-2009	27.5 (15–42)	8.2 (4.8–14.7)	Not Sampled	Not Sampled
	2011	23.3	8.1 (5.4-10)	Not Sampled	Not Sampled
White Oak Creek	2005-2007	15.8 (8–27)	5.8 (<1-12.8)	Not Sampled	7.1 (2.5–12.8)

[1] Water samples collected between 2005 and 2008.
[2] Water samples collected in 2009 and 2010.
[3] Water samples collected in 2009 and 2010.

Study Summary:

Samples in various environmental media (water, sediment, algae, macroinvertebrates, fish) were collected by USGS (2010-2011), and others (e.g. WVDEP, Potesta) between 2005 and 2011. The stream segments presented here represent a subset of the stream segments with available data. Only streams with water [Se] > 5.0 ug/L are presented to facilitate comparison with other studies with Se-impacted streams. Overarching observations include:

1) [Se] in water averaged from 5.3 ug/L – 31.4 ug/L with a high of 90 ug/L (Connor Run, 2009).
2) [Se] in fish tissue: creek chub – averaged from 5.8 mg/kg wb to 28 mg/kg wb, with a maximum whole body concentration of 80 mg/kg wb (Little Scary Creek, 2009).
3) [Se] in fish tissue: blacknose dace – averaged from 10.7 mg/kg wb to 66 mg/kg wb, with a maximum whole body concentration of 113 mg/kg wb (Bull push fork w/pond, 2010)
4) [Se] in fish tissue: central stoneroller – averaged from 6.9 mg/kg wb to 12.4 mg/kg wb, with a maximum whole body concentration of 34.5 mg/kg wb (Hughes Fork, 2005). Note also, that central stoneroller, although common through stream segments samples, were not ubiquitous, as was observed in the study conducted by NAMC in the Arkansas River near Pueblo CO.

5. Selenium concentrations in fish tissue collected from the Gunnison River.
http://pubs.usgs.gov/of/2012/1235/of12-1235.pdf

Approach: In sampling conducted in summer 2010, muscle tissue plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and whole body tissue samples were collected from speckled dace (*Rhinichthys osculus)* inhabiting critical habitat in the Gunnison River in Western Colorado (Table E-21). Total selenium in fish muscle plugs (mg/kg dw) for roundtail chub, or in whole body (speckled dace) was calculated for all tissues. In follow-up sampling conducted in the summer of 2011, muscle plugs were collected from common carp (*Cyprinus Linnaeus)*, roundtail chub (*Gila robusta; listed*), and bonytail chub (*Gila elegans, listed*) inhabiting critical habitat in the Gunnison River in Western Colorado.

This study was intended to document any changes in selenium concentration in fish over the last 20 years based on remediation efforts that have been completed to date.

Table E-21. Fish tissue concentrations observed in Cyprinids.

Species	**Year**	**Mean (Range) [Se]**	**# > muscle = 11***	**# > whole body = 8**
Roundtail Chub	2010	9.7 mg/kg dw (5.2-32.4)	2/15	
	2011	7.33 mg/kg dw (5.6-11.2)	1/15	
Speckled Dace	2010	7.46 mg/kg dw (5.7-9.7)		6/15

* Muscle plugs were collected since this species is large enough for non-destructive sampling, and b) a listed species.

5.0 OTHER DATA – CHRONIC STUDIES WITH INVERTEBRATE SPECIES

A limited number of studies have evaluated the effects of selenite on invertebrate species, an important prey item for fish and birds as summarized by Debruyn and Chapman (2007). The following **studies** with a rotifer, and annelid, and an insect (mayfly) were found suitable for establishing species sensitivity.

5.1 Rotifers

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

5.2 Aquatic Worms

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

5.3 Aquatic Insects (Plecoptera: Mayfly)

Conley, J.M., D.H. Funk and D.B. Buchwalter. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ. Sci. Technol. 43:7952-7957.

Conley, J.M., D.H. Funk, N.J. Cariello and D.B. Buchwalter. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicol. 20:1840-1851.

Conley, J.M., D.H. Funk, D.H. Hesterberg, L-C. Hsu, J. Kan, Y-T. Liu and D.B. Buchwalter. 2013. Bioconcentration and biotransformation of selenite versus selenite exposed to periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ. Sci. Technol. 47:7965-7973.

Conley et al. (2009) exposed mayfly larvae (*Centroptilum triangulifer*) to dietary selenium contained in natural periphyton biofilms to eclosion. The periphyton fed to the mayfly larvae were exposed to dissolved selenite (radiolabeled ^{75}Se) in November 2008 (12.6 and 13.9 µg/L) and in January 2009 (2.4, 2.4, 4.9, 10.3, and 10.7 µg/L). Periphyton bioconcentrated Se an average of 1113-fold over the different aqueous Se concentrations (Table E-22). Twenty 4 to 6-day old mayfly larvae were exposed for 4.5 to 6 weeks to each of the periphyton diets until the larvae eclosed to subimagos. The subimagos were allowed to emerge to the adult imago stage which deposited their egg masses in Petri dishes. Selenium was measured in postpartum adults along with their dry weights and clutch size.

Table E-22. Selenium concentrations in water exposed to periphyton, periphyton and mayfly adults.

Treatment	Dissolved [Se] exposed to periphyton, µg/L	[Se] in periphyton, mg/kg dw	[Se] in mayfly adult, mg/kg dw
5A	2.4	2.2	4.2
5B	2.4	2.0	5.7
10A	4.9	4.4	9.7
20C	10.3	8.7	16.2
20D	10.7	11.3	27.5
20A	12.6	25.5	56.7
20B	13.9	17.5	34.8

Selenium increased in concentration from periphyton to the adult mayflies (trophic transfer factor) an average of 2.2-fold (Table E-22). The authors observed a decrease in fecundity as maternal postpartum Se concentrations increased. Fecundity was also related to growth of the mayflies. The authors observed a reduction in fecundity for this mayfly when they were fed diets containing more than 11 mg Se/kg dw. This threshold is considered the effect value for this study. Using the trophic transfer factor of 2.2, the periphyton Se concentration of 11 mg/kg dw translates to an adult mayfly Se concentration of 24.2 mg/kg dw.

Conley et al. (2011) exposed larval *C. triangulifer* similar to Conley et al. (2009) to two different rations of periphyton (1x and 2x) to evaluate the effect of feeding ration on the bioaccumulation and life cycle performance of the mayfly. Periphyton (on plates) was initially exposed to low (1.1 to 3.4 µg/L), medium (5.9 – 8.9 µg/L) and high (19.2 – 23.1 µg/L) selenite. Fifteen 1-2 day-old mayfly larvae were then fed either 1 plate (1x ration) or 2 plates (2x ration) in bottles containing 1.8 L water to eclosion to subimagos (25-29 days). Subimagos were induced to emerge to adults in petri dishes and their clutch size measured through digital imaging. Selenium measurements from this study are given in Table E-23.

Table E-23. Selenium concentrations in water, periphyton and mayfly tissues for two feeding rations.
(Adapted from Table 1 in Conley et al. 2011)

Feeding ration – Se level	Mean dissolved Se exposed to periphyton, µg/L	Mean periphyton, mg Se/kg dw	Mean mayfly tissue, mg Se/kg dw
1x – low	1.1	4.2 ± 0.6 (4)	12.8 ± 3.6 (28)
1x – medium	5.9	11.9 ± 2.1 (4)	31.7 ± 7.5 (15)
1x - high	21.4	27.2 ± 4.2 (4)	68.4 ± 24.0 (9)
2x – low	2.7/3.4[a]	9.5 ± 0.9 (3)	14.1 ± 3.8 (19)
2x – medium	7.1/8.9[a]	19.9 ± 1.6 (3)	21.6 ± 2.8 (22)
2x - high	19.2/23.1[a]	40.9 ± 1.7 (3)	37.3 ± 6.7 (13)

[a] Two values represent two different loading exposures, September and October. The plates were combined for mayfly exposure.

Mayflies fed the 1x ration had 54% and 72% reductions in survival relative to controls in the medium and high Se treatment levels, respectively, both significant ($p<0.05$). The mayflies fed the 1x ration also had significant reductions in fecundity in the low (44% reduction), medium (63% reduction) and high (77% reduction) Se treatment levels. However, for the mayflies fed the 2x ration, there were no significant differences between the controls and any of the three Se treatment levels for any of the endpoints measured including survival and fecundity. The 2x ration mayflies had 60% more biomass than the 1x ration mayflies. This growth difference explains why the 1x ration mayflies had higher concentrations of Se in their tissues. The two different rations resulted in vastly different effect levels for Se, <12.8 mg/kg dw in the 1x ration test and >37.3 mg/kg dw in the 2x ration. It is apparent from this study that if the mayflies do not obtain sufficient nutrition, they are more sensitive to selenium. Although reduced feeding levels occur in nature, it is a confounding variable in this study that cannot be used to set a chronic effect level for selenium.

Conley et al. (2013) evaluated the accumulation of selenite and selenate into periphyton with subsequent feeding exposure to mayfly larvae. As in his previous studies, *C. triangulifer* larvae were fed periphyton previously exposed to different concentrations of selenium. In this study, periphyton plates were first exposed to low (10 µg/L) and high (30 µg/L) concentrations of either selenite or selenate and then fed to mayfly larvae to ecolsion to subimagos. The selenite and selenate treatment exposures resulted in similar levels of selenium in the subimagos. Since no differences in selenium accumulation was observed, the selenite and selenate treatments could be pooled for measuring the endpoints, survival and secondary production (total mayfly biomass produced). Mean selenium concentrations fed the mayflies were 2.2, 12.8 and 37 mg/kg Se dw in the control, low and high treatments, respectively. Mayfly tissue (subimago) concentrations (extrapolated from Figure 4a in Conley et al. 2013) were approximately 4-7, 20-35, and 45-75 mg/kg Se dw, in the control, low and high treatments, respectively. The authors reported significant reductions in survival from the control in the high Se treatment (both pooled data and individual selenite and selenate treatments) but no significant differences were observed in the low Se treatments. Secondary production was significantly reduced relative to the control in the high Se treatment for both selenium species. For the low Se exposure treatment, secondary production was not significantly different than the control for the selenite treated periphyton exposure, but was for the selenate and pooled data suggesting an effect level between 20 and 35 mg/kg Se dw. These results as well as those observed in 2x ration exposures in Conley et al. (2011) where no effects were observed at 37.3 mg/kg Se dw generally support the chronic value determined for Conley et al. (2009) of 24.2 mg/kg Se dw.

The following invertebrate studies were inconclusive for establishing species sensitivity because of limitations in the experimental designs, as explained for each.

5.4 Aquatic Insect (Midge: Chironimids)

Malchow et al. (1995) fed fourth instar *Chironomus decorus* midge larvae a diet of seleniferous algae under laboratory conditions for 96 hours. For algae cultured with selenite, a larval tissue concentration of 4.05 µg Se/g dry weight resulted in a 46% reduction in growth relative to the controls. At a larval tissue concentration of 8.6 µg Se/g dry weight, larval growth was reduced by only 39%. Since the study only reported two exposure concentrations, it is unclear if the tissue effect concentration at 4.05 µg Se/g dry weight is real or an anomaly. Additional exposure concentrations and subsequent effect levels are needed to resolve this issue.

Malchow et al. (1995) also fed fourth instar *Chrionomus decorus* midge larvae a diet of algae cultured with selenate, and the midge larvae were exposed under laboratory conditions for 96 hours. A dietary exposure of 2.11 µg Se/g dry weight significantly reduced larval growth (15% reduction) at tissue concentrations of 2.55 µg Se/g dry weight. At a larval tissue concentration of 6.62 µg Se/g dry weight, growth was reduced 20% relative to the controls. The 15-20% reduced growth at larval tissue concentrations 2.55 µg Se/g dry weight may be statistically significant, but not biologically meaningful. In addition, exposure to only two selenium concentrations precludes confirmation of a dose-response.

Alaimo et al. (1994) also exposed *2010* midge larvae to selenite diet, but the selenium source was from field contaminated widgeongrass (*Ruppia maritima*). *Ruppia* stems and leaves were collected from four selenium contaminated evaporation ponds located in the San Joaquin Valley of California. Three-day old larvae were exposed to each of the four treatment diets (*Ruppia* from each pond) plus a Cerophyll control for 14 days (egg to pupation), with the moderately hard reconstituted water renewed at day 7 and every three days thereafter. The growth (weight) of exposed larvae was significantly reduced in all of the selenium treatments when compared to the controls. The lowest effect level was observed for the Westlake pond (primarily selenite), where growth was reduced 40 percent relative to the controls at a larval tissue concentration below the detection level (1.0 ppm dry weight, or 1.0 µg Se/g dry weight). These results are suspect because the field collected *Ruppia* likely contained contaminants other than selenium, the control organisms were fed a different diet (Cerophyll), and the single concentration exposure is difficult to defend.

6.0 OTHER DATA – FIELD STUDY WEST VIRGINIA IMPOUNDMENTS

In response to the USEPA (2004) draft whole fish tissue criterion for selenium, the West Virginia Department of Environmental Protection (2010) initiated a study to assess selenium bioaccumulation among fishes residing in the State's lakes and streams. A focus of the study was the collection and evaluation of bluegill, *Lepomis macrochirus*, larvae (ichthyoplankton) from selected waterbodies since

2007, based on concerns regarding fish population health at locations subjected to elevated selenium inputs, particularly during the more sensitive developmental life stages of fishes (e.g. yolk-sac larvae). Also, in 2009, WVDEP began acquiring data about selenium concentrations within fish eggs of various species within reference and selenium-impacted waters. WVDEP also conducted deformity surveys of adult fishes in selenium enriched waters as well as at reference locations in 2008-2009.

WVDEP scientists found that larval deformity rates were variable throughout the study duration but were nonetheless correlated with waterborne selenium exposure. Reference locations produced age-based larval bluegill subsamples (24-168 hours) with low deformity rates (0 - 1.27%); whereas, locations with seleniferous inputs exhibited bluegill deformity rates ranging from 0% to 47.56% in developmental stages up to 312 hours. Maximum deformity rates among staged bluegill subsamples as determined through these evaluations were 19.28%, representing specimens collected from selenium-enriched waters. Concentrations of selenium within fish eggs also varied according to study location and ranged from <0.8 mg/kg dry weight among bluegill eggs at the control site to 64.62 mg/kg dry weight among largemouth bass, *Micropterus salmoides,* eggs collected from selenium-enriched waters. Searches for more mature, yet developmentally-deformed fishes revealed increased deformity rates (14%) among largemouth bass residing in a selenium impacted reservoir as compared to deformity rates among largemouth bass found in the reference lake (0%). The data on egg selenium concentrations are not adequate for constructing a concentration-response curve. Nevertheless, the overall deformity rate in the contaminated Upper Mud River Reservoir was 5% among 10,000 individual fish, average egg selenium concentration 9.8 mg/kg dw. The overall deformity rate in the reference Plum Orchard Lake was 0.5% among 13,000 individuals, average egg selenium concentration nondetectable or <0.8 mg/kg dw.

7.0 OTHER DATA - NUTRITIONAL DEFICIENCY/SUFFICIENCY STUDIES CONTAINING MEASURED SELENIUM IN THE DIET AND WHOLE BODY FISH TISSUE

Ingested dietary dose studies in fish designed to identify nutritionally deficient and/or nutritionally sufficient selenium doses in fish food or prey primarily describe selenium effects on growth, with survival reductions and effects on antioxidant enzyme activity also occasionally reported. A number of the dietary studies have measured a range of dietary doses that maximize fish growth, as opposed to a single dietary dose associated with nutritional sufficiency for growth. Regardless of whether nutritionally sufficient dietary doses are reported as a single concentration or as a range of concentrations, reduced growth or survival is observed at both lower dietary doses (nutritional deficiency) and at higher dietary doses (toxicity).

Although dietary doses are normally presented as selenium concentrations in food, expressed in terms of mg/kg Se in the diet, several studies have also concurrently presented nutritionally deficient/sufficient Se levels in terms of the whole body Se concentration in the fish. These studies permit a comparison of nutritionally deficient/sufficient whole body Se residues in fish to the national criterion for Se in whole bodies of fish. When combined with measured whole body fish tissue residues associated with toxicity, a complete picture of the range of Se residues in whole body fish tissue associated with nutritional deficiency, nutritional sufficiency and toxicity emerges.

Eight fish species have information on both nutritionally deficient dietary doses and whole body concentrations of selenium measured in the same study (Table E-24). Six of the eight species are native to North America. Nutritionally deficient dietary doses of Se range between 0.03 mg/kg dw in Atlantic salmon (*Salmo salar*, Poston et al. 1976) associated with reduced survival to 1.4 mg/kg dw in Atlantic cod (*Gadus morhua*, Hamre et al. 2008), also associated with reduced survival. Whole body Se residues identified as nutritionally deficient range between 0.64 mg/kg dw in Malabar grouper (*Epinephelus malabaricus*) associated with suboptimal weight gain and feed efficiency (Lin and Shiau 2005) and 4.72 mg/kg dw in North African catfish (*Clarias gariepinus*), also associated with suboptimal weight gain (Abdel-Tawwab et al. 2007). The whole body Se residues associated with growth and/or survival reductions due to nutritional deficiency of the six North American species (Prussian carp, Han et al. 2011; common carp, Gaber 2007; Atlantic cod, Hamre et al. 2008; Coho salmon, Felton et al. 1990; cobia, Lin et al. 2010; Atlantic salmon, Poston et al. 1976) all range between 1.0 and 2.7 mg/kg dw.

Ten fish species have information on both nutritionally sufficient dietary doses and whole body concentrations of selenium measured in the same study (Table D-23). Eight of the 10 species are native to North America. Nutritionally sufficient dietary doses of Se for the North American resident species, all but one of which are based on maximum growth of fish, range between 0.1 mg/kg dw in hybrid striped

bass (Jaramillo 2006) and 6.6 mg/kg dw in rainbow trout (Hilton and Hodson 1983). Several studies have identified a range of dietary doses and associated whole body residues that maximize growth and survival relative to that of fish fed lower dietary doses and which subsequently contain lower whole body selenium residues. Whole body Se residues associated with nutritional sufficiency based on maximal growth and/or survival of all North American species except for hybrid striped bass (Jaramillo 2006) range between 0.2 – 3.63 mg/kg dw (Table D-23). For hybrid striped bass, Jaramillo (2006) observed that maximum weight gain occurred in selenite supplemented diets containing 1.19 mg/kg dw Se, which resulted in whole body Se residues of 5.13 mg/kg dw. Jaramillo (2006) also exposed hybrid striped bass to seleno-DL-methionine supplemented diets containing 0.90 mg/kg dw, which resulted in the maximum weight gain of all seleno-DL-methionine supplemented diets tested, and a whole body Se residue of 7.2 mg/kg dw.

The nutritional sufficiency study of Rider et al. (2009) with rainbow trout is unique in that it determined dietary and whole body selenium requirements for both stressed and unstressed fish. Rider et al. (2009) observed that rainbow trout stressed by a combination of low water levels in holding tanks and twice daily handling of fish by 30 second aerial exposure in dip nets resulted in a higher nutritional requirement for selenium than was observed in fish not subjected to the stress routine. They concluded that trout exposed to physical stressors could benefit from an additional 0.3 – 2.0 mg/kg dw additional selenium supplementation over and above the Se content of nutritionally Se sufficient diets for fish not undergoing stress.

The fish with the highest known nutritional requirement for selenium is the non-North American resident North African catfish (*Clarias gariepinus*). Abdel-Tawwab et al. (2007) determined in a 12 week study with fingerlings that Se dietary doses of 1.04 mg/kg dw and 3.67 mg/kg dw were associated with suboptimal and maximum weight gains of the catfish, respectively. Catfish survival was 100% in both the Se-deficient and Se-sufficient dietary dose exposures during the 12 week study period. The respective whole body selenium tissue residues at the end of the 12 week study were 4.72 mg/kg dw in the Se-deficient fish and 15.43 mg/kg dw in the fish fed the nutritionally sufficient Se diet. North African catfish (Abdel-Tawwab et al. 2007) is the only known fish species with an identified whole body nutritional requirement for Se higher than the national aquatic life criterion for whole body Se in fish.

Table E-24. Studies with both empirically measured selenium dietary doses and whole body residues associated with nutritional deficiency and sufficiency in fish.

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.21	Basal diet	0.64	Deficiency	Suboptimal weight gain and feed efficiency	Lin and Shiau 2005
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	0.47	Seleno-methionine	1.0	Deficiency	Suboptimal growth, feeding rate and feed conversion rate	Han et al. 2011
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.04	Basal diet	1.04	Deficiency	Reduced growth and survival	Gaber 2007
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	1.4	Basal diet	1.1	Deficiency	Larval survival 32% lower compared to larvae fed selenium-enriched diet	Hamre et al. 2008
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.21 - 0.62	0.21 = Basal diet, 0.62 = seleno-DL-methionine	1.13 - 2.11	Deficiency	Statistically significantly reduced specific growth rate and survival	Liu et al. 2010
Coho salmon (*Oncorhynchus kisutch*)	Smolt 22.7 g	Hatchery reared	0.7 - 0.9	Not given	1.974	Deficiency	Survival of hatchery reared smolts 1.5 - 2.0x lower than wild smolts	Felton et al. 1990
Atlantic salmon (*Salmo salar*)	Fry 0.1 g	4 weeks	0.03 - 0.04	Basal diet	2.7	Deficiency	Decreased survival relative to fry fed diet supplemented with 0.1 µg/g Se and 0.5 IU/g vitamin E	Poston et al. 1976
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	1.04	Organic Se	4.72	Deficiency	Suboptimal weight gain and specific growth rate	Abdel-Tawwab et al. 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 0.6 g	16 weeks	0.6 - 6.6	Selenite $Na_2SeO_3 \cdot 5H_2O$	0.2 - 1.0	Sufficiency	No deficiency or toxicity signs on growth	Hilton and Hodson 1983

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	1.2	Basal diet	0.58 - 0.70	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	0.77	Basal diet	0.9	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Malabar grouper (*Epinephelus malabaricus*)	Juvenile 12.2 g	8 weeks	0.77	Seleno-methionine	0.92	Sufficiency	Maximal weight gain and feed efficiency	Lin and Shiau 2005
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.4	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.13	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Common carp (*Cyprinus carpio*)	Juvenile 26.9 g	120 days	0.24 - 0.32	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.23 - 1.29	Sufficiency	Maximal growth and survival	Gaber 2007
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.3 - 3.9	Selenite $Na_2SeO_3 \cdot 5H_2O$	1.6 - 2.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Prussian carp (*Carassius gibelio*)	Juvenile 2.74 g	100 days	1.23 - 2.77	Seleno-methionine	1.7 - 3.4	Sufficiency	Maximal growth, no effect on survival, no increase in oxidative stress	Han et al. 2011
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	0.10	Basal diet	2.01	Sufficiency	Minimum dietary requirement for acceptable survival and growth	Jaramillo 2006
Atlantic salmon (*Salmo salar*)	Parr 4.5 g	8 weeks	3.1	Seleno-methionine	2.06	Sufficiency	No deficiency signs on growth, survival or glutathione peroxidase activity	Lorentzen et al. 1994
Cobia (*Rachycentron canadum*)	Juvenile 6.27 g	10 weeks	0.85 - 1.36	Seleno-DL-methionine	2.58 - 2.62	Sufficiency	Maximal and statistically identical specific growth rate and survival	Liu et al. 2010

Species	Lifestage / Size Wet wt	Exposure duration	Ingested dietary dose Se mg/kg dry wt.	Se chemical form	Whole body Se mg/kg dry wt	Deficiency or Sufficiency	Deficiency symptoms Basis for sufficiency determination	Reference
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile 26.3 g	11 weeks	2.4 - 4.1	Organic Se - yeast	2.8 - 4.8	Sufficiency	Optimal growth, survival and antioxidant status	Rider et al. 2009
Atlantic cod (*Gadus morhua*)	Larvae 0.16 g (estimated from dry wt of larvae	23 days	4.8	Selenite $Na_2SeO_3 \cdot 5H_2O$	3.5	Sufficiency	Larval survival increased 32%, growth essentially unchanged relative to survival of larvae fed basal diet	Hamre et al. 2008
Coho salmon (*Oncorhynchus kisutch*)	Smolt 14.28 g	Wild smolts	Se in natural diet unknown	Unknown	3.63	Sufficiency	Survival of wild smolts 1.5 - 2.0x higher than hatchery reared smolts	Felton et al. 1990
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.94 g	12 weeks	1.19	Selenite $Na_2SeO_3 \cdot 5H_2O$	5.13	Sufficiency	Highest weight gain of any selenite diet test, significantly higher than basal diet weight gain	Jaramillo 2006
Hybrid striped bass (wiper, *Morone chrysops* x *Morone saxatilis*)	Juvenile 2.92 g	12 weeks	0.90	Seleno-DL-methionine	7.2	Sufficiency	Highest survival and weight gain of any seleno-DL-methionine diet tested	Jaramillo 2006
North African catfish (*Clarias gariepinus*)	Fingerling 68.6 g	12 weeks	3.67	Organic Se	15.43	Sufficiency	Maximal weight gain, specific growth rate and survival	Abdel-Tawwab et al. 2007

APPENDIX F: TOXICITY OF SELENIUM TO AQUATIC PLANTS

1.0 SELENITE

Data are available on the toxicity of selenite to 13 species of freshwater algae and plants (Table F-1). Results ranged from an LC_{50} of 70,000 µg/L for the green alga, *Chlorella ellipsoidea* (Shabana and El-Attar 1995) to 522 µg/L for incipient inhibition of the green alga, *Scenedesmus quadricauda* (Bringmann and Kuhn 1977a, 1978a,b, 1979, 1980b). Foe and Knight (Manuscript) found that 75 µg/L decreased the dry weight of *Selenastrum capricornutum* (Table F-1). Wehr and Brown (1985) reported that 320 µg/L increased the growth of the alga *Chrysochromulina breviturrita*.

The 96-hr EC_{50} for the saltwater diatom, *Skeletonema costatum*, is 7,930 µg/L, based on reduction in chlorophyll *a* (Table F-1). Growth of *Chlorella* sp., *Platymonas subcordiformis*, and *Fucus spiralis* increased at selenite concentrations from 2.6 to 10,000 µg/L (Table F-1). Other marine algae exposed to selenite from 14 to 60 days had no observed effect concentrations (NOAEC) that ranged from 1,076 to 107,606 µg/L. These data suggest that saltwater plants will not be adversely affected by concentrations of selenite that do not affect saltwater animals.

2.0 SELENATE

Growth of several species of green algae was affected by concentrations ranging from 100 to 40,000 µg/L (Table F-1). Blue-green algae appear to be more tolerant to selenate with 1,866 µg/L being the lowest concentration reported to affect growth (Kiffney and Knight 1990). Kumar (1964) found that a blue-green alga developed and lost resistance to selenate. The difference in the sensitivities of green and blue-green algae to selenate might be of ecological significance, particularly in bodies of water susceptible to nuisance algal blooms. For example, Patrick et al. (1975) reported that a concentration of 1,000 µg/L caused a natural assemblage of algae to shift to a community dominated by blue-green algae.

The saltwater coccolithophore, *Cricosphaera elongata*, had reduced growth when exposed to 41,800 µg/L selenate for 14 days (Boisson et al. 1995). Seven other saltwater algal species investigated by Wong and Oliveira (1991a) exhibited NOEC growth values that ranged from 1,043 to 104,328 µg/L. At 10,000 µg/L, selenate is lethal to four species of saltwater phytoplankton and lower concentrations increase or decrease growth (Table F-1). Wheeler et al. (1982) reported that concentrations as low as 10 µg/L reduced growth of *Porphyridium cruentum* (Table F-1).

Although selenite appears to be more acutely toxic than selenate to most aquatic animals, this does not seem to be true for aquatic plants. Selenite and selenate are about equally toxic to the freshwater algae *Anabaena cylindrica*, *Anabaena flos-aquae*, *Anabaena variabilis*, *Anacystis nidulans*, and *Scenedesmus dimorphus* (Kiffney and Knight 1990; Kumar and Prakash 1971; Moede et al. 1980) and the saltwater algae *Agemenellum quadroplicatum*, *Chaetoceros vixvisibilis* and *Amphidinium carterae* (Wong and Oliveira 1991a). The two oxidation states equally stimulated growth of *Chrysochromulina*

breviturrita (Wehr and Brown 1985). On the other hand, selenate is more toxic than selenite to the freshwater *Selenastrum capricornutum* (Richter 1982; Ibrahim and Spacie 1990) and the saltwater *Chorella* sp., *Platymonas subcordiformis* and *Nannochloropsis oculata* (Wheeler et al. 1982; Wong and Oliveira 1991a). In addition, Fries (1982) found that growth of thalli of the brown macroalga, *Fucus spiralis*, was stimulated more by exposure to selenite at 2.605 μg/L than to the same concentration of selenate.

A Final Plant Value, as defined in the Guidelines, cannot be obtained because no test in which the concentrations of selenite or selenate were measured and the endpoint was biologically relevant has been conducted with an important aquatic plant species.

Table F-1. Toxicity of selenium to aquatic plants.

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
FRESHWATER SPECIES						
Selenium (IV)						
Green alga, *Chlorella vulgaris*	Sodium selenite	-	90-120	Reduced growth	5,480	De Jong 1965
Green alga, *Chlorella ellipsoidea*	Sodium selenite	-	7	EC50	70,000	Shabana and El-Attar 1995
Green alga, *Scenedesmus dimorphus*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	522	Bringmann and Kuhn 1977a; 1978a,b; 1979; 1980b
Green alga, *Scenedesmus quadricauda*	Sodium selenite	-	8	Incipient inhibition	2,500	Bringmann and Kuhn 1959a
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	4	EC50	2,900	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenite	-	6	EC50	65,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena constricta*	Sodium selenite	-	7	EC50	67,000	Shabana and El-Attar 1995
Blue-green alga, *Anabaena cylindrica*	Sodium selenite	-	14	Reduced growth	24,000	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenite	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anabaena variabilis*	Sodium selenite	-	6-18	LC50	15,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anacystis nidulans*	Sodium selenite	-	10-18	LC50	30,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcystis aeruginisa*	Sodium selenite	-	8	Incipient inhibition	9,400 (9,300)	Bringmann and Kuhn 1976; 1978a,b
Alga, *Euglena gracilis*	-	-	15	Reduced growth	5,920	Bariaud and Mestre 1984
Duckweed, *Lemna minor*	-	-	4	EC50	2,400	Wang 1986
Duckweed, *Lemna minor*	Sodium selenite	-	14	EC50 (mult. rate)	3,500	Jenner and Janssen-Mommen 1993

Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Duckweed, *Lemna minor*	Sodium selenite	-	14	NOEC (mult. rate)	800	Jenner and Janssen-Mommen 1993
Selenium (VI)						
Green alga, *Ankistrodesmus falcatus*	Sodium selenate	-	14	Did not reduce growth	10	Vocke et al. 1980
Green alga, *Scenedesmus dimorphus*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Green alga, *Scenedesmus obliquus*	Sodium selenate	-	14	Reduced growth	100	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	14	Reduced growth	300	Vocke et al. 1980
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	4	EC50	199	Richter 1982
Green alga, *Selenastrum capricornutum*	Sodium selenate	-	6	EC50	<40,000	Ibrahim and Spacie 1990
Blue-green alga, *Anabaena cylindrica*	Sodium selenate	-	14	Reduced growth	22,100	Moede et al. 1980
Blue-green alga, *Anabaena flos-aquae*	Sodium selenate	-	10	Reduced chlorophyll a	1,866	Kiffney and Knight 1990
Blue-green alga, *Anacystis nidulans*	Sodium selenate	-	6-18	EC50	39,000[b]	Kumar and Prakash 1971
Blue-green alga, *Anabaena viriabilis*	Sodium selenate	-	10-18	EC50	17,000[b]	Kumar and Prakash 1971
Blue-green alga, *Microcoleus vaginatus*	Sodium selenate	-	14	Reduced growth	10,000	Vocke et al. 1980
Duckweed, *Lemna minor*	Sodium selenate	-	14	EC50 (mult. rate)	11,500	Jenner and Janssen-Mommen 1993
Duckweed, *Lemna minor*	Sodium selenate	-	14	NOEC (mult. rate)	>2,400	Jenner and Janssen-Mommen 1993

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
SALTWATER SPECIES						
Selenium (IV)						
Green alga, *Dunaliella tertiolecta*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Diatom, *Skeletonema costatum*	Selenious acid[c]	-	4	EC50 (reduction in chlorophyll a)	7,930	U.S. EPA 1978
Coccolithophore, *Cricosphaera elongata*	Sodium selenite	-	14	Reduced growth	4,570	Boisson et al. 1995
Dinoflagellate, *Amphidinium carterae*	Sodium selenite	-	60	NOEC growth	10,761	Wong and Oliveira 1991a
Dinoflagellate, *Peridinopsis borgei*	Selenium oxide	-	70-75	Maximum growth	0.01-0.05	Lindstrom 1985
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenite	-	60	NOEC growth	107,606	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodiun selenite	-	60	NOEC growth	1,076	Wong and Oliveira 1991a
Selenium (VI)						
Green alga, *Dunaliella tertiolecta*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a
Cyanophyceae alga, *Agemenellum quadruplicatum*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Diatom, *Chaetoceros vixvisibilis*	Sodium selenate	-	60	NOEC growth	1,043	Wong and Oliveira 1991a
Coccolithophore, *Cricosphaera elongate*	Sodium selenate	-	14	Reduced growth	41,800	Boisson et al. 1995
Dinoflagellate,	Sodium	-	60	NOEC	10,433	Wong and

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Amphidinium carterae	selenate			growth		Oliveira 1991a
Eustigmatophyceae alga, *Nannochloropsis oculata*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Isochrysis galbana*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a
Pyrmnesiophyceae alga, *Pavlova lutheri*	Sodium selenate	-	60	NOEC growth	104,328	Wong and Oliveira 1991a

[a] Concentration of selenium, not the chemical.
[b] Estimated from published graph.
[c] Reported by Barrows et al. (1980) in work performed under the same contract.

APPENDIX G: UNUSED DATA

Based on the requirements set forth in the guidelines (Stephen et al. 1985) the following studies are not acceptable for the following reasons and are classified as unused data. Note the acceptance of chronic toxicity data included diet and field exposures where selenium was the dominant toxicant.

Studies Were Conducted with Species That Are Not Resident in North America

Ahsanullah and Brand (1985)
Ahsanullah and Palmer (1980)
Baker and Davies (1997)
Barghigiani et al. (1993)
Chidambaram and Sastry (1991a,b)
Congiu et al. (1989)
Cuvin and Furness (1988)
Fowler and Benayoun (1976a,b)
Gaikwad (1989)
Gotsis (1982)
Hiraika et al. (1985)
Juhnke and Ludemann (1978)
Kitamura (1990)
Manoharan and Prabakaran (1994)
Minganti et al. (1994, 1995)
Niimi and LaHam (1975, 1976)
Regoli (1998)
Regoli and Principato (1995)
Rhodes et al. (1994)
Ringdal and Julshamn (1985)
Rouleau et al. (1992)
Sastry and Shukla (1994)
Savant and Nilkanth (1991)
Shultz and Ito (1979)
Srivastava and Tyagi (1985)
Takayanagi (2001)
Tomasik et al. (1995b)
Tian and Liu (1993)
Wrench (1978)

Deelstra et al. (1989), Forsythe and Klaine (1994), Okasako and Siegel (1980) and Petrucci et al. (1995) conducted tests with brine shrimp species that are too atypical to be used in derving national criteria.

These Studies or Reviews Contain Relevant Data That Have Been Published Elsewhere

Adams and Johnson (1981)
Biddinger and Gloss (1984)
Bowie et al. (1996)
Brandao et al. (1992)
Brooks (1984)
Burton and Stemmer (1988)
Chapman et al. (1986)
Davies (1978)
Debruyn and Chapman (2007)
Devillers et al. (1988)
Eisler (1985)
Hall and Burton (1982)
Hodson and Hilton (1983)
Hodson et al. (1984)
Jenkins (1980)
Kaiser et al. (1997)
Kay (1984)
LeBlanc (1984)
Lemly (1993c, 1996ab, 1997d)
Lemly and Smith (1987)
McKee and Wolf (1963)
National Research Council (1976) Neuhold (1987)
NCDNR&CD (1986)
Peterson and Nebeker (1992)
Phillips and Russo (1978)
Presser (1994)
Roux et al. (1996)
Swift (2002)
Thompson et al. (1972)
Versar (1975)

Authors Did Not Specify the Oxidation State of Selenium Used in Study

Greenberg and Kopec (1986)
Hutchinson and Stokes (1975)
Kapu and Schaeffer (1991)
Kramer et al. (1989)
Mahan et al. (1989)
Rauscher (1988)
Snell et al. (1991b)

Not Useful Because of No Effects Observed at Exposure Concentrations or Insufficient Number of Treatments

Muscatello and Janz (2009)
Pyle et al. (2005)
Schlenk et al (2003)

Chronic Study with no Dietary Exposure

Hopkins et al. (2002)
Oti (2005)
Rowe (2003)
Teh et al. (2002)

Selenium Was a Component of an Effluent, Fly Ash, Formulation, Mixture, Sediment or Sludge

Apte et al. (1987)
Baer et al. (1995)
Baker et al. (1991)
Berg et al. (1995)
Besser et al. (1989)
Biedlingmaier and Schmidt (1989)
Bjoernberg (1989)
Bjoernberg et al. (1988)
Bleckmann et al. (1995)
Boisson et al. (1989)
Bondavalli et al. (1996)
Bowmer et al. (1994)
Brieger et al. (1992)
Burton and Pinkney (1984)
Burton et al. (1983, 1987a)
Cherry et al. (1987)
Cieminski and Flake (1995)
Clark et al. (1989)
Cooke and Lee (1993)
Cossu et al. (1997)
Coyle et al. (1993)
Crane et al. (1992)
Crock et al. (1992)
Cushman et al. (1977)
Davies and Russell (1988)
de Peyster et al. (1993)
Dickman and Rygiel (1996)
Dierenfeld et al. (1993)
Doebel et al. (2004)
Drndarski et al. (1990)
Eriksson and Forsberg (1992)
Eriksson and Pedros-Alio (1990)
Fairbrother et al. (1994)
Fava et al. (1985a,b)
Feroci et al. (1997)
Finger and Bulak (1988)
Finley (1985)
Fisher and Wente (1993)
Fjeld and Rognerud (1993)
Fletcher et al. (1994)
Follett (1991)
Gerhardt (1990)
Gerhardt et al. (1991)
Gibbs and Miskiewicz (1995)
Graham et al. (1992)

Gunderson et al. (1997)
Hall (1988)
Hall et al. (1984, 1987, 1988,1992)
Hamilton et al. (1986, 2000)
Harrison et al. (1990)
Hartwell et al. (1987ab, 1988, 1997)
Hatcher et al. (1992)
Haynes et al. (1997)
Hayward et al. (1996)
Hellou et al. (1996b)
Henebry and Ross (1989)
Henny et al. (1989, 1990, 1995)
Hildebrand et al. (1976)
Hjeltnes and Julshman (1992)
Hockett and Mount (1996)
Hodson (1990)
Hoffman et al. (1988, 1991)
Homziak et al. (1993)
Hopkins et al. (2000)
Hopkins et al. (2004)
Hothem and Welsh (1994a)
Jackson (1988)
Jackson et al. (1990)
Jacquez et al. (1987)
Jay and Muncy (1979)
Jayasekera (1994)
Jayasekera and Rossbach (1996)
Jenner and Bowmer (1990) (1992)
Jin et al. (1997)
Jorgensen and Heisinger (1987)
Karlson and Frankenberger (1990)
Kemble et al. (1994)
Kennedy (1986)
Kersten et al. (1991)
King and Cromartie (1986)
King et al. (1991, 1994)
Klusek et al. (1993)
Koh and Harper (1988)
Koike et al. (1993)
Krishnaja et al. (1987)
Kruuk and Conroy (1991)
Kuehl and Haebler (1995)
Kuehl et al. (1994)
Kuss et al. (1995)
Landau et al. (1985)
Livingston et al. (1991)
Lobel et al. (1990)
Luoma and Phillips (1988)
Lundquist et al. (1994)
Lyle (1986)
MacFarlane et al. (1986)
Mann and Fyfe (1988)
Marcogliese et al. (1987)
Marvin and Howell. (1997)Maurer et al (1999)
McCloskey and Newman (1995)
McCloskey et al. (1995)
McCrea and Fischer (1986)
McLean et al. (1991)
Mehrle et al. (1987)
Metcalf-Smith (1994)
Micallef and Tyler (1989)
Mikac et al. (1985)
Miles and Tome (1997)
Miller et al. (1996)
Misitano and Schiewe (1990)
Moore (1988)
Munawar and Legner (1993)
Muskett et al. (1985)
Naddy et al. (1995)
Nielsen and Bjerregaard (1991)
Norman et al. (1992)
Nuutinen & Kukkonen (1998)
Oberbach and Hartfiel (1987, 1988)
Oberbach et al. (1989)
Ohlendorf et al. (1989, 1990, 1991)
Olson and Welsh (1993)
Peters et al.(1999)
Phillips and Gregory (1980)
Pratt and Bowers (1990)
Presser and Ohlendorf (1987)
Prevot and Sayer-Gobillard (1986)
Pritchard (1997)
Pyle et al. (2001)
Reash et al. (1988, in press)
Rhodes and Burke (1996)

Ribeyre et al. (1995)
Rice et al. (1995)
Riggs and Esch (1987)
Riggs et al. (1987)
Robertson et al. (1991)
Roper et al. (1997)
Rowe et al. (1996)
Russell et al. (1994)
Ryther et al. (1979)
Saiki and Jenings (1992)
Saiki and Ogle (1995)
Saleh et al. (1988)
Seelye et al. (1982)
Sevareid and Ichikawa (1983)
Skinner (1985)
Somerville et al. (1987)
Sorenson and Bauer (1983)
Specht et al. (1984)
Steele et al. (1992)
Stemmer et al. (1990)
Summers et al. (1995)
Thomas et al. (1980b)
Timothy et al. (2001)
Trieff et al. (1995)
Turgeon and O=Conner (1991)
Twerdok et al. (1997)
Unsal (1987)
Van Metre and Gray (1992)
Wahl et al. (1994)
Wandan and Zabik (1996)
Wang et al. (1992, 1995)
Welsh (1992)
Weres et al. (1990)
White and Geitner (1996)
Wiemeyer et al. (1986)
Wildhaber and Schmitt (1996)
Williams et al. (1989)
Wolfe et al. (1996)
Wolfenberger (1987)
Wong and Chau (1988)
Wong et al. (1982)
Wu et al. (1997)
Yamaoka et al. (1994)
Zagatto et al. (1987)
Zaidi et al. (1995)
Zhang et al. (1996)

Exposed enzymes, excised tissue or tissue extractor

Tripathi and Pandey (1985) and Heinz (1993b) used test organisms that had been previously exposed to pollutants in food or water.

Albers et al. (1996)
Al-Sabti (1994, 1995)
Arvy et al. (1995)
Augier et al. (1993a, b)
Avery et al. (1996)
Baatrup (1989)
Baatrup and Dansher (1987)
Baatrup et al. (1986)
Babich et al. (1986, 1989)
Barrington et al. (1997)
Becker et al. (1995a,b)
Bell et al. (1984, 1985, 1986a,b, 1987ab)
Berges and Harrison (1995)
Blondin et al. (1988)
Boisson et al. (1996)
Bottino et al. (1984)
Braddon (1982)
Braddon-Galloway and Balthrop (1985)
Bradford et al. (1994a,b)
Brandt et al. (1990)
Byl et al. (1994)
Chandy and Patel (1985)
Chen et al. (1997)
Cheng et al. (1993)
Christensen and Tucker (1976)
Dabbert and Powell (1993)
DeQuiroga et al. (1989)
Dierickx (1993)
Dietrich et al. (1987)
DiIlio et al. (1986)

Doyotte et al. (1997)
Drotar et al. (1987)
Dubois and Callard (1993)
Ebringer et al. (1996)
Engberg and Borsting (1994)
Engberg et al. (1993)
Eun et al. (1993)
Foltinova and Gajdosova (1993)
Foltinova et al. (1994)
Freeman and Sanglang (1977)
Grubor-Lajsic et al. (1995)
Hait and Sinha (1987)
Hanson (1997)
Heisinger and Scott (1985)
Heisinger and Wail (1989)
Henderson et al. (1987)
Henny and Bennett (1990)
Hoffman and Heinz (1988, 1998)
Hoffman et al. (1989, 1998)
Hoglund (1991)
Hontela et al. (1995)
Hsu et al. (1995)
Hsu and Goetz (1992)
Ishikawa et al. (1987)
James et al. (1993)
Jovanovic et al. (1995, 1997)
Kai et al. (1995)
Kedziroski et al. (1996)
Kelly et al. (1987)
Kralj and Stunja (1994)
Lalitha and Rani (1995)
Lan et al. (1995)
Lemaire et al. (1993)
Livingstone et al. (1992)
Low and Sin (1995, 1996)
Micallef and Tyler (1990)
Montagnese et al. (1993)
Murata et al. (1996)
Nakonieczny (1993)
Neuhierl and Boeck (1996)
Nigro (1994)
Nigro et al (1992)
Norheim and Borch-Iohnsen (1990)
Norheim et al. (1991)
O=Brien et al. (1995)
Olson and Christensen (1980)
Overbaugh and Fall (1985)
Palmisano et al. (1995)
Patel et al. (1990)
Patel and Chandy (1987)
Perez Campo et al. (1990)
Perez-Trigo et al. (1995)
Phadnis et al. (1988)
Price and Harrison (1988)
Rady et al. (1992)
Rani and Lalitha (1996)
Regoli et al. (1997)
Schmidt et al. (1985)
Schmitt et al. (1993)
Segner et al. (1994)
Sen et al. (1995)
Shigeoka et al. (1990, 1991)
Siwicki et al. (1994)
Srivastava and Srivastava (1995)
Sun et al. (1995)
Takeda et al. (1992a,b,(1993, 1997)
Treuthardt (1992)
Vazquez et al. (1994)
Veena et al. (1997)
Wise et al. (1993a,b)
Wong and Oliveira (1991b)
Yokota et al. (1988)

Test procedures test material or results were not adequately described by Botsford (1997), Botsford et al. (1997, 1998), Bovee (1978), Gissel-Nielsen and Gissel-Nielsen (1973, 1978), Greenberg and Kopec (1986), Mauk (2001), and Nassos et al. (1980) or when the test media contained an excessive amount (>200 μg/L) of EDTA (Riedel and Sanders (1996).

Some data obtained from tests conducted with just one exposure concentration to evaluate acute or chronic toxicity were not used (e.g., Bennett 1988; Heinz and Hoffman 1998; Munawar et al. 1987; Pagano et al. 1986; Wolfenberger 1986).

Kaiser (1980) calculated the toxicities of selenium(IV) and selenium(VI) to *Daphnia magna* based on physiochemical parameters. Kumar (1964) did not include a control treatment in the toxicity tests. The daphnids were probably stressed by crowding in the tests reported be Schultz et al. (1980). Siebers and Ehlers (1979) exposed too few test organisms as did Owsley (1984) in some tests.

Selenium Concentrations Reported in Wild Aquatic Organisms Were Insufficient to Calculate BAF

Abdel-Moati and Atta (1991)
Adeloju and Young (1994)
Aguirre et al. (1994)
Akesson and Srikumar (1994)
Aksnes et al. (1983)
Allen and Wilson (1990)
Ambulkar et al. (1995)
Amiard et al. (1991, 1993)
Andersen and Depledge (1997)
Andreev and Simeonov (1992)
Angulo (1996)
Arrula et al. (1996)
Arway (1988)
Ashton (1991)
Augier et al. (1991, 1993, 1995a,b)
Augspurger et al. (1998)
Avery et al. (1996)
Badsha and Goldspink (1988)
Baines and Fisher (2001)
Baldwin and Maher (1997)
Baldwin et al. (1996)
Barghigiani (1993)
Barghigiani et al. (1991)
Baron et al. (1997)
Batley (1987)
Baumann and Gillespie (1986)
Baumann and May (1984)
Beal (1974)
Beck et al. (1997)
Beland et al. (1993)
Beliaeff et al. (1997)
Bell and Cowey (1989)
Benemariya et al. (1991)
Berry et al. (1997)
Bertram et al. (1986)
Besser et al. (1994, 1993)
Birkner (1978)
Boisson and Romeo (1996)
Bowerman et al. (1994)
Braune et a. (1991)
Brezina and Arnold (1977)
Brugmann and Hennings (1994)
Brugmann and Lange (1988)
Brumbaugh and Walther (1991)
Burger (1992, 1994, 1995, 1996, 1997a,b)
Burger and Gochfeld (1992a,b, 1993, 1995 ab, 1996, 1997)
Burger et al. (1992a,b,c,1993, 1994a,b)
Byrne and DeLeon (1986)
Byrne et al. (1985)
Cantillo et al. (1997)
Capar and Yess (1996)
Capelli et al. (1987, 1991)
Cappon (1984)
Cappon and Smith (1981) (1982a,b)
Cardellicchio (1995)
Carell et al. (1987)
Carter and Porter (1997)
Caurant et al. (1994, 1996)
Chau and Riley (1965)
Chiang et al. (1994)

Chou and Uthe (1991)
Chvojka (1988)
Chvojka et al. (1990)
Clifford and Harrison (1988)
Collins (1992)
Combs et al. (1996)
Cosson et al. (1988)
Courtney et al. (1994)
Cruwys et al. (1994)
Crutchfield (2000)
Cumbie and Van Horn (1978)
Currey et al. (1992)
Custer and Hohman (1994)
Custer and Mitchell (1991, 1993)
Custer et al. (1997)
Dabeka and McKenzie (1991)
Davoren (1986)
Deaker and Maher (1997)
Demon et al. (1988)
Dietz et al. (1995, 1996)
Doherty et al. (1993)
Elliott and Scheuhammer (1997)
Eriksson et al. (1989)
Evans et al. (1993)
Felton rt al. (1990)
Felton et al. (1994)
Fitzsimons et al. (1995)
Focardi et al. (1985, 1988)
Fowler (1986)
Fowler et al. (1975, 1985)
France (1987)
Friberg (1988)
Froslie et al. (1985, 1987)
Gabrashanske and Daskalova (1985)
Gabrashanska and Nedeva (1994)
Galgan and Frank (1995)
Garcia - Hermandez et al. (2000)
Giardina et al. (1997)
Gillespie and Baumann (1986)
Gochfeld (1997)
Goede (1985, 1991, 1993a,b)
Goede et al. (1989, 1993)
Goede and DeBruin (1984, 1985)
Goede and Wolterbeek (1993, 1994a,b)
Gras et al. (1992)
Greig and Jones (1976)
Gutenmann et al. (1988)
Gutierrez-Galindo et al. (1994)
Guven et al. (1992)
Halbrook et al. (1996)
Hall and Fisher (1985)
Hamilton and Waddell (1994)
Hamilton and Wiedmeyer (1990)
Hansen et al. (1990)
Hardiman and Pearson (1995)
Hargrave et al. (1992)
Harrison and Klaverkamp (1990)
Hasunuma et al. (1993)
Haynes et al. (1995)
Hein et al. (1994)
Heiny and Tate (1997)
Heinz (1993a)
Heinz and Fitzgerald (1993a,b)
Heit (1985)
Heit and Klusek (1985)
Heit et al. (1980, 1989)
Hellou et al. (1992a,b) (1996a,b)
Henny and Herron (1989)
Hodge et al. (1996)
Hilton et al. (1982)
Honda et al. (1986)
Hothem and Ohlendorf (1989)
Hothem and Welsh (1994b)
Hothem and Zador (1995)
Hothem et al. (1995)
Houpt et al. (1988)
Hunter et al. (1995, 1997)
Ibrahim and Farrag (1992)
Ibrahim and Mat (1995)
Ishikawa et al. (1993)
Itano et al. (1984, 1985a,b)
Jarman et al. (1996)
Johns et al. (1988)
Johnson (1987)
Jop et al. (1997)

Jorhem et al. (1994)
Julshamn et al. (1987)
Kai et al. (1986a,b, 1988, 1992a,b, 1996)
Kaiser et al. (1979)
Kalas et al. (1995)
Kidwell et al. (1995)
Koeman et al. (1973)
Kovacs et al. (1984)
Krogh and Scanes (1997)
Krushevska et al. (1996)
Lakshmanan and Stephen (1994)
Lalitha et al. (1994)
LamLeung et al. (1991)
Lan et al. (1994a,b)
Langlois and Langis (1995)
Larsen and Stuerup (1994)
Larsen et al. (1997)
Lauchli (1993)
Law et al. (1996)
Lee and Fisher (1992a,b, 1993)
Leighton and Wobeser (1994)
Leland and Scudder (1990)
Lemly (1985a, 1994)
Leonzio et al. (1986, 1989, 1992)
Leskinen et al. (1986)
Li et al. (1996)
Lie et al. (1994)
Liu et al. (1987)
Lizama et al. (1989)
Lobel et al. (1989, 1991, 1992a,b)
Lonzarich et al. (1992)
Lourdes et al. (1990)
Lowe et al. (1985)
Lucas et al. (1970)
Lytle and Lytle (1982)
Mackey et al. (1996)
Maher (1987)
Maher et al. (1992, 1997)
Mann et al. (1988)
Mason et al. (2000)
Masuzawa et al. (1988)
Matsumoto (1991)
Maven et al. (1995)
May and McKinney (1981)
Mcdowell et al. (1995)
McKenzie-Parnell et al. (1988)
Meador et al. (1993)
Mehrle et al. (1982)
Meltzer et al. (1993)
Metcalfe-Smith et al. (1992, 1996)
Michot et al. (1994)
Mills et al. (1993)
Moharram et al. (1987)
Moller (1996)
Mora and Anderson (1995)
Morera et al. (1997)
Muir et al. (1988)
Mutanen et al. (1986)
Nadkarni and Primack (1993)
Nakamoto and Hassler (1992)
Narasaki and Cao (1996)
Navarrete et al. (1990)
Nettleton et al. (1990)
Nicola et al. (1987)
Nielsen and Dietz (1990)
Norheim (1987)
Norheim et al. (1992)
Norrgren et al. (1993)
Norstrom et al. (1986)
O=Conner (1996)
O=Shea et al. (1984)
Ober et al. (1987)
Oehlenschlager (1997)
Ohlendorf (1986)
Ohlendorf and Harrison (1986)
Ohlendorf and Marois (1990)
Ohlendorf et al. (1986a,b, 1987, 1988a,b)
Okazaki and Panietz (1981)
Ostapczuk et al. (1997)
Pakkala et al. (1972)
Pal et al. (1997) Palawski et al. (1991)
Palmer-Locarnini and Presley (1995)
Paludan-Miller et al. (1993)
Papadopoulou and Andreotis (1985)
Park and Presley (1997)
Park et al. (1994)

Paveglio et al. (1994)
Payer and Runkel (1978)
Payer et al. (1976)
Pennington et al. (1982)
Presley et al. (1990)
Quevauviller et al. (1993a,b)
Ramos et al. (1992)
Rao et al. (1996)
Reinfelder and Fisher (1991)
Reinfelder et al. (1993, 1998)
Renzoni et al. (1986)
Riget et al. (1996)
Risenhoover (1989)
Roditi (2000)
Roux et al. (1994)
Ruelle and Keenlyne (1993)
Sager and Cofield (1984)
Saiki (1986 ab, 1987, 1990)
Saiki and Lowe (1987)
Saiki and May (1988)
Saiki and Palawski (1990)
Saiki et al. (1992, 1993)
Sanders and Gilmour (1994)
Scanes (1997)
Scheuhammer et al. (1988)
Schantz et al. (1997)
Schmitt and Brumbaugh (1990)
Schramel and Xu (1991)
Schuler et al. (1990)
Scott and Latshaw (1993)
Secor et al. (1993)
Seelye et al. (1982)
Sharif et al. (1993)
Shen et al. (1997)
Shirasaki et al. (1996)
Shultz and Ito (1979)
Simopoulos (1997)
Skaare et al. (1990, 1994)
Smith and Flegal (1989)
Smith et al. (1992)
Sorensen (1988)
Sorensen and Bauer (1984a,b) Sorensen and Bjerregaard (1991)
Sorensen et al. (1982, 1983, 1984)
Southworth et al. (2000)
Sparling and Lowe (1996)
Speyer (1980)
Steimle et al. (1994)
Stoeppler et al. (1988)
Stone et al. (1988)
Stripp et al. (1990)
Sundarrao et al. (1991) (1992)
Svensson et al. (1992)
Tabaka et al. (1996)
Talbot and Chang (1987)
Tallandini et al. (1996)
Tan and Marshall (1997)
Tang et al. (1997)
Tao et al. (1993)
Teherani (1987)
Teigen et al. (1993)
Thomas et al. (1999)
Tilbury et al. (1997)
Topcuoglu et al. (1990)
TranVan and Teherani (1988)
Trocine and Trefry (1996)
Uthe and Bigh (1971)
Vanderstoep et al. (1990)
Varanasi et al. (1993, 1994)
Vitaliano and Zdanowicz (1992)
Vlieg (1990)
Vlieg et al. (1993)
Vos et al. (1986)
Waddell and May (1995)
Wagemann (1988)
Wagemann and Stewart (1994)
Wagemann et al. (1988) (1996) Walsh et al. (1977)
Wang (1996)
Ward and Flick (1990)
Warren et al. (1990)
Weber (1985)
Welsh and Maughan (1994)
Wen et al. (1997)
Wenzel and Gabrielsen (1995)
Whyte and Boutillier (1991)
Williams et al. (1994)
Wilson et al. (1992, 1997)
Winger and Andreasen (1985)
Winger et al. (1984, 1990)
Woock and Summers (1984)
Wren et al. (1987)
Wu and Huang (1991)
Yamaoka et al. (1996)

Yamazaki et al. (1996)
Yoshida and Yasumoto (1987)
Zatta et al. (1985)
Zeisler et al. (1988, 1993)
Zhou and Liu (1997)

APPENDIX H: CALCULATION OF EF VALUES

EPA calculated EF values by searching its database of selenium measurements and identifying all the selenium measurements from algae, detritus, or sediment. EPA then searched for corresponding water column measurements from samples collected at the same aquatic site within one year of the particulate sample. If more than one water measurement was available for any given particulate measurement, the median was used. For each of these matched pairs of particulate and water measurements, EPA calculated the ratio of particulate concentration to water concentration. If more than one ratio for any given category of particulate material (algae, detritus, or sediment) was calculated at an aquatic site, EPA used the median ratio. The geometric mean of the algae, detritus, and sediment ratios was used as the site *EF*. Because there were at most only 3 possible values (one for algae, one for detritus, and one for sediment), EPA used the geometric mean in order to reduce the potential for one of the values to have excessive influence on the final site *EF* value. Sites with insufficient data to fulfill these criteria are left blank.

EPA evaluated differences in bioaccumulation between different categories of aquatic systems by analyzing EF values for different categories. EPA sequentially consolidated categories and examined differences in the distribution of EF values between categories. See text for a complete description of this analysis.

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Birkner 1978	East Allen Reservoir, Medicine Bow WY	20	Reservoir	Lentic	3.00		41.00	11.09	4.80	2.31
Birkner 1978	Galett Lake, Laramie WY	7	Lake	Lentic	0.18		2.80	0.70	0.80	0.88
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	30	Pond	Lentic	15.50		47.30	27.08	15.90	1.70
Birkner 1978	Meeboer Lake, Laramie WY	3	Lake	Lentic	0.10		0.30	0.17	0.30	0.58
Birkner 1978	Miller's Lake, Wellington CO	22	Lake	Lentic	4.60		44.00	14.23	6.00	2.37
Birkner 1978	Sweitzer Lake, Delta CO	27	Lake	Lentic	10.35		6.50	8.20	9.40	0.87
Birkner 1978	Twin Buttes Reservoir, Laramie WY	23	Reservoir	Lentic	7.80		10.80	9.18	7.60	1.21

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Bowie et al. 1996	Hyco Reservoir		Reservoir	Lentic	27.00			27.00	11.50	2.35
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	N2	Reservoir	Lentic	2.65		0.60	1.26	1.00	1.26
Butler et al. 1997	Large pond on Dove Creek	DCP1	Pond	Lentic	1.00		2.10	1.45	2.00	0.72
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	MNP2	Pond	Lentic	5.40		6.70	6.01	3.00	2.00
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	MNP3	Pond	Lentic	4.50		5.90	5.15	1.00	5.15
Butler et al. 1997	Pond on Cahone Canyon, west of 1 5 Road	CHP	Pond	Lentic	4.00		2.10	2.90	5.00	0.58
Butler et al. 1997	Pond on Woods Canyon at 15 Road	WCP	Pond	Lentic	2.30		3.20	2.71	3.00	0.90
Butler et al. 1997	West pond at CC Road	PVP1	Pond	Lentic	1.50		1.40	1.45	2.00	0.72
Grasso et al. 1995	Arapahoe Wetlands Pond	17	Pond	Lentic	1.87		0.40	0.86	1.00	0.86
Lemly 1985	Badin Lake		Lake	Lentic	7.70		2.07	3.99	0.32	12.48
Lemly 1985	Belews Lake		Lake	Lentic	44.10		8.27	19.10	10.91	1.75
Lemly 1985	High Rock Lake		Lake	Lentic	6.20		1.80	3.34	0.67	4.99
Muscatello and Janz 2009	Vulture Lake		Lake	Lentic	0.35		0.54	0.43	0.43	1.01
Orr et al. 2006	Barns Lake Wetland	BLW	Lake	Lentic	4.40		2.00	2.97	0.50	5.93

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Orr et al. 2006	Fording River Oxbow	FRO	Oxbow	Lentic	5.55		7.90	6.62	5.04	1.37
Orr et al. 2006	Fording Settling Pond (Clode Pond)	FSP	Pond	Lentic	5.49		2.80	3.92	42.99	0.09
Orr et al. 2006	Goddard Marsh	GM	Marsh	Lentic	3.21		26.00	9.14	90.95	0.10
Orr et al. 2012	Clode Pond 11	CL11	Pond	Lentic	25.80			25.80	36.10	0.71
Orr et al. 2012	Elk Lakes 14	EL14	Lake	Lentic	0.66			0.66	0.40	1.64
Orr et al. 2012	Flathead Wetland 17	FL17	Marsh	Lentic	1.42			1.42	0.20	7.10
Orr et al. 2012	Fording River Oxbow 10	FO10	Oxbow	Lentic	67.31			67.31	50.10	1.34
Orr et al. 2012	Goddard Marsh 13	GO13	Marsh	Lentic	18.15			18.15	16.30	1.11
Orr et al. 2012	Henretta Lake 27	HE27	Lake	Lentic	4.30			4.30	8.60	0.50
Saiki and Lowe 1987	Kesterson Pond 11		Pond	Lentic	18.15	47.95	8.56	19.53	38.60	0.51
Saiki and Lowe 1987	Kesterson Pond 2		Pond	Lentic	152.70	44.65	34.82	61.92	195.85	0.32
Saiki and Lowe 1987	Kesterson Pond 8		Pond	Lentic	136.50	92.00	6.05	42.34	70.35	0.60
Saiki and Lowe 1987	Volta Pond 26		Pond	Lentic	0.42	1.01	0.29	0.50	0.53	0.93
Saiki and Lowe 1987	Volta Pond 7		Pond	Lentic		1.39	0.39	0.74	0.63	1.17
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 7	Pond	Lentic	87.10		5.90	22.67	100.00	0.23

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 2	Pond	Lentic	52.50		9.30	22.10	90.00	0.25
Schuler et al. 1990	Kesterson National Wildlife Refuge	Kesterson Pond 11	Pond	Lentic	53.70		11.50	24.85	40.00	0.62
Stephens et al. 1988	Marsh 4720	*	Marsh	Lentic	2.10		4.20	2.97	31.00	0.10
Butler et al. 1991	Uncompahgre River at Colona	4	River	Lotic	0.95			0.95	1.50	0.63
Butler et al. 1993	Spring Cr. at La Boca	SP2	Creek	Lotic	1.60		0.50	0.89	5.00	0.18
Butler et al. 1995	Cahone Canyon at Highway 666	CH	Creek	Lotic	2.50		4.30	3.28	12.00	0.27
Butler et al. 1995	Hartman Draw near mouth, at Cortez	HD2	Draw	Lotic	0.45		0.20	0.30	2.00	0.15
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	ME1	Creek	Lotic	1.80			1.80	2.00	0.90
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	ME2	Creek	Lotic	1.11		1.10	1.10	3.00	0.37
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	ME4	Creek	Lotic	1.04		0.50	0.72	6.00	0.12
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	ME3	Creek	Lotic	0.82		0.40	0.57	6.00	0.10
Butler et al. 1995	Navajo Wash near Towaoc	NW	Wash	Lotic	3.45		1.60	2.35	12.00	0.20
Butler et al. 1995	San Juan River at Four Comers	SJ1	River	Lotic	0.52		0.30	0.39	1.50	0.26
Butler et al. 1995	San Juan River at Mexican Hat Utah	SJ3	River	Lotic	0.94		0.20	0.43	1.50	0.29
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	WC	Creek	Lotic	3.30		1.50	2.22	5.50	0.40

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Butler et al. 1997	Cahone Canyon at Highway 666	CH1	Creek	Lotic	2.05			2.05	10.50	0.20
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	MUD2	Creek	Lotic	1.30			1.30	18.50	0.07
Butler et al. 1997	Tributary of Cahone Canyon at 13 Road	CH2	Creek	Lotic	1.75			1.75	5.50	0.32
Butler et al. 1997	Tributary of Yellow Jacket Canyon at Highway 666	YJ1	Creek	Lotic	1.85			1.85	7.00	0.26
Butler et al. 1997	Unnamed tributary of Cow Canyon at 8 Road	COW	Creek	Lotic	1.45			1.45	3.50	0.41
Butler et al. 1997	Unnamed tributary of Cross Canyon upstream from Alkali Canyon	CCTR	Creek	Lotic	1.75			1.75	4.50	0.39
Casey 2005	Deerlick Creek		Creek	Lotic		1.00	0.20	0.45	0.20	2.24
Casey 2005	Luscar Creek		Creek	Lotic	5.50	3.20	2.40	3.48	10.70	0.33
Formation 2012	Crow Creek - 1A	CC-1A	Creek	Lotic	3.64		1.20	2.09	2.45	0.80
Formation 2012	Crow Creek - 3A	CC-3A	Creek	Lotic	3.10		0.83	1.60	2.20	0.81
Formation 2012	Crow Creek - CC150	CC-150	Creek	Lotic	1.20		0.63	0.87	0.80	1.04
Formation 2012	Crow Creek - CC350	CC-350	Creek	Lotic	1.50		0.70	1.02	0.86	1.16
Formation 2012	Crow Creek - CC75	CC-75	Creek	Lotic	1.01		0.54	0.74	0.52	1.19
Formation 2012	Deer Creek	DC-600	Creek	Lotic	4.55		1.40	2.52	1.62	1.55

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Formation 2012	Hoopes Spring - HS	HS	Spring	Lotic	12.00		2.30	5.25	20.95	0.24
Formation 2012	Hoopes Spring - HS3	HS-3	Spring	Lotic	12.00		7.00	9.17	17.05	0.54
Formation 2012	Sage Creek - LSV2C	LSV-2C	Creek	Lotic	8.09		4.60	6.10	13.80	0.45
Formation 2012	Sage Creek - LSV4	LSV-4	Creek	Lotic	9.56		3.60	5.87	8.45	0.69
Formation 2012	South Fork Tincup Cr.	SFTC-1	Creek	Lotic	0.73		0.31	0.47	0.44	1.32
Golder 2011; Teck Coal 2013	McLeod River below Cheviot Creek	MR-2	River	Lotic	1.47			1.47	2.38	0.62
Golder 2011; Teck Coal 2013	McLeod River below Luscar Dreek	MR-6	River	Lotic	0.86			0.86	4.29	0.20
Golder 2011; Teck Coal 2013	McLeod River below Whitehorse Creek	MR-4	River	Lotic	0.68			0.68	1.07	0.64
Golder 2011; Teck Coal 2013	McLeod River reference	MR-1	River	Lotic	0.75			0.75	0.30	2.50
Golder 2011; Teck Coal 2013	Prospect Creek far field	PC-3	Creek	Lotic	0.37			0.37	0.63	0.59
Golder 2011; Teck Coal 2013	Prospect Creek reference	PC-1	Creek	Lotic	0.86			0.86	0.40	2.15
Hamilton and Buhl 2004	lower East Mill Creek	LEMC	Creek	Lotic	25.70		38.90	31.62	24.00	1.32

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	ER 745	River	Lotic	0.31		1.28	0.63	0.10	6.30
McDonald and Strosher 1998	Elk R. above Fording R.	ER 750	River	Lotic	0.78		0.70	0.74	0.40	1.85
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	ER 746	River	Lotic	1.56		2.41	1.94	8.60	0.23
McDonald and Strosher 1998	Michel Cr. at Highway 3	ER 751	Creek	Lotic	1.26		2.32	1.71	7.10	0.24
Orr et al. 2006	Alexander Creek	AC	Creek	Lotic	4.49		0.90	2.01	0.90	2.23
Orr et al. 2006	Fording River	FR	River	Lotic	3.27		2.10	2.62	20.10	0.13
Orr et al. 2006	Line Creek	LC	Creek	Lotic	2.19		2.10	2.14	20.90	0.10
Orr et al. 2012	Elk River 1	EL1	River	Lotic	2.30			2.30	4.20	0.55
Orr et al. 2012	Elk River 12	EL12	River	Lotic	2.00			2.00	0.75	2.67
Orr et al. 2012	Fording River 23	FO23	River	Lotic	6.35			6.35	30.60	0.21
Orr et al. 2012	Michel Creek 2	MI2	Creek	Lotic	2.10			2.10	7.40	0.28
Presser and Luoma 2009	Upper Peters canyon (dry)	U PCW dry	Wash	Lotic	1.20		0.60	0.85	3.20	0.27
Saiki and Lowe 1987	San Luis Drain		Drain	Lotic	67.00	275.00	79.90	113.76	316.50	0.36
Saiki and Lowe 1987	Volta Wasteway		Wasteway	Lotic	0.87	2.03	0.24	0.76	0.74	1.03
Saiki et al. 1993	Mud Slough at Gun Club Road	GT5	Slough	Lotic	4.50	14.95		8.20	6.00	1.37

Reference	Site description	Site ID	Specific waterbody type - original	Specific waterbody type - Lentic or Lotic	C_{algae} (mg/kg)	$C_{detritus}$ (mg/kg)	C_{sed} (mg/kg)	$C_{particulate}$ (mg/kg)	C_{water} (µg/L)	Site EF (L/g)
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	GT4	Slough	Lotic	1.39	8.40		3.42	8.00	0.43
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	SJR2	River	Lotic	1.25	5.00		2.50	7.00	0.36
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	SJR3	River	Lotic	0.45	1.25		0.75	1.00	0.75
Stephens et al. 1988	Drain J3	*	Drain	Lotic	24.00		48.00	33.94	110.00	0.31

APPENDIX I: OBSERVED VERSUS PREDICTED EGG-OVARY CONCENTRATIONS

The following table includes data for 317 individual fish tissue selenium measurements from the 64 sites where EFs could be calculated. Observed egg-ovary fish tissue measurements were compared to predicted egg-ovary fish tissue measurements calculated using equation 22 of the main text, also shown here for convenience.

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

These data were used to generate the observed to predicted egg-ovary concentration Figure 6.3 of the main text. When the measured tissue type was either muscle or whole body, it was converted to egg-ovary using taxa specific conversion factors. The predicted and measured concentrations are highly correlated ($r = 0.82$, $t_{(315)} = 25.30$, $P < 0.001$).

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Birkner 1978	East Allen Reservoir, Medicine Bow WY	Iowa darter	4.80	2.31	2.87	1.45	46.14	52.68	WB
Birkner 1978	Galett Lake, Laramie WY	Iowa darter	0.80	0.88	2.87	1.45	2.91	3.05	WB
Birkner 1978	Larimer Highway 9 Pond, Fort Collins CO	northern plains killifish	15.90	1.70	2.44	1.20	79.04	68.71	WB
Birkner 1978	Meeboer Lake, Laramie WY	northern plains killifish	0.30	0.58	2.44	1.20	0.51	9.22	WB
Birkner 1978	Miller's Lake, Wellington CO	fathead minnow	6.00	2.37	2.78	1.40	55.31	15.37	WB
Birkner 1978	Miller's Lake, Wellington CO	Iowa darter	6.00	2.37	2.87	1.45	59.18	33.38	WB
Birkner 1978	Sweitzer Lake, Delta CO	northern plains killifish	9.40	0.87	2.44	1.20	23.94	38.18	WB
Birkner 1978	Sweitzer Lake, Delta CO	fathead minnow	9.40	0.87	2.78	1.40	31.89	110.38	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	northern plains killifish	7.60	1.21	2.44	1.20	26.79	27.65	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	fathead minnow	7.60	1.21	2.78	1.40	35.69	48.20	WB
Birkner 1978	Twin Buttes Reservoir, Laramie WY	Iowa darter	7.60	1.21	2.87	1.45	38.18	60.81	WB
Bowie et al. 1996	Hyco Reservoir	bluegill	11.50	2.35	2.00	2.13	114.97	87.47	WB
Butler et al. 1991	Uncompahgre River at Colona	flannelmouth sucker	1.50	0.63	1.52	1.41	2.03	2.40	WB
Butler et al. 1991	Uncompahgre River at Colona	white sucker	1.50	0.63	1.58	1.38	2.07	7.32	WB
Butler et al. 1991	Uncompahgre River at Colona	bluehead sucker	1.50	0.63	1.24	1.82	2.13	3.27	WB
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	3.77	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1991	Uncompahgre River at Colona	mottled sculpin	1.50	0.63	2.72	1.45	3.72	6.39	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	4.77	WB
Butler et al. 1991	Uncompahgre River at Colona	brown trout	1.50	0.63	2.78	1.45	3.80	5.06	WB
Butler et al. 1991	Uncompahgre River at Colona	rainbow trout	1.50	0.63	2.33	2.44	5.39	6.88	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	brown trout	1.00	1.26	2.78	1.45	5.08	6.20	E-O
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	channel catfish	1.00	1.26	1.35	1.45	2.47	2.32	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	2.03	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	bullhead	1.00	1.26	1.62	1.45	2.96	3.05	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	5.19	WB
Butler et al. 1993	Navajo Reservoir, Piedra River Arm, near La Boca	common carp	1.00	1.26	1.58	1.92	3.82	6.15	WB
Butler et al. 1993	Spring Cr. at La Boca	white sucker	5.00	0.18	1.58	1.38	1.96	4.83	WB
Butler et al. 1993	Spring Cr. at La Boca	bluehead sucker	5.50	0.18	1.24	1.82	2.22	12.91	WB
Butler et al. 1993	Spring Cr. at La Boca	speckled dace	5.00	0.18	1.36	1.95	2.37	23.45	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.00	0.18	2.78	1.40	3.48	11.46	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.00	0.18	2.78	1.45	3.60	1.74	WB
Butler et al. 1993	Spring Cr. at La Boca	fathead minnow	5.50	0.18	2.78	1.40	3.83	8.38	WB
Butler et al. 1993	Spring Cr. at La Boca	brown trout	5.50	0.18	2.78	1.45	3.96	4.92	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	1.07	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	sucker	2.00	0.15	1.25	1.41	0.53	0.96	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.69	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.76	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	0.87	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	flannelmouth sucker	2.00	0.15	1.52	1.41	0.64	1.35	WB
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.10	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	Hartman Draw near mouth, at Cortez	fathead minnow	2.00	0.15	2.78	1.40	1.16	2.24	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	speckled dace	2.00	0.90	1.36	1.95	4.77	12.51	WB
Butler et al. 1995	McElmo Cr. at Hwy. 160, near Cortez	fathead minnow	2.00	0.90	2.78	1.40	7.00	7.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.25	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	1.97	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	2.82	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	flannelmouth sucker	3.00	0.37	1.52	1.41	2.37	3.10	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	1.51	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	bluehead sucker	3.00	0.37	1.24	1.82	2.49	2.36	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	speckled dace	3.00	0.37	1.36	1.95	2.92	11.92	WB
Butler et al. 1995	McElmo Cr. downstream from Alkali Cyn.	fathead minnow	3.00	0.37	2.78	1.40	4.29	6.71	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.11	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	1.83	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	2.68	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	3.38	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.12	1.52	1.41	1.54	4.23	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.49	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.11	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	common carp	6.00	0.12	1.58	1.92	2.18	7.30	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	1.96	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.12	2.78	1.40	2.80	8.24	WB
Butler et al. 1995	McElmo Cr. downstream from Yellow Jacket Cyn.	red shiner	6.00	0.12	2.27	1.95	3.20	9.97	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.40	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	2.96	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	3.38	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	flannelmouth sucker	6.00	0.10	1.52	1.41	1.23	5.07	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.27	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bluehead sucker	6.00	0.10	1.24	1.82	1.29	3.09	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	bullhead	6.00	0.10	1.62	1.45	1.34	4.35	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	5.47	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	13.68	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	speckled dace	6.00	0.10	1.36	1.95	1.52	10.75	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	8.45	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	common carp	6.00	0.10	1.58	1.92	1.74	9.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	green sunfish	6.00	0.10	2.29	1.45	1.91	7.26	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.01	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	7.41	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	fathead minnow	6.00	0.10	2.78	1.40	2.23	6.15	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.99	WB
Butler et al. 1995	McElmo Cr.upstream from Yellow Jacket Cyn.	red shiner	6.00	0.10	2.27	1.95	2.55	8.21	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	16.91	WB
Butler et al. 1995	Navajo Wash near Towaoc	bluehead sucker	12.00	0.20	1.24	1.82	5.30	13.09	WB
Butler et al. 1995	Navajo Wash near Towaoc	speckled dace	12.00	0.20	1.36	1.95	6.23	17.00	WB
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	2.98	M
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.70	M
Butler et al. 1995	San Juan River at Four Comers	channel catfish	1.50	0.26	1.35	1.45	0.77	5.95	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	2.11	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	3.10	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	0.86	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	1.55	WB
Butler et al. 1995	San Juan River at Four Comers	flannelmouth sucker	1.50	0.26	1.52	1.41	0.84	5.92	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	1.71	WB
Butler et al. 1995	San Juan River at Four Comers	bluehead sucker	1.50	0.26	1.24	1.82	0.89	2.18	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	8.40	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	9.97	WB
Butler et al. 1995	San Juan River at Four Comers	speckled dace	1.50	0.26	1.36	1.95	1.04	5.67	WB
Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	10.18	WB

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Butler et al. 1995	San Juan River at Four Comers	common carp	1.50	0.26	1.58	1.92	1.19	6.53	WB
Butler et al. 1995	San Juan River at Four Comers	red shiner	1.50	0.26	2.27	1.95	1.75	6.84	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	channel catfish	1.50	0.29	1.35	1.45	0.85	10.88	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.68	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	1.97	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	2.40	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	flannelmouth sucker	1.50	0.29	1.52	1.41	0.93	4.23	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.18	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.36	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	bluehead sucker	1.50	0.29	1.24	1.82	0.98	4.91	WB
Butler et al. 1995	San Juan River at Mexican Hat Utah	common carp	1.50	0.29	1.58	1.92	1.31	7.49	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	25.71	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	32.00	WB
Butler et al. 1995	Woods Cyn. Near Yellow Jacket	fathead minnow	5.50	0.40	2.78	1.40	8.65	36.89	WB
Butler et al. 1997	Cahone Canyon at Highway 666	green sunfish	10.50	0.20	2.29	1.45	6.83	13.79	WB
Butler et al. 1997	Large pond south of G Road, southern Mancos Valley	fathead minnow	3.00	2.00	2.78	1.40	23.39	15.37	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	4.55	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	9.45	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	bluehead sucker	18.50	0.07	1.24	1.82	2.94	10.18	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	11.03	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	green sunfish	18.50	0.07	2.29	1.45	4.33	10.16	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	10.76	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	16.77	WB
Butler et al. 1997	Mud Creek at Highway 32, near Cortez	fathead minnow	18.50	0.07	2.78	1.40	5.05	9.08	WB
Butler et al. 1997	Pond downstream from site MNP2, southern Mancos Valley	smallmouth bass	1.00	5.15	1.93	1.42	14.09	17.03	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	13.97	WB
Butler et al. 1997	Pond on Woods Canyon at 15 Road	fathead minnow	3.00	0.90	2.78	1.40	10.55	20.96	WB
Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	3.14	M

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Casey 2005	Deerlick Creek	rainbow trout	0.20	2.24	2.33	2.44	2.55	8.16	E-O
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	16.79	M
Casey 2005	Luscar Creek	rainbow trout	10.70	0.33	2.33	2.44	19.85	33.48	E-O
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	14.43	WB
Formation 2012	Crow Creek - 1A	sculpin	2.20	0.80	2.78	1.45	7.08	12.10	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	15.20	WB
Formation 2012	Crow Creek - 1A	brown trout	2.20	0.80	2.96	1.45	7.52	13.49	WB
Formation 2012	Crow Creek - 1A	sculpin	2.45	0.80	2.78	1.45	7.89	11.29	WB
Formation 2012	Crow Creek - 1A	brown trout	2.45	0.80	2.96	1.45	8.37	14.39	WB
Formation 2012	Crow Creek - 1A	sculpin	2.90	0.80	2.78	1.45	9.34	25.35	WB
Formation 2012	Crow Creek - 1A	brown trout	2.90	0.80	2.96	1.45	9.91	24.36	WB
Formation 2012	Crow Creek - 1A	sculpin	4.80	0.80	2.78	1.45	15.45	18.33	WB
Formation 2012	Crow Creek - 1A	brown trout	4.80	0.80	2.96	1.45	16.40	20.29	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	20.97	WB
Formation 2012	Crow Creek - 3A	sculpin	1.80	0.81	2.78	1.45	5.86	16.91	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	15.09	WB
Formation 2012	Crow Creek - 3A	brown trout	1.80	0.81	2.97	1.45	6.22	13.30	WB
Formation 2012	Crow Creek - 3A	sculpin	2.20	0.81	2.78	1.45	7.17	16.65	WB
Formation 2012	Crow Creek - 3A	brown trout	2.20	0.81	2.97	1.45	7.60	16.27	WB
Formation 2012	Crow Creek - 3A	brown trout	2.60	0.81	2.97	1.45	8.99	22.24	WB
Formation 2012	Crow Creek - 3A	sculpin	4.20	0.81	2.78	1.45	13.68	29.32	WB
Formation 2012	Crow Creek - 3A	brown trout	4.20	0.81	2.97	1.45	14.52	28.45	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	8.72	WB
Formation 2012	Crow Creek - CC150	sculpin	0.68	1.04	2.74	1.45	2.81	7.31	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	8.43	WB
Formation 2012	Crow Creek - CC150	brown trout	0.68	1.04	2.91	1.45	2.98	12.54	WB
Formation 2012	Crow Creek - CC150	sculpin	0.80	1.04	2.74	1.45	3.31	7.46	WB
Formation 2012	Crow Creek - CC150	brown trout	0.80	1.04	2.91	1.45	3.51	7.52	WB
Formation 2012	Crow Creek - CC150	sculpin	1.40	1.04	2.74	1.45	5.79	15.57	WB
Formation 2012	Crow Creek - CC150	brown trout	1.40	1.04	2.91	1.45	6.14	14.66	WB
Formation 2012	Crow Creek - CC150	sculpin	1.50	1.04	2.74	1.45	6.20	10.67	WB
Formation 2012	Crow Creek - CC150	brown trout	1.50	1.04	2.91	1.45	6.58	11.32	WB

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Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	9.39	WB
Formation 2012	Crow Creek - CC350	sculpin	0.82	1.16	2.79	1.45	3.86	10.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.86	1.16	2.79	1.45	4.02	7.66	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	9.08	WB
Formation 2012	Crow Creek - CC350	brown trout	0.82	1.16	2.97	1.45	4.09	12.33	WB
Formation 2012	Crow Creek - CC350	sculpin	0.89	1.16	2.79	1.45	4.19	14.56	WB
Formation 2012	Crow Creek - CC350	brown trout	0.86	1.16	2.97	1.45	4.27	8.36	WB
Formation 2012	Crow Creek - CC350	brown trout	0.89	1.16	2.97	1.45	4.44	16.63	WB
Formation 2012	Crow Creek - CC350	sculpin	1.10	1.16	2.79	1.45	5.15	13.83	WB
Formation 2012	Crow Creek - CC350	brown trout	1.10	1.16	2.97	1.45	5.47	11.49	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	8.10	WB
Formation 2012	Crow Creek - CC75	sculpin	0.46	1.19	2.69	1.45	2.13	7.30	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	5.86	WB
Formation 2012	Crow Creek - CC75	brown trout	0.46	1.19	2.87	1.45	2.26	7.74	WB
Formation 2012	Crow Creek - CC75	sculpin	0.52	1.19	2.69	1.45	2.39	5.47	WB
Formation 2012	Crow Creek - CC75	brown trout	0.52	1.19	2.87	1.45	2.54	4.60	WB
Formation 2012	Crow Creek - CC75	sculpin	0.85	1.19	2.69	1.45	3.94	10.43	WB
Formation 2012	Crow Creek - CC75	brown trout	0.85	1.19	2.87	1.45	4.18	14.92	WB
Formation 2012	Crow Creek - CC75	sculpin	1.00	1.19	2.69	1.45	4.64	10.28	WB
Formation 2012	Crow Creek - CC75	brown trout	1.00	1.19	2.87	1.45	4.92	9.54	WB
Formation 2012	Deer Creek	sculpin	1.45	1.55	2.81	1.45	9.17	11.07	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	12.34	WB
Formation 2012	Deer Creek	sculpin	1.50	1.55	2.81	1.45	9.49	11.42	WB
Formation 2012	Deer Creek	brown trout	1.45	1.55	3.00	1.45	9.73	8.46	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	12.35	WB
Formation 2012	Deer Creek	brown trout	1.50	1.55	3.00	1.45	10.07	8.96	WB
Formation 2012	Deer Creek	sculpin	2.00	1.55	2.81	1.45	12.65	11.55	WB
Formation 2012	Deer Creek	brown trout	2.00	1.55	3.00	1.45	13.43	18.55	WB
Formation 2012	Deer Creek	sculpin	2.40	1.55	2.81	1.45	15.18	12.51	WB
Formation 2012	Deer Creek	brown trout	2.40	1.55	3.00	1.45	16.11	15.24	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.71	WB
Formation 2012	Hoopes Spring - HS	sculpin	20.50	0.24	3.63	1.45	26.38	33.74	WB

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Formation 2012	Hoopes Spring - HS	sculpin	20.95	0.24	3.63	1.45	26.96	15.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	23.89	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.50	0.24	3.86	1.45	27.99	36.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	20.95	0.24	3.86	1.45	28.61	36.00	WB
Formation 2012	Hoopes Spring - HS	sculpin	27.30	0.24	3.63	1.45	35.13	52.15	WB
Formation 2012	Hoopes Spring - HS	brown trout	27.30	0.24	3.86	1.45	37.28	47.18	WB
Formation 2012	Hoopes Spring - HS	sculpin	40.45	0.24	3.63	1.45	52.05	59.94	WB
Formation 2012	Hoopes Spring - HS	brown trout	40.45	0.24	3.86	1.45	55.23	32.97	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	31.71	WB
Formation 2012	Hoopes Spring - HS3	sculpin	16.10	0.54	2.47	1.45	30.96	26.95	WB
Formation 2012	Hoopes Spring - HS3	sculpin	17.05	0.54	2.47	1.45	32.79	38.65	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	29.78	WB
Formation 2012	Hoopes Spring - HS3	brown trout	16.10	0.54	2.63	1.45	32.85	27.23	WB
Formation 2012	Hoopes Spring - HS3	brown trout	17.05	0.54	2.63	1.45	34.79	25.87	WB
Formation 2012	Hoopes Spring - HS3	sculpin	26.00	0.54	2.47	1.45	49.99	34.73	WB
Formation 2012	Hoopes Spring - HS3	brown trout	26.00	0.54	2.63	1.45	53.05	34.24	WB
Formation 2012	Hoopes Spring - HS3	sculpin	31.75	0.54	2.47	1.45	61.05	34.37	WB
Formation 2012	Hoopes Spring - HS3	brown trout	31.75	0.54	2.63	1.45	64.78	41.89	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	25.35	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.50	0.45	2.83	1.45	24.76	16.52	WB
Formation 2012	Sage Creek - LSV2C	sculpin	13.80	0.45	2.83	1.45	25.31	27.36	WB
Formation 2012	Sage Creek - LSV2C	sculpin	14.30	0.45	2.83	1.45	26.23	37.66	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	28.12	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.50	0.45	3.01	1.45	26.27	18.48	WB
Formation 2012	Sage Creek - LSV2C	brown trout	13.80	0.45	3.01	1.45	26.86	32.78	WB
Formation 2012	Sage Creek - LSV2C	brown trout	14.30	0.45	3.01	1.45	27.83	28.24	WB
Formation 2012	Sage Creek - LSV2C	sculpin	18.75	0.45	2.83	1.45	34.39	29.49	WB
Formation 2012	Sage Creek - LSV2C	brown trout	18.75	0.45	3.01	1.45	36.49	30.30	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	29.04	WB
Formation 2012	Sage Creek - LSV4	sculpin	8.45	0.69	2.70	1.45	23.02	26.53	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	23.42	WB
Formation 2012	Sage Creek - LSV4	brown trout	8.45	0.69	2.88	1.45	24.43	21.95	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Formation 2012	South Fork Tincup Cr.	sculpin	0.32	1.32	2.86	1.45	1.73	8.24	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.32	1.32	3.05	1.45	1.84	5.32	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.43	1.32	2.86	1.45	2.37	5.44	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.44	1.32	2.86	1.45	2.42	13.51	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.43	1.32	3.05	1.45	2.51	3.25	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.44	1.32	3.05	1.45	2.57	9.69	WB
Formation 2012	South Fork Tincup Cr.	sculpin	0.56	1.32	2.86	1.45	3.06	8.52	WB
Formation 2012	South Fork Tincup Cr.	brown trout	0.56	1.32	3.05	1.45	3.24	3.82	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	3.92	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.41	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	4.75	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.03	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.52	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	white sucker	1.00	0.86	1.58	1.38	1.89	5.54	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.21	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	9.22	WB
Grasso et al. 1995	Arapahoe Wetlands Pond	fathead minnow	1.00	0.86	2.78	1.40	3.36	10.20	WB
Hamilton and Buhl 2004	lower East Mill Creek	cutthroat trout	24.00	1.32	2.29	1.96	142.01	102.73	WB
Lemly 1985	Badin Lake	black bullhead	0.32	12.48	1.72	1.45	9.99	4.44	M
Lemly 1985	Badin Lake	western mosquitofish	0.32	12.48	2.37	1.20	11.33	5.77	M
Lemly 1985	Badin Lake	common carp	0.32	12.48	1.58	1.92	12.10	5.81	M
Lemly 1985	Badin Lake	green sunfish	0.32	12.48	2.29	1.45	13.30	3.25	M
Lemly 1985	Badin Lake	fathead minnow	0.32	12.48	2.78	1.40	15.52	3.17	M
Lemly 1985	Badin Lake	red shiner	0.32	12.48	2.27	1.95	17.74	4.45	M
Lemly 1985	Belews Lake	black bullhead	10.91	1.75	1.72	1.45	47.79	29.84	M
Lemly 1985	Belews Lake	western mosquitofish	10.91	1.75	2.37	1.20	54.18	46.86	M
Lemly 1985	Belews Lake	common carp	10.91	1.75	1.58	1.92	57.86	38.97	M
Lemly 1985	Belews Lake	green sunfish	10.91	1.75	2.29	1.45	63.60	20.84	M
Lemly 1985	Belews Lake	fathead minnow	10.91	1.75	2.78	1.40	74.25	28.75	M
Lemly 1985	Belews Lake	red shiner	10.91	1.75	2.27	1.95	84.87	38.59	M
Lemly 1985	High Rock Lake	black bullhead	0.67	4.99	1.72	1.45	8.36	5.58	M

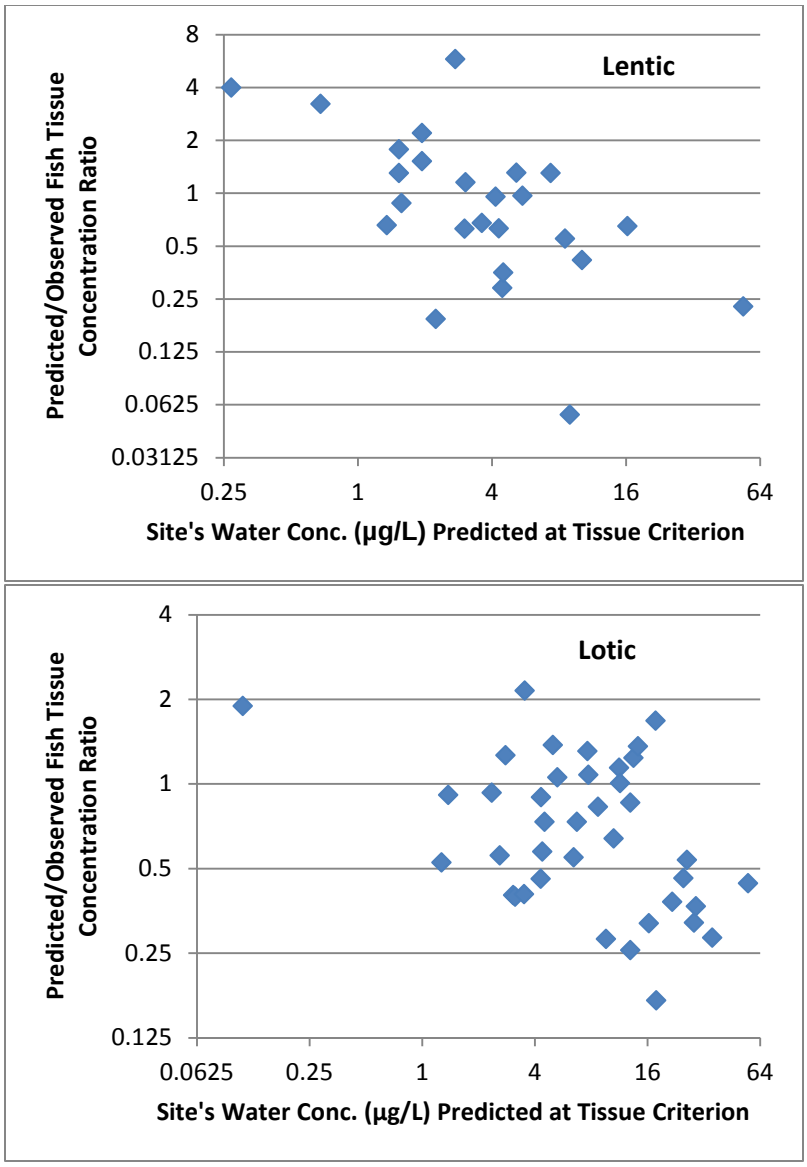
Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Lemly 1985	High Rock Lake	western mosquitofish	0.67	4.99	2.37	1.20	9.48	6.10	M
Lemly 1985	High Rock Lake	common carp	0.67	4.99	1.58	1.92	10.12	4.49	M
Lemly 1985	High Rock Lake	green sunfish	0.67	4.99	2.29	1.45	11.13	3.13	M
Lemly 1985	High Rock Lake	fathead minnow	0.67	4.99	2.78	1.40	12.99	4.00	M
Lemly 1985	High Rock Lake	red shiner	0.67	4.99	2.27	1.95	14.85	4.62	M
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	cutthroat trout	0.10	6.30	2.29	1.96	2.83	10.61	WB
McDonald and Strosher 1998	Elk R. above Cadorna Cr. (745)	mountain whitefish	0.10	6.30	2.97	7.39	13.83	7.11	WB
McDonald and Strosher 1998	Fording R. above Swift Cr. (746)	cutthroat trout	8.60	0.23	2.29	1.96	8.71	24.96	WB
Muscatello and Janz 2009	Vulture Lake	white sucker	0.43	1.01	1.58	1.38	0.95	4.65	WB
Muscatello and Janz 2009	Vulture Lake	burbot	0.43	1.01	2.45	1.45	1.54	15.91	WB
Muscatello and Janz 2009	Vulture Lake	ninespine stickleback	0.43	1.01	3.22	1.45	2.03	6.02	WB
Muscatello and Janz 2009	Vulture Lake	northern pike	0.43	1.01	4.02	2.39	4.17	1.83	WB
Orr et al. 2012	Clode Pond 11	cutthroat trout	36.10	0.71	2.29	1.96	115.88	81.06	E-O
Orr et al. 2012	Elk Lakes 14	cutthroat trout	0.40	1.64	2.29	1.96	2.95	14.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	11.02	E-O
Orr et al. 2012	Elk River 1	cutthroat trout	4.20	0.55	2.29	1.96	10.33	15.60	E-O
Orr et al. 2012	Elk River 12	cutthroat trout	0.75	2.67	2.29	1.96	8.98	9.00	E-O
Orr et al. 2012	Fording River 23	cutthroat trout	30.60	0.21	2.29	1.96	28.52	15.56	E-O
Orr et al. 2012	Fording River Oxbow 10	cutthroat trout	50.10	1.34	2.29	1.96	302.30	47.81	E-O
Orr et al. 2012	Henretta Lake 27	cutthroat trout	8.60	0.50	2.29	1.96	19.33	13.56	E-O
Orr et al. 2012	Michel Creek 2	cutthroat trout	7.40	0.28	2.29	1.96	9.43	10.07	E-O
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	155.61	WB
Saiki and Lowe 1987	Kesterson Pond 11	western mosquitofish	38.60	0.51	2.37	1.20	55.41	124.49	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	268.13	WB
Saiki and Lowe 1987	Kesterson Pond 2	western mosquitofish	195.85	0.32	2.37	1.20	175.68	295.66	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	196.31	WB
Saiki and Lowe 1987	Kesterson Pond 8	western mosquitofish	70.35	0.60	2.37	1.20	120.13	266.93	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	178.36	WB
Saiki and Lowe 1987	San Luis Drain	western mosquitofish	316.50	0.36	2.37	1.20	322.76	397.41	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.53	WB
Saiki and Lowe 1987	Volta Pond 26	western mosquitofish	0.53	0.93	2.37	1.20	1.41	1.48	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.62	WB
Saiki and Lowe 1987	Volta Wasteway	western mosquitofish	0.74	1.03	2.37	1.20	2.15	1.63	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	10.67	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	bluegill	6.00	1.37	1.47	2.13	25.69	13.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.65	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	largemouth bass	6.00	1.37	2.04	1.42	23.73	9.79	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	13.17	WB
Saiki et al. 1993	Mud Slough at Gun Club Road	western mosquitofish	6.00	1.37	2.10	1.20	20.61	19.15	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.17	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	bluegill	8.00	0.43	1.47	2.13	10.70	9.60	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	5.68	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	largemouth bass	8.00	0.43	2.04	1.42	9.89	6.67	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.39	WB
Saiki et al. 1993	Salt Slough at the San Luis National Wildlife Refuge	western mosquitofish	8.00	0.43	2.10	1.20	8.59	5.87	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	5.76	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	bluegill	7.00	0.36	1.47	2.13	7.83	7.04	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.12	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	largemouth bass	7.00	0.36	2.04	1.42	7.23	3.41	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	2.63	WB
Saiki et al. 1993	San Joaquin R. above Hills Ferry Road	western mosquitofish	7.00	0.36	2.10	1.20	6.28	5.39	WB

Study	Site	Species	Site Water (µg/l)	EF (l/g)	TTF^{comp}	CF	Pred. E/O (mg/kg)	Obs. E/O (mg/kg)	Obs. tissue type
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.05	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	bluegill	1.00	0.75	1.47	2.13	2.34	4.27	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.41	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	largemouth bass	1.00	0.75	2.04	1.42	2.16	2.55	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.03	WB
Saiki et al. 1993	San Joaquin R. at Durham Ferry State Recereation Area	western mosquitofish	1.00	0.75	2.10	1.20	1.87	2.39	WB
Stephens et al. 1988	Marsh 4720	black bullhead	31.00	0.10	1.72	1.45	7.43	10.16	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	36.49	WB
Stephens et al. 1988	Marsh 4720	common carp	31.00	0.10	1.58	1.92	9.00	40.33	WB

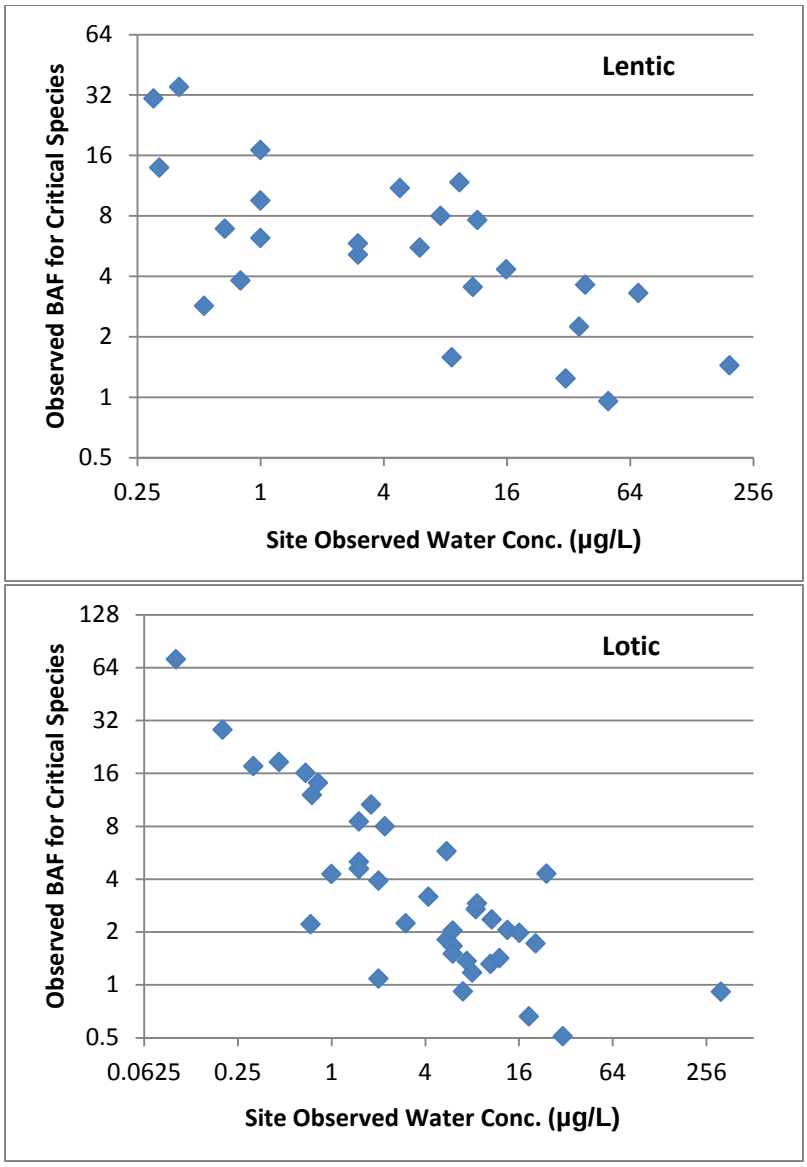
The ratio of predicted versus observed tissue concentrations in the above table can be compared against the main text Table 3.13 water concentrations that would be predicted to occur if each site's egg-ovary tissue concentration were at the criterion level. Figure I-1 shows the results. It can be seen that for those sites (in the left portion of each graph) where tissue concentrations equal to the egg-ovary criterion would be predicted to yield water concentrations not far on either side of the water criteria values, the predicted-to-observed tissue concentration ratios are not particularly biased relative to a ratio of 1.0. This indicates that the model is performing reasonably well for those sites strongly influencing the derived values of the water criteria.

The derivation of the water criteria concentrations involves an assumption of linearity in projecting the water concentration that would correspond to a tissue concentration equal to the tissue criterion. Figure I-2 suggests that high BAFs tend to be associated with low water concentrations, and low BAFs with high water concentrations. At the low concentrations associated with the 20^{th} percentile model-predicted BAF, the linearity assumption would appear to be environmentally conservative. At high concentrations, the opposite situation would occur, but overall, because the criterion is based on the 20^{th} percentile, the linearity assumption appears to be protective.



Figures I-1. For lentic (left panel) and lotic (right panel) waters, predicted-to-observed fish-tissue concentration ratio for each of the 65 sites, plotted versus each site's Table 3.13 water concentration that would be predicted to occur if its tissue levels were at the egg-ovary tissue criterion level.

Corresponding to how the water criteria concentrations were derived, for sites with multiple fish species, the plotted ratio is for the species having the highest predicted tissue-to-water ratio (i.e., highest predicted BAF). For sites having multiple samples of that species, the plotted value is the average predicted-to-observed ratio for that species.



Figures I-2. For lentic (left panel) and lotic (right panel) waters, observed BAFs (egg-ovary tissue-to-water concentration ratios) versus observed water concentration (both from the above table), for each site's fish species used in Table 3.13 (that is, for the species used in the water criteria calculations).

For sites having multiple samples of such species, tissue concentrations were averaged. Because nearly all samples were either whole body or muscle, the graphed BAFs include application of the CF, to normalize all samples to egg-ovary tissue. Since the CFs have been are assumed to be independent of concentration, the graphs do not reflect any potential CF nonlinearities, if they exist.

APPENDIX J: SUPPLEMENTARY INFORMATION ON SELENIUM BIOACCUMULATION IN AQUATIC ANIMALS

1.0 EFFECTS OF GROWTH RATE ON THE ACCUMULATION OF SELENIUM IN FISH

EPA analyzed the effect of the growth rate parameter *g* when estimating selenium bioaccumulation using the mechanistic bioaccumulation modeling described in Equation 1 of the main text. Because the addition of tissue associated with growth could have a dilution effect on the chemicals present in tissue, a parameter representing growth rate is present in the denominator of Equation 1. Indeed, growth can be an important factor in the bioaccumulation of very hydrophobic chemicals with low excretion rates such as polychlorinated biphenyls, (Connolly and Pedersen 1988). However, the effect of growth may not be as important for selenium because of its unique biogeochemical characteristics, route of exposure, and role as a micronutrient.

EPA tested the effect of the growth rate parameter *g* on estimates of selenium bioaccumulation using Equation 1 with different food web scenarios. Increasing growth rates from 0 (no growth) to 0.2/day (a relatively high rate of growth) reduced selenium concentrations in trophic level 2 and 3 organisms by as much as a factor of 10 to 20. Thus incorporating growth rate in Equation 1 could result in significant dilution of selenium and lower estimates of selenium bioaccumulation.

Although increasing the value of the growth parameter g in Equation 1 reduces estimates of selenium bioaccumulation, this simple analysis neglects an important physiological linkage between growth and food consumption. Organisms must consume enough food to support growth and meet their energy requirements for respiration, specific dynamic action, waste loss, and reproduction. These physiological requirements suggest that higher growth rates are associated with greater rates of food consumption. Because food consumption is the primary route of selenium exposure in aquatic organisms, increased selenium exposure associated with higher food consumption could counterbalance the dilution of selenium in tissue associated with higher growth rates.

EPA tested the effects of growth on estimates of selenium bioaccumulation using Equation 1 when increased food consumption was associated with higher growth rates. EPA modified Equation 1 to incorporate a simple relationship for bioenergetics (Thomann et al. 1992) and applied the model to reexamine the sensitivity of selenium bioaccumulation to growth rates in trophic level 2 and 3 organisms. The results of this analysis showed that increasing growth rates over two orders of magnitude increased selenium concentrations in trophic level 2 by a factor of 2, and decreased selenium concentrations in trophic level 3 by 10%. When growth rates were increased simultaneously in trophic levels 2 and 3, the selenium concentrations increased by less than a factor of 2. This analysis suggests that when bioenergetics is considered, selenium bioaccumulation is generally insensitive to organism growth rates. EPA believes that uncertainties in the toxicokinetic parameters of selenium far outweigh the effects on growth rate on selenium bioaccumulation. Thus, the growth rate parameter *g* was removed from Equation

1 for the purpose of deriving a translation equation that could be used to implement a tissue-based selenium water quality criterion.

2.0 Analysis of the Relative Contribution of Aqueous and Dietary Uptake on the Bioaccumulation of Selenium

EPA analyzed the relative contributions of direct aqueous uptake versus ingestion of selenium in consideration of removing the uptake rate constant k_u from Equation 1 in Section 3.2 of the main text. Because an important exposure route for some chemicals is direct contact with water, an uptake rate constant k_u is present in the numerator of Equation 1. However, fish and invertebrate organisms absorb selenium primarily through the consumption of food rather than from direct aqueous uptake (Forester 2007; Lemly 1985; Luoma et al. 1992). Thus, removing the uptake rate constant k_u could simplify Equation 1 while maintaining the key determinants of selenium bioaccumulation.

EPA tested the relative contribution of aqueous versus dietary uptake of selenium using a version of Equation 1 that incorporates both exposure pathways (Thomann et. al. 1992). For trophic level 2, selenium bioaccumulation was estimated for a range of uptake rates that varied according to the respiration rate and aqueous transfer efficiency of selenium relative to dissolved oxygen. For trophic level 3, uptake rates were varied within a range of values reported in Besser et al. (1993) and Bertram and Brooks (1986).

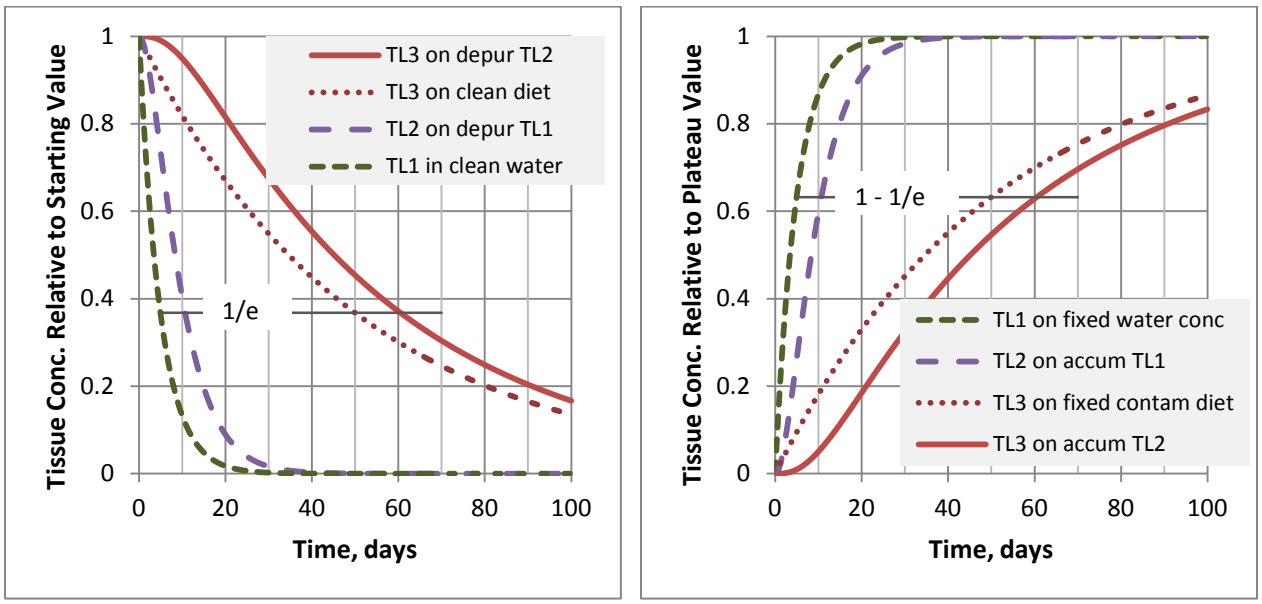
EPA's analysis showed that diet accounted for 34% - 92% of selenium bioaccumulation at trophic level 2, with a median of 74%. At trophic level 3, diet accounted for 62% - 100% of tissue selenium, with a median of 95%. Thus, disregarding aqueous uptake of selenium only resulted in a small (~5%) reduction in estimated selenium bioaccumulation in trophic level 3 organisms. These results are consistent with previous studies indicating that diet is the primary exposure route of selenium, and suggests that the uptake rate constant for selenium can be removed from Equation 1 with negligible effect for higher trophic levels organisms.

3.0 Kinetics of Accumulation and Depuration: Averaging Period

3.1 Background

For setting averaging periods for aquatic life criteria, U.S. EPA (1995b) used the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action. In the context of the water-borne direct toxicity of metals, characteristic time = 1/k, where k is the first-order kinetic coefficient in a toxico-kinetic model fitted to the relationship between LC50 and exposure duration.

In the context of selenium bioaccumulation in a single trophic level, k would the first-order depuration coefficient, and 1/k would equal the time needed to depurate to a concentration of 1/e times the initial concentration (where e=2.718). For depuration of multiple trophic levels sequentially, the characteristic time is likewise the time needed for c/c_o to reach a value of 1/e, as shown in Figure J-1a. The accumulation curve is the inverted depuration curve, as shown in Figure J-1b.



Figures J-1 a & b. Depuration and accumulation behavior for algae-detritus-sediment k=0.2/day, invertebrate k=0.2/day and fish k=0.02/day, calculated with time step = 0.1 day.
Concentration is expressed as a dimensionless ratio: concentration at time t divided by either starting concentration (J1a) or plateau concentration (J1b).

In the Figures J-1 a & b examples, the characteristic time for algae-detritus-sediment is 5 days, the characteristic time for invertebrates on an invariant diet is 5 days, the characteristic time for fish on an invariant diet is 50 days, and the characteristic time for fish on an invertebrate diet that is itself depurating or accumulating is the approximate sum of the individual characteristic times, or ~60 days.

In contrast to the model depuration rate, k, the model uptake rate (AE, assimilation efficiency, multiplied by IR, intake rate) does not affect the characteristic response time. Rather it affects the magnitude of the accumulation plateau. Uptake rate thus affects the TTF value itself but is not relevant to setting an averaging period.

Because short averaging periods are more environmentally conservative than long averaging periods, selecting parameter values for fast kinetics is more environmentally conservative. Figure J1 reflects environmentally conservative choices for k values.

3.2 Approach for Modeling Effects of Time-Variable Se Concentrations

Expression of concentrations. None of the concentrations in this analysis are expressed in ordinary units of concentration. All concentrations are modeled as values normalized to their allowable benchmark concentration – that is, concentration = 1 for a particular medium (water, algae-detritus-sediment, invertebrates, or fish) means that the medium is at its criterion concentration or corresponding benchmark. It is assumed that the benchmarks correctly align – water held at its benchmark concentration will ultimately yield Trophic Levels 1, 2, and 3 at their respective benchmark concentrations. The Trophic Level 3 benchmark is the reproductive EC10 for the 5th percentile taxon: i.e., the fish tissue criterion.

Formulation of the bioaccumulation model for kinetic analysis. For algae-detritus-sediment, for invertebrates, and for fish, accumulation at time t equals accumulation at time t-1 plus intake minus depuration, as follows:

Algae-detritus-sediment:

$$C_{TL1}[t] = C_{TL1}[t\text{-}1] + k_{uptake}\, C[t\text{-}1]water - k_{TL1}\, C_{TL1}[t\text{-}1]$$

Invertebrates:

$$C_{TL2}[t] = C_{TL2}[t\text{-}1] + AE_{TL2}\, IR_{TL2}\, C_{TL1}[t\text{-}1] - k_{TL2}\, C_{TL2}[t\text{-}1]$$

Fish:

$$C_{TL3}[t] = C_{TL3}[t\text{-}1] + AE_{TL3}\, IR_{TL3}\, C_{TL2}[t\text{-}1] - k_{TL3}\, C_{TL3}[t\text{-}1]$$

For algae-detritus-sediment, the depuration rate k is assigned a value of 0.2/day, similar to the sum of depuration and growth-dilution rate coefficients used by Brix and DeForest (2008). Because a lentic system would involve the slower kinetics of sediment exchange, the rapid rate used here implies a lotic system.

For invertebrates, a value of 0.2/day was assigned, considerably higher than those for *Lumbriculus*, Asian clam, zebra mussel, but close to those of mayfly and copepods, which are very small in size. As previously mentioned, higher k (more rapid kinetics) is an environmentally conservative assumption in this context.

For fish, the median depuration coefficient measured by Bertram and Brooks (1986) for 6-9 month-old (early adult) fathead minnows was used, providing a k_{TL3} value of 0.02/day. Because of the small size of adults of this species, this represents faster kinetics than would likely be applicable the salmonids and centrarchids of greatest concern for selenium toxicity. The striped bass k value of Baines et al. (2002) is inapplicable here because it was measured in the early juvenile life stage, a size that is too small to be relevant to reproductive impairment stemming from exposure of adult females. The concentration in fish could be equivalently viewed as either whole body or egg-ovary, relative to their

respective benchmarks. That is, partitioning within body of the fish is assumed not to involve a time delay.

The value of a TTF is given by AE x IR/k (or k_{uptake}/k for algae-detritus-sediment). Concentrations in TL1, TL2, and TL3 are normalized to their benchmarks, meaning that all benchmark concentrations have a value of 1.0. In this normalized context, the TTFs must also equal 1.0, since upon reaching steady state, TL1 at its benchmark will yield TL2 at its benchmark, which in turn will yield TL3 at its benchmark. Again, the analysis is not intended to reflect actual concentrations, merely portray temporal behavior. Since 1 = TTF = AE x IR/k, it follows that AE x IR = k within this normalized framework. Although only the product AE x IR is relevant, they are retained as distinct parameters to maintain parallelism with remainder of the criterion document. AE was assigned a value of 0.5 for fish and invertebrates, and IR = k/AE in the normalized framework.

Time step durations of 0.1-1.0 day were considered. Short time steps increase accuracy by decreasing the numerical dispersion inherent in expressing C[t] = f(C[t-1]). A time step of 0.5 day was found to yield sufficient accuracy, as measured by predicted values at the characteristic time for depuration or accumulation (per Figure J-1).

Prediction of Effects. The effect level associated with the tissue concentration at any time t is calculated via the log probit concentration-response curve, one of the commonly used sigmoid curves. It assumes that the sensitivities in the underlying population are log-normally distributed such that the concentration yielding effects on k percentage of the population is given by:

$$EC_k = EC50 \exp(\sigma z)$$

where σ is the inverse of the concentration-response curve slope and z is the normal deviate corresponding to k percent (e.g., for k=10%, z=NORMSINV(0.1)=−1.28155). Among the reproductive impairment studies presented in Appendix C, an approximate median ratio for EC50/EC10 is 1.5. This translates to σ=0.3164.

Since the fish tissue criterion concentration equals 1.0 in this normalized framework, at any time t, the fractional level of effect corresponding to any value of C_{TL3} is given by:

Fractional Effect[t] = NORMSDIST(z[t])

where z[t] is given by:

$z[t] = LN(C_{TL3}[t]/1.5)/0.3164$

Exposure Scenarios. Three exposure scenarios were evaluated under which the water criterion was just barely attained. The first two are absolute worst case scenarios, in which the 30-day average water concentration remains continuously at the criterion concentration at all times. The third is a realistic scemario.

1. Steady concentrations at the criterion: this is worst-case continuous exposure. In the real world this could not occur because water concentrations vary substantially over time. For the 30-day average concentration not to exceed more than once in three years, the realistically varying daily concentrations must remain well below the criterion concentration a large majority of the time.

2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals (i.e., separated by 29 days of zero concentration) such that the 30-day average always equals the criterion. This is the worst-case intermittent scenario, attaining the criterion through a time series that continually maximizes the 30-day average exposure at the water criterion concentration while also imposing the highest variability possible from spikes of 1-day duration. In the real world intermittent runoff sources do not occur at uniform intervals: merely averaging 30-days between discharges would yield an exceedance each time the discharge occurred with less than 30-days spacing. Further, the once-per-month peak concentrations could never be controlled at exactly 30X the chronic water criterion per the above discussion of the first scenario.

   It is because they lack real-world random variability that the above two scenarios are not realistic. They are used as absolute worst cases for purposes of comparison. The following third scenario represents a realistic and indeed typical situation for continuous exposure:

3. Log-normally distributed, smoothly variable concentrations with the 30-day average exceeding the criterion once in three years when counted using the procedure of EPA (1986). The log

standard deviation of 0.5 applied here represents typical real-world time variability for continuously flowing waters. The log serial correlation coefficient $\rho = 0.8$ represents that typical of smaller streams.

With respect to maximizing toxic effects while attaining the criterion, Scenarios #1 and #2 are absolute worst cases. In contrast, Scenario #3 represents typical time variability in ambient waters. This third scenario requires randomly generated concentrations (having specified target statistical characteristics). Multiple runs of long series are therefore needed to assure some reasonable degree of accuracy. A minimum of 20 runs of random series of 3000 days were used. The concentrations at each half-day time step were generated by the following formula:

*C[t]water = C[t-1]water^(ρ′) * GM^(1-ρ′) * EXP{σ * SQRT(1-ρ′^2)*NORMSINV(RAND)}*

where ρ′ (rho prime) is the desired serial correlation coefficient between half-day time steps: ρ′=SQRT(ρ) [approximation], where ρ (rho) is the desired serial correlation coefficient between daily values; GM is the desired geometric mean or median, and σ is the desired log standard deviation. The above formula allows a time series with the desired statistical characteristics to be generated.

3.2.1 Model Results

3.2.1.1 Steady concentrations at the water criterion concentration.

No graphic is needed to explain this scenario. With water steady at its criterion, algae-detritus-sediment and invertebrates are likewise steady at their benchmark concentrations, and fish tissue is at its criterion concentration. For the 5th percentile taxon, the effect would thus be 10% since the concentration is steady at the EC10.

3.2.1.2 Uniformly spaced spikes at maximum concentrations

Figure J-2. Scenario 2, uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion. Read invertebrate and fish tissue concentrations on left scale, water concentrations on right scale. Time=0 does not represent the beginning of exposure; prior to Time=0 the same exposure pattern had been going on for a long time (e.g., 10,000 days).

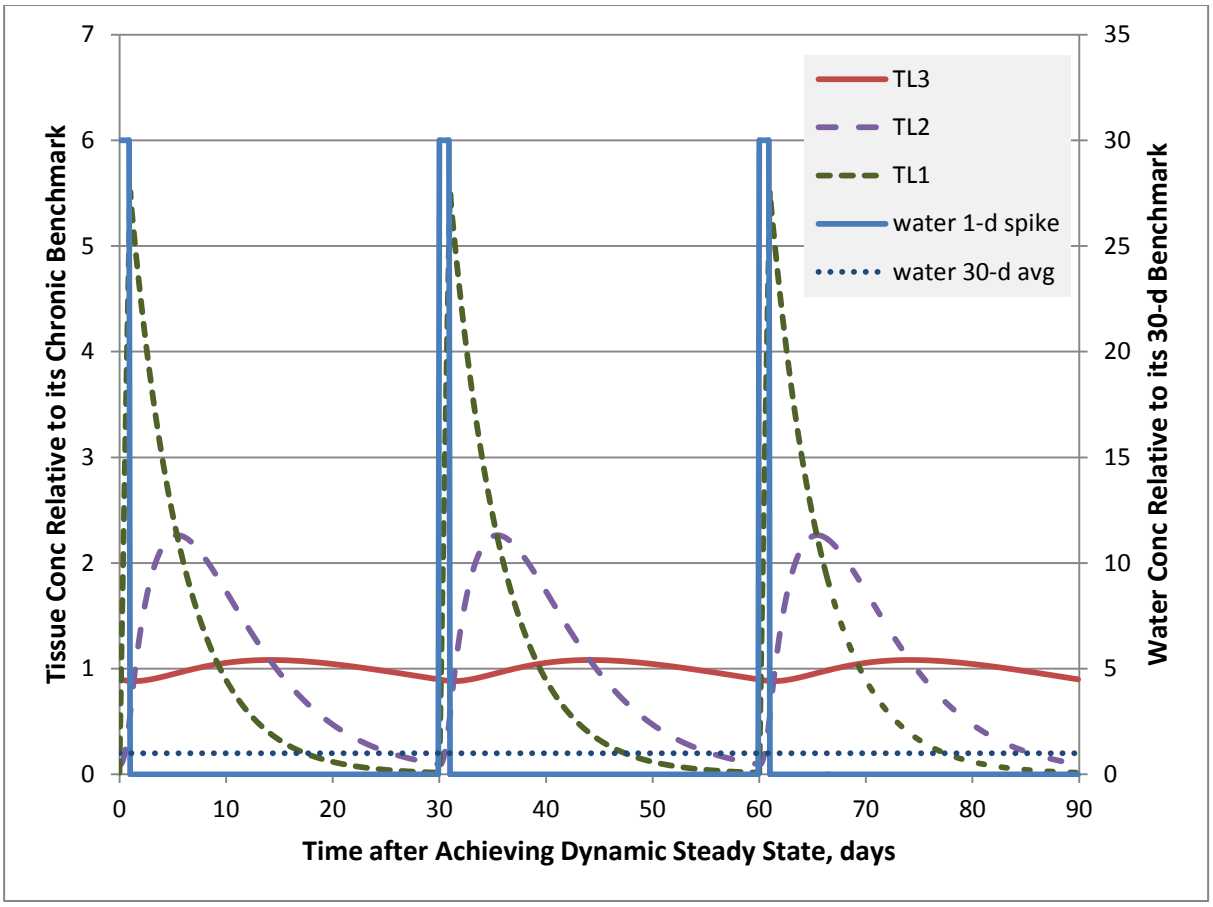


Figure J-2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion.
Tissue and water concentrations are expressed as dimensionless ratios relative to their respective criteria or benchmarks, as explained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

With their more rapid kinetics, TL1 and TL2 tissue concentration swings are much more drastic than TL3 (fish) tissue concentration swings, but were the spike to continue as a steady exposure 30-fold above the water benchmark, TL1, TL2, and TL3 would all ultimately plateau at 30-fold above their respective benchmarks.

The key point here is that attaining the 30-day average via 1-day spikes spaced 30 days apart generates a small oscillation in fish tissue concentrations. Averaged over the 30-days, the fish tissue concentrations exactly attain their criterion and the predicted effect is 10%.

3.2.1.3 Log-normally distributed, smoothly varying concentrations

This is the most realistic scenarios, corresponding to typical variability observed in streams.

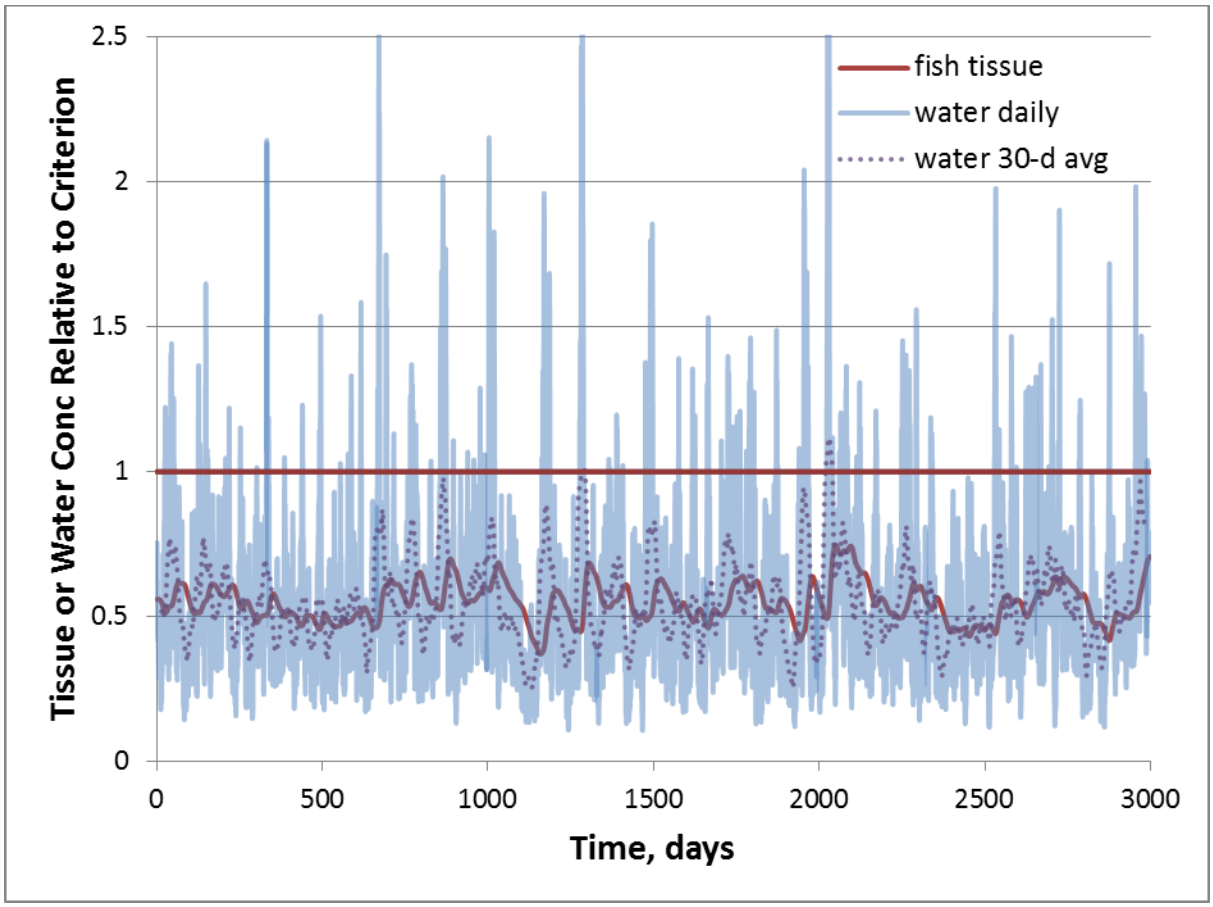


Figure J-3. A typical example of log-normally distributed, smoothly variable concentrations. The standard deviation of natural logs is 0.5 and the serial correlation coefficient of logs is 0.8 for daily values, both typical real-world situations. (The compression of 3000 days into the graph might make it difficult to recognize that the time series is smoothly varying – it has serial correlation.) At time=0, TL1, TL2, and TL3 begin at their average concentrations.

In the Figure J-3 example run, instantaneous water concentrations exceed the 30-day average criterion 7% of the time. The 30-day average concentrations exceed the criterion 1.05 times per 3 year period, counted per the EPA (1986) counting method. Tissue concentrations do not exceed their criterion at any time, and the aggregate effect is 0.12%.

In contrast to the previous scenario, the elevated concentrations here are random in their magnitude, duration, and spacing. This randomness reduces the average exposure (and aggregate effect) compatible with attainment of the 30-day average water target.

3.2.2 Summary of Scenario Results

Because Scenario 3 involves generation of random concentrations, the above graphs show just one run (3000 days) for each. Full results for the 20 runs of that scenario are shown below.

Scenario	Water: # 30-day avg. exceedances / 3-yr 1	Water: % of time exceeding	Tissue: % of time exceeding	Mean effect for 5th %ile Taxon	Comment
1. Steady	0.00	0.00	0.00	10.0	Steady at water and tissue benchmarks
2. Uniform spikes	0.00	3.33	56.7	10.0	30-d avg water conc. remains steady at benchmark (Fig. J2)
3. Smooth variable	1.01	7.8	0.00	0.18	Median=0.49 x benchmark, log stdev=0.5, rho(daily)=0.8 (e.g., Fig. 5) 2
1. Counting procedure for 30-d avg. exceedances is that of U.S. EPA (1986). 2. Results for Scenario 3 are average of 20 runs of 3000 days, each run with 0.6-1.4 exceedances / 3 yr. Runs not yielding exceedances within these bounds were not used. Among the 20 runs used, the effect CV=0.35.					

It can be concluded that the kinetics of selenium accumulation and depuration are sufficiently slow that applying a 30-day averaging period to the water criterion concentration affords protection even under unrealistic worst case conditions.

3.2.3 Example Responses to Increases in Water Concentrations

The previous Figures J-2 and J-3 illustrate situations after achievement of a dynamic steady state, where daily water concentrations change but longer-term mean water concentrations do not change. Given the same kinetic parameters as used above (i.e., yielding a 60-day characteristic time), this section addresses the rate at which tissue concentrations respond to increases in mean water concentrations, for example as would result from a new source. This is similar to the rising curve previously shown in Figure J-1b. The rapid kinetics used here for the water-TL1 step imply a small lotic system having little involvement of the bed sediments.

3.2.3.1 Step-function example

This example addresses the question: If water concentrations are increased to a level that is slightly too high, ultimately (at Time=∞) yielding fish-tissue concentrations at the EC20 instead of the EC10, how long would it take for those tissue concentrations to rise to a level that exceeds the (EC10-based) criterion?

Prior to Time=0 in this example the concentrations in TL3 had been at a moderate background concentration of 0.406 times the criterion, corresponding to the median West Virginia reference-site egg concentrations tabulated by West Virginia Department of Environmental Protection (2010). The concentrations in TL1 and TL2 are likewise assumed to have been at 0.406 normalized to their corresponding benchmarks. At Time=0 the water concentrations increase such that ultimately they will

produce an effect 10% higher than the target, thus at the EC20 of the hypothetical 5th percentile sensitive species. For typical selenium concentration-response slopes, this is 1.15-fold above the EC10. Figure J4 illustrates this scenario, which shows that 90 days are needed for TL3 concentrations to rise above the criterion.

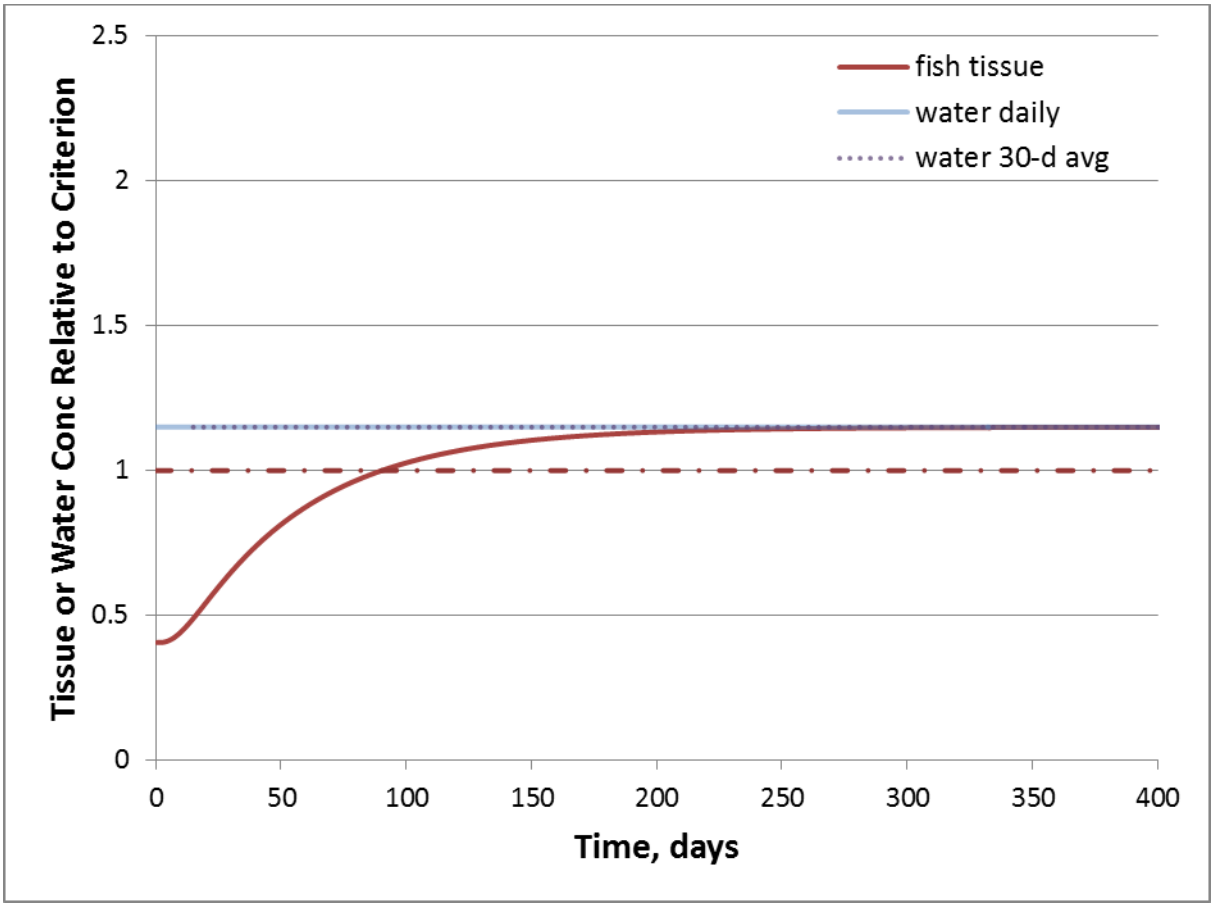


Figure J-4. TL3 concentration responding to a Time=0 step-function increase in water concentration that remains time-invariant thereafter.
Given that the water concentration is too high, ultimately yielding tissue concentrations at the hypothetical sensitive species EC20, 1.15-fold above the criterion, and given the previously presented kinetic parameters, it is calculated to take 90 days for TL3 concentrations to rise above the criterion.

3.2.3.2 Continuously time-variable example for flowing waters

To provide more realism, this example considers typical time variability, following up on Figure J3. In this example, prior to Time=0, TL1, TL2, and TL3 concentrations were at a low background concentration, 0.1 normalized to their criterion or respective benchmark. At Time=0 begin water concentrations having median = geometric mean = 0.49 normalized as a dimensionless ratio, concentration/criterion. Because the water concentrations are log-normally distributed, with log standard deviation = 0.5, the arithmetic mean is higher than the median and has the normalized value 0.56. If the simulation went on for a very long time, this time series (designed to have geometric mean 0.49 times the criterion, log standard deviation 0.5, and log serial correlation coefficient 0.8) would average one exceedance every three years, when exceedances are counted using the EPA (1986) approach. Figure J-5 shows a typical short series of 400 days.

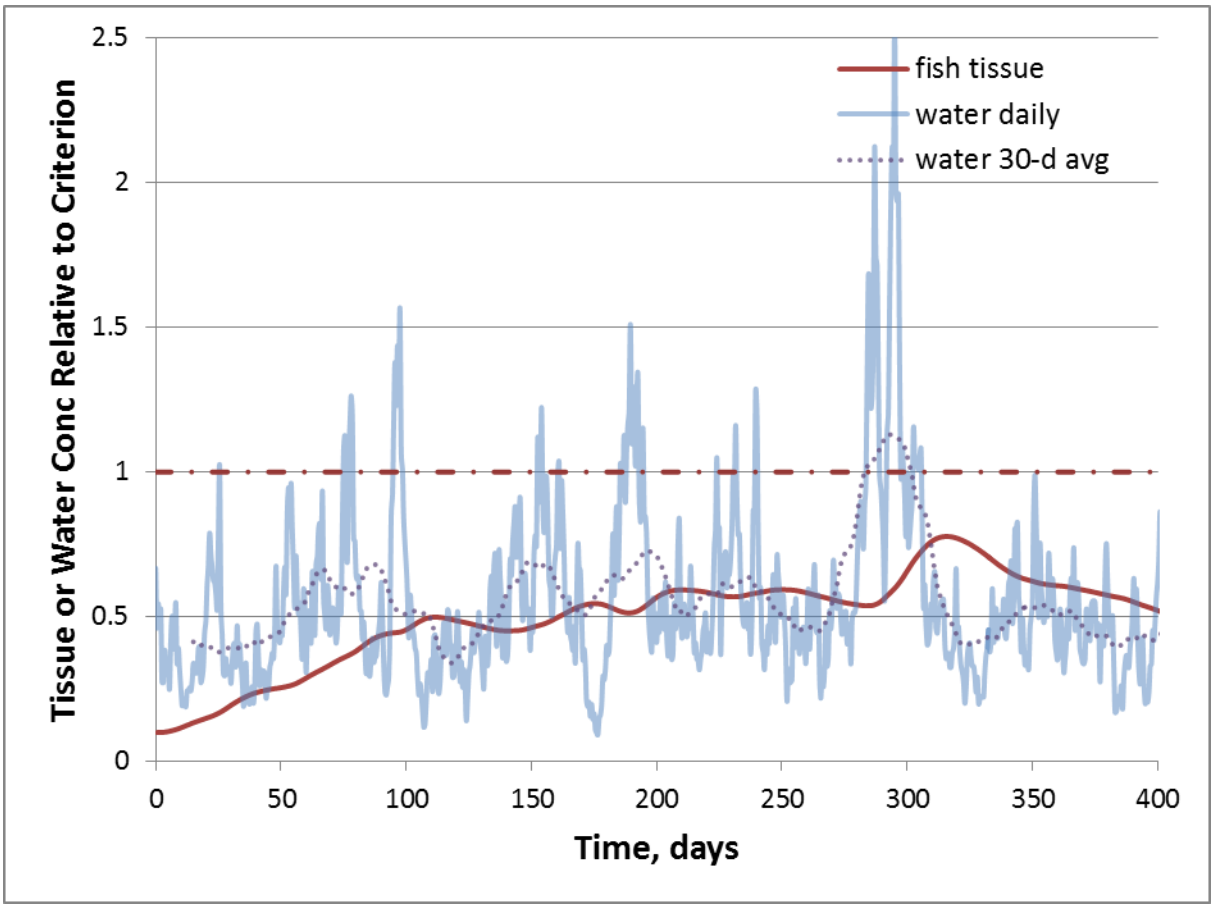


Figure J-5. Flowing water example of TL3 concentration starting at a concentration of 0.1 normalized to the criterion, and responding to randomly varying log-normally distributed water concentrations having median 0.49 (expressed as a dimensionless ratio: concentration/criterion), log standard deviation 0.5, and log serial correlation coefficient 0.8.
Again, all concentrations are as dimensionless ratios relative to the criteria concentrations.

Several points are worth noting. Because the water concentrations happen (by chance) to be below average for the first 50 days, the TL3 concentrations rise somewhat slowly during that period. Were they to be above average during that period, the TL3 concentrations would more rapidly approach their dynamically varying plateau. In such a short time series it is not graphically apparent what the long-term average TL3 concentration will be; however, because the long-term arithmetic mean water concentration would be 0.56 (normalized the its criterion), the TL3 concentration would likewise end up averaging 0.56 normalized to its criterion, if tracked for many years.

It is also worth noting that most 400-day series of the type shown in Figure J-5 would not have occurrences of 30-day average concentrations above the criterion (as suggested by Figure J-3). This particular random series does have a period of 30-day average exceedances, near Day 300, but it does not persist long enough to cause the TL3 concentration to approach its criterion.

Lastly, it should be noted that when concentrations are randomly varying as in Figure J-5, the water concentrations that one observes are highly dependent on when the samples are taken. The TL3 concentrations observed are far less dependent on when the samples are taken (after the plateau is approached), but time variations, although muted, are still present.

The example scenarios depicted here show lotic time to steady state of approximately 3 months to less than 1 year under different discharge scenarios including both continuous and intermittent discharges. The scenarios also assume that the new selenium input is from one source; multiple new sources particularly with varying discharge patterns, might have a different response time and pattern for various trophic levels.

The example is likely not appropriate for lentic systems, because they would not be expected to have the rapidly varying water concentrations of Figure J-5. In addition, the water-to-TL1 kinetics would likely be slower in lentic systems with new or time-varying sources because of the role of bottom sediments acting as a reservoir in recycling selenium. Ultimately this should yield slower rising and smoother TL3 concentrations compared to those in Figure J-5.

APPENDIX K: TRANSLATION OF A SELENIUM FISH TISSUE CRITERION ELEMENT TO A SITE-SPECIFIC WATER COLUMN VALUE

1.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING MECHANISTIC BIOACCUMULATION MODELING

Introduction:

EPA recommends fish tissue elements of the selenium criterion supersede water column elements under steady state conditions because the selenium concentration in fish tissue is a more sensitive and reliable indicator of the negative effects of selenium in aquatic life. However, implementation of a fish tissue criterion element can be challenging because many state and tribal Clean Water Act (CWA) programs prefer the expression of water quality criteria as an ambient concentration in the water-column. Therefore, EPA also recommends two monthly average water-column criterion elements, one for lotic (flowing) waters, and the other for lentic (still) waters. EPA derived all water column criterion elements from the egg/ovary criterion element representing a protective selenium concentration for fish species populations. Thus the water column criterion elements also represent protective selenium concentrations for fish species populations. If threatened or endangered fish species are present, states and tribes may need to derive alternative water column elements with a refined protection goal that account for site-specific bioaccumulation characteristics.

EPA derived water-column criterion elements by modeling selenium bioaccumulation in aquatic systems. The EPA worked with the United States Geological Survey to derive a translation equation utilizing a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al., 1992; Wang et. al., 1996; Luoma and Fisher, 1997; Wang, 2001; Schlekat et al. 2002b; Luoma and Rainbow 2005; Presser and Luoma 2006; Presser and Luoma 2010; Presser 2013). EPA translated the selenium egg-ovary criterion element into two set(s) of site-specific water concentration values (lentic and lotic), and used the distribution(s) of those water column values to derive the respective water-column criterion elements. This appendix describes approaches that states and tribes may choose to use regarding application of this same mechanistic modeling approach (or alternatively an empirical bioaccumulation factor (BAF) approach) to translate a fish tissue criterion element (egg-ovary, whole body, or muscle) into site-specific water-column concentrations to more precisely manage selenium in specific aquatic systems.

The relationship between the concentration of selenium in the tissues of fish and the concentration of selenium in the water column can vary substantially among aquatic systems. The species of fish, the species and proportion of prey, and a variety of site-specific biogeochemical factors affect selenium bioaccumulation and thus determine the allowable concentration of selenium in ambient water protective of aquatic life. States and tribes may choose to adopt the results of site-specific water column translations as site-specific criteria (SSC) or adopt a translation procedure into state or tribal water quality

standards. Under both options, the water quality standards revisions must be approved by EPA under Section 303(c) of the Clean Water Act. If a state or tribe adopts a translation procedure that will be implemented by other CWA programs, it must be scientifically defensible, produce repeatable, predictable outcomes, and result in criteria that protect the applicable designated use. Examples of such approaches include the mechanistic modeling approach and the empirical BAF approach described within this Appendix.

EPA considered both mechanistic and empirical modeling approaches to translate the selenium egg-ovary criterion element into water column concentration elements. A mechanistic modeling approach uses scientific knowledge of the physical and chemical processes underlying bioaccumulation to establish a relationship between the concentrations of selenium in the water column and the concentration of selenium in the tissue of aquatic organisms. The mechanistic modeling approach enables formulation of site-specific models of trophic transfer of selenium through aquatic food webs and translation of the egg-ovary criterion element into an equivalent site-specific water concentration. The empirical modeling approach establishes a relationship between concentrations of selenium in fish tissue and ambient water directly by measuring selenium concentrations in both media and calculating the ratio of the two concentrations. The ratio (BAF) can then be used to estimate the target concentration of selenium in the water column as related to the adopted fish tissue element.

Both the mechanistic and empirical modeling approaches have advantages and disadvantages that should be considered before deciding which approach to use. On the one hand, the mechanistic modeling approach has the advantage of not requiring extensive fish tissue sampling and analysis by using knowledge of aquatic system food webs. However, uncertainty in the selection of model parameters increases uncertainty in the outcome leading to a reduction in defensibility. Of particular concern with respect to the mechanistic model EPA developed is the selection of the value for the enrichment factor parameter *EF* (discussed in more detail below). On the other hand, the empirical BAF approach is conceptually and computationally simpler because it relies only on field measurements and does not require extensive knowledge of the physical, chemical, or biological characteristics of the aquatic system. However, obtaining a sufficient number of measurements in fish tissue and water may be logistically difficult and/or more expensive.

The appropriate modeling approach to use when translating the selenium egg-ovary criterion element to a site-specific water-column concentration depends on individual circumstances and site-specific characteristics. The mechanistic modeling approach may be a useful method in situations where there is little or no data on the amount of selenium in an aquatic system, the empirical BAF approach may be desirable in circumstances where in fish tissue and water data are available. Below is a description of

methodology than can be used to translate the egg-ovary criterion element to a site-specific water-column concentration for site-specific management of selenium.

1.1 Relating the Concentration of Selenium in Fish Tissue and Water using the Mechanistic Modeling Approach

The relationship between the concentration of selenium in the eggs or ovaries of fish and the concentration of selenium in the water column is given in Equation K-1 (Equation 18 from the main text):

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \textit{(Equation K-1)}$$

Where:

C_{water} = the concentration of selenium in water (µg/L),

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

$TTF^{composite}$ = the product of the trophic transfer factor (*TTF*) values of the fish species that is the target of the egg-ovary criterion element and the *TTF* values of all lower trophic levels in its food web (no units of measurement, see explanation below).

EF = the steady state proportional bioconcentration of dissolved selenium at the base of the aquatic food web (L/g),

CF = the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues (no units of measurement).

The basic principles expressed in Equation K-1 are illustrated in the conceptual model shown in Figure K-1.

Selenium dissolved in surface water enters aquatic food webs by becoming associated with trophic level 1 primary producer organisms (e.g., algae) and other biotic (e.g., detritus) and abiotic (e.g., sediment) particulate material. An enrichment function (*EF*) quantifies the bioconcentration of selenium in particulate material and thus its bioavailability in the aquatic system. The parameter *EF* is a single value that represents the steady state proportional concentration of selenium in particulate material relative to the concentration of selenium dissolved in water.

Organic particulate material is consumed by trophic level 2 organisms (usually aquatic invertebrates, but also some fish species that are herbivores/detritivores) resulting in the accumulation of selenium in the tissues of those organisms. Trophic level 2 invertebrates are consumed by trophic level 3 fishes resulting in further accumulation of selenium in the tissues of those fish. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer factor (*TTF).* A *TTF* is a single value that represents the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. Different species of organisms metabolize selenium in different ways. Thus each species is associated with a specific *TTF* value. Because the trophic transfer of selenium through all trophic levels is mathematically equal to the product of the individual *TTF* values, all consumer-resource interactions in a particular aquatic ecosystem are simplified in Equation K-1 by representing the product of all the individual *TTF* values as the single parameter $TTF^{composite}$.

Fish accumulate selenium in different tissues of the body in differing amounts. Species physiology, age, diet, sex, and spawning status are some of the factors that affect selenium partitioning in body tissues. Because the primary selenium criterion element is expressed as a concentration in the eggs and/or ovaries, a conversion factor (*CF*) quantifies the relationship between the concentration of selenium in the eggs and/or ovaries and the average concentration of selenium in the whole body or muscle tissues. The parameter *CF* in Equation K-1 is a single value that represents the steady state proportional concentration of selenium in the eggs and/or ovaries relative to the average concentration of selenium in all body tissues. Different species of fish accumulate selenium in their eggs and ovaries to different degrees. Thus each species of fish is associated with a specific *CF* value.

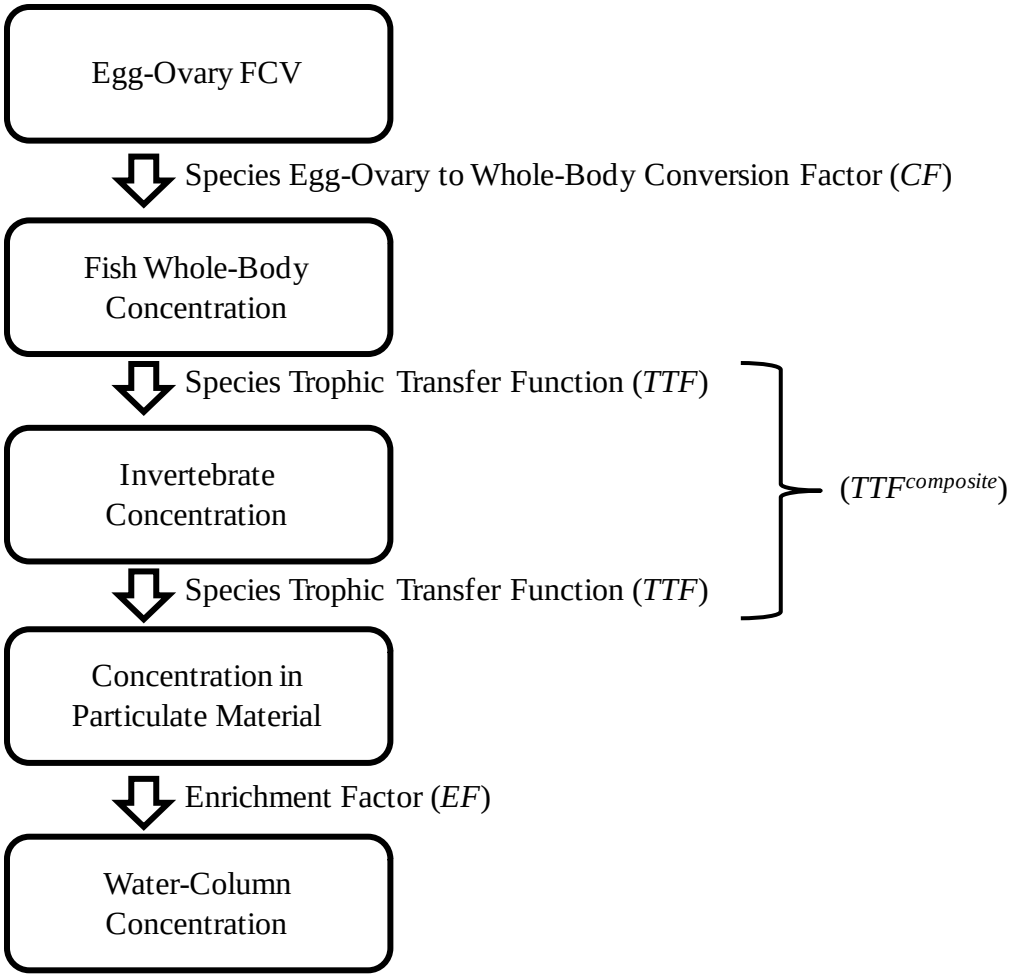


Figure K-1. Conceptual model for translating the egg-ovary FCV to a water-column concentration.
Note: States may want to use the whole body or muscle criterion elements as the starting point for site specific translation to a water column concentration.

Once the parameters that quantify the transfer of selenium through each step in this pathway are identified, they can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific concentration of selenium in the water column (i.e., target water column concentration).

Because each *TTF* value is species-specific, it is possible to differentiate bioaccumulation in different aquatic systems by modeling the food web of the target fish species. For example, where the food web contains more than 3 trophic levels, $TTF^{composite}$ can be represented as the product of all *TTF* values for each trophic level given in Equation K-2, which is a generalization of Equation 10 from the main text:

$$TTF^{composite} = TTF^{TL2} \times TTF^{TL3} \times \ldots \times TTF^{TLn} \qquad \textit{(Equation K-2)}$$

Where:

$TTF^{composite}$ = the product of all *TTF* values at all trophic levels.

TTF^{TLn} = the *TTF* value of the highest trophic level.

The consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* value at a particular trophic level as the average *TTF* values of all species at that trophic level weighted by the proportion of species consumed given as Equation K-3 (Equation 11 in the main text):

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \textit{(Equation K-3)}$$

Where:

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

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

These concepts can be used to formulate a mathematical expression of $TTF^{composite}$ that models selenium bioaccumulation in a variety of aquatic ecosystems. Figure K-2 illustrates five hypothetical food web scenarios and the formulation of $TTF^{composite}$ for each of them. For each scenario, the value of $TTF^{composite}$, the *CF* value associated with the targeted fish species, and the site-specific *EF* value can be used with Equation K-1 to translate the egg-ovary criterion element to a site-specific water concentration value. The hypothetical food web models in Figure K-2 are a few possible examples of food web models for illustrative purposes. It is desirable to derive and use of a food web model that best represents the aquatic system for which the water column translation will apply. The general steps for deriving a site-specific translation of the egg-ovary criterion element to a water concentration value are described below.

A) Three trophic levels (simple):

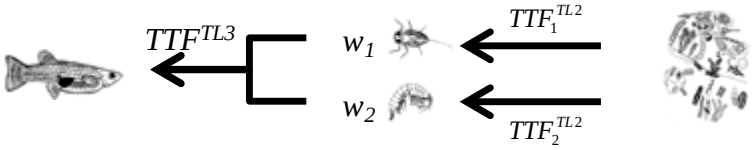
$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$

B) Four trophic levels (simple):

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

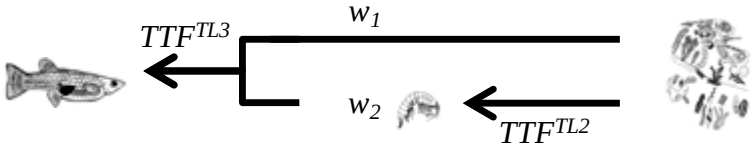
C) Three trophic levels (mix within trophic levels):

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



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

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



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

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

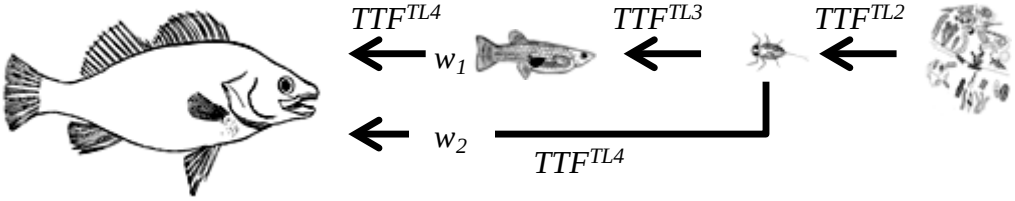


Figure K-2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios.

$TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food web model is used to calculate a value for $TTF^{composite}$ using appropriate species-specific *TTF* values and the proportions of each species consumed at each trophic level. See text for further explanation.

1.2 Steps for Deriving a Site-Specific Water Concentration Value from the Egg-Ovary Criterion Element

Below are the steps for deriving a site-specific water concentration value from the selenium egg-ovary criterion element using EPA's mechanistic model approach:

1) Identify the appropriate target fish species.
2) Model the food web of the targeted fish species.
3) Identify appropriate *TTF* values by either:
    a. selecting the appropriate *TTF* values from a list of EPA-derived values, or
    b. deriving *TTF* values from existing data, or
    c. deriving *TTF* values by conducting additional studies, or
    d. extrapolating *TTF* values from existing values.
4) Determine the appropriate value of *EF* by either
    a. deriving a site-specific *EF* value from field measurements, or
    b. deriving an appropriate *EF* value from existing data, or
    c. extrapolating from *EF* values of similar waters.
5) Determine the appropriate *CF* value by either,
    a. selecting the appropriate *CF* value from a list of EPA-derived values, or
    b. deriving a *CF* value from existing data, or
    c. deriving a *CF* value by conducting additional studies, or
    d. extrapolating a *CF* value from existing values.
6) Translate the selenium egg-ovary criterion element into a site-specific water concentration value using Equation K-1.

Below are detailed descriptions of each step followed by example calculations using a variety of hypothetical scenarios. EPA is providing this information to support help states and tribes that choose to develop selenium water column values from the egg-ovary criterion element or develop translation procedures. Successful application of the mechanistic approach described here requires use of particular food web models and parameter values that are appropriate for particular aquatic systems.

1.2.1 Identify the Appropriate Target Fish Species

1.2.1.1 When fish are present

In developing a site-specific translation of the egg-ovary criterion element, the user wshould select whether to use a mechanistic model or empricial (BAF) approach. This decision will in large part

determine the data and information requirements. A mechanistic model approach will likely require information on the spatial and temporal distribution of aquatic organisms, and may require measurements of selenium in ambient water and particulate material. An empirical model approach will use measurements of selenium is fish tissue and ambient water.

Developing a site-specific translation of the egg-ovary criterion element will also entail selection of which species of fish to target. The concentration of selenium in eggs and ovaries is the most sensitive and consistent indicator of toxicity. However, toxicity and bioaccumulation potential can vary among species. Species in the families Acipenseridae, Centrarchidae, and Salmonidae are particularly sensitive to selenium (Table 3.3 in the main document), whereas species such as stoneroller species, creek chub, blackside dace, and white sucker have documented tolerance to selenium and can be found in selenium contaminated systems (NAMC 2008, Presser 2012). Green sunfish accumulate less selenium than other species with comparable exposures in the same aquatic system (Hitt and Smith 2015). Selection of the fish species in the aquatic system with the greatest selenium sensitivity and bioaccumulation potential is recommended.

Several additional factors should also be considered in deciding which species to target when developing a site-specific translation of the egg-ovary criterion element. Anadromous species (species that migrate from salt water to spawn in fresh water) should generally avoided because selenium exposure and bioaccumulation occurs over a relatively long period through the consumption of locally contaminated aquatic organisms. Additionally considerations include whether the fish species selected typically consume organisms known or suspected to readily bioaccumulate selenium (e.g., mollusks). For example, high concentrations of selenium in San Francisco Bay white sturgeon are associated with their consumption of *Potamocorbula amurensis*, a bivalve in close proximity to selenium-contaminated sediments that rapidly and efficiently accumulates selenium (Stewart et al. 2004). In contrast, striped bass from the same aquatic system have substantially lower concentration of selenium in their tissues because their zooplankton-based food web has substantially lower selenium bioaccumulation characteristics (Schlekat et al. 2004; Stewart et al. 2004). The 2016 selenium criterion was developed for freshwater, but if considering other ecosystems, it may be worth noting that salinity may also affect bioaccumulation of selenium. Freshwater mollusks tend to have relatively higher *TTF* values when compared to other freshwater invertebrate taxa (e.g., aquatic insects), but they are lower than mollusks in marine or brackish systems (and particularly *P. amurensis*, an invasive clam in the San Francisco Bay). In aquatic systems with resident fish species of unknown selenium sensitivity and bioaccumulation potential, other factors such as ecological significance could be considered when choosing a target species.

Data from fisheries or biological surveys or other biological assessments could be considered to determine the fish species that reside in specific surface waters. State and tribal resource agency personnel

familiar with fish sampling activities could also be a source of information on resident fish species. General information on the fish species present in state and tribal surface waters may also be found at:

- State Fish and Game agencies
- U.S. Fish and Wildlife Service (http://www.fws.gov)
- U.S. Geological Survey (http://www.usgs.gov)
- NatureServe.org (http://www.natureserve.org)
- Fishbase (http://www.fishbase.org)
- State or local sources of biological information (e.g. Biota Information System of New Mexico at http://www.bison-m.org)

Measurements of selenium in fish tissue would most reflect the ecosystem if adult (reproductively mature) fish are sampled. Selenium measurements in fish tissue will likely be more stable in adult fish because they are more likely to have a stable prey base. Reproductively mature (ripe or gravid) females would be needed for measures selenium in eggs and/or ovary tissue for comparison to the the egg-ovary tissue criterion element. It would be prudent to avoid sampling ovary tissue "post-spawn" due to a potential decrease in selenium concentration presumably due to the loss of selenium through spawning and release of eggs with relatively high concentrations of selenium. Consideration of closely related taxonomic surrogates (same genus or family) for threatened or endangered species may be useful.

Figure K-3 shows an example decision tree that may help in selection of the appropriate fish species for deriving a site-specific water concentration value from the selenium egg-ovary, whole-body, or muscle FCV. The use of taxonomic hierarchies for anlysis utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993). Additional information on fish tissue sampling (e.g., species selection, temporal and spatial considerations) is under development and will be published in the form of a technical support document (TSD) by the EPA in the near future.

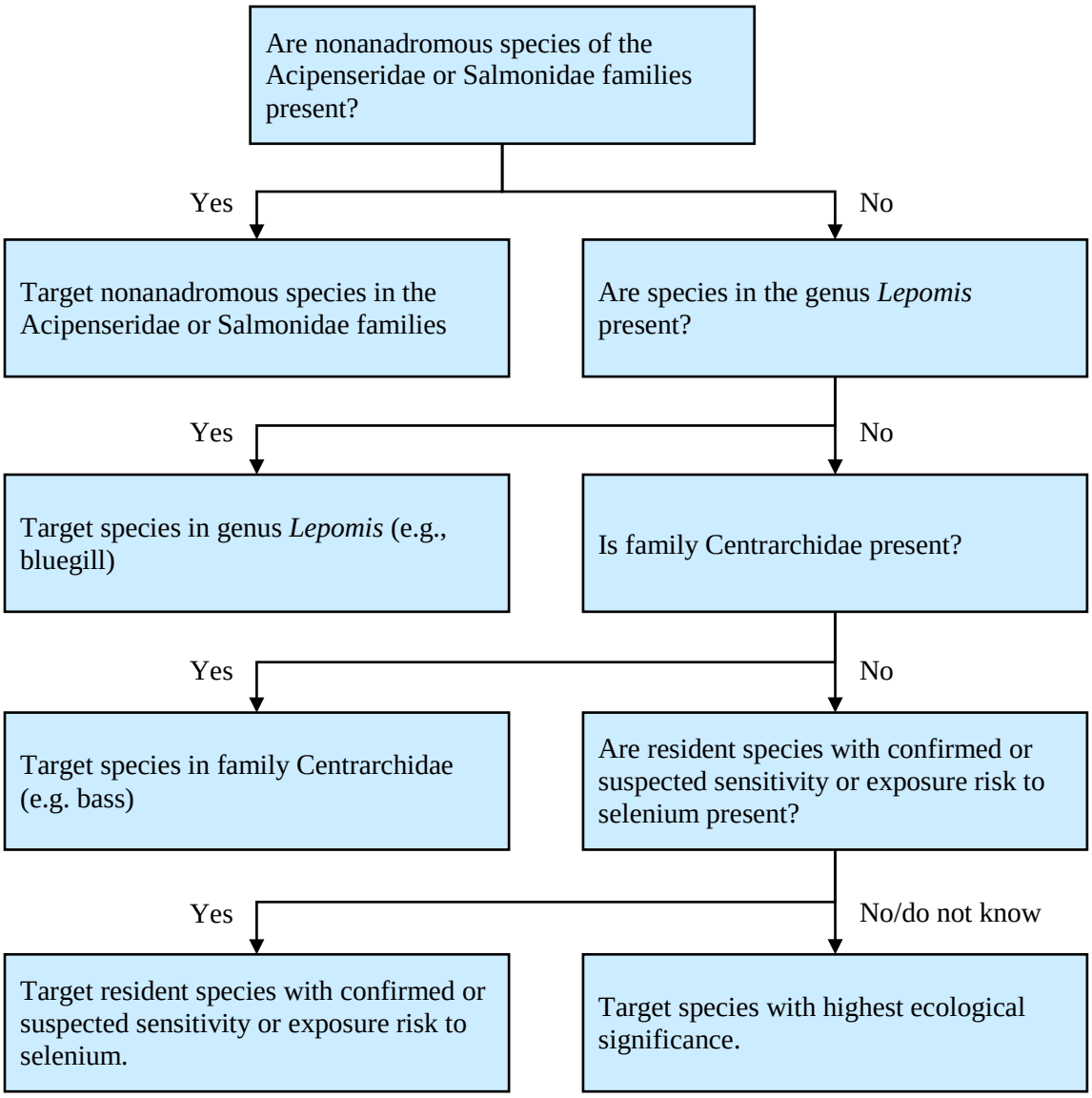


Figure K-3. Recommendeed decision process for selection of the fish species to use when deriving a water concentration from the selenium egg-ovary FCV.
This decision tree is also generally applicable when using the whole body or muscle tissue as the starting point for development of SSC, particularly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

1.2.1.2 When fish are absent from a site

Some aquatic systems do not contain resident fish. Fish may be absent from a waterbody because of intermittent or persistent low flows, physical impediments such as waterfalls or impoundments, lack of adequate habitat for feeding and/or spawning, or intolerable aquatic conditions related to pH, turbidity, temperature, salinity, total dissolved solids, chemical contaminants, or pathogens. These conditions could be due to natural or anthropogenic causes. Some streams may be naturally intermittent or ephemeral, or

they might exhibit low or intermittent flows because of impoundments or water draw-down for agricultural irrigation, industrial uses, drinking water supply, or other uses.

When fish are absent from a waterbody, consideration of sampling the most sensitive fish species inhabiting nearby, most proximate downstream waters may be useful in order to understand selenium bioaccumulation potential in such systems. Although the upper reaches of some aquatic systems may not support fish communities, the invertebrate organisms that reside there may tolerate high concentrations of selenium and pose a selenium risk to predator fish if transported downstream. Users may choose to evaluate upstream waters without fish by measuring the selenium concentration in water, biotic and/or abiotic particulate material, and/or the tissues of invertebrate aquatic organisms that reside there. Because selenium associated with particulate material and invertebrate organisms can be transported downstream during intermittent high flows, elevated concentrations of selenium in the tissues of downstream fish could indicate upstream sources of selenium that require a more detailed evaluation of upstream conditions.

1.2.2 Model the Food-Web of the Targeted Fish Species

After selecting the target fish species, model users should formulate a mathematical expression of the target species food-web that will be used to calculate the value of $TTF^{composite}$. As discussed previously, $TTF^{composite}$ is the product of the *TTF* values across trophic levels of the target fish species food-web. The complexity of the food-web model will depend on the species of fish that is targeted, the diversity of prey species in the aquatic system, and the amount of information that is available. Many of the same information sources used to identify the targeted fish species in a waterbody could also be used to obtain information about its food web. The types and proportions of food organisms the targeted fish species consumes can be directly assessed through studies that examine stomach contents or from information gathered through biological assessments. If site-specific information is not available, model users could estimate the target fish species food-web using publicly available databases such as NatureServe (http://www.natureserve.org). For example, the NatureServe database record for fathead minnow in the HUC watershed #5040004 in Ohio indicates under the heading: “Ecology and Life History - Food Comments,” the fathead minnow “feeds opportunistically in soft bottom mud; eats algae and other plants, insects, small crustaceans, and other invertebrates (Becker 1983, Sublette et al. 1990).”

Additional sources of information include:

- FishBase (http://www.fishbase.org). FishBase is a relational database developed at the World Fish Center in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and many other partners.

- Carlander, K.D. Handbook of Freshwater Fishery Biology, volumes 1, 2 and 3. Iowa state University Press, Ames, Iowa. 1969-1997.

1.2.3 Identify Appropriate *TTF* Values

The food-web model uses appropriately selected species-specific *TTF* values (and, if appropriate, proportions within the same trophic level). Model users identify the appropriate *TTF* values by using one of the following four procedures, or by using other scientifically defensible methods.

1.2.3.1 Select the appropriate *TTF* values from the provided list of EPA-derived values

Species-specific TTF values represent the steady state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. EPA-derived *TTF* values for aquatic invertebrates and fish are provided in Tables K-1 and K-2 (Tables 3.10 and 3.11 in main text; see also main text for a complete explanation of the procedure EPA used to derive these values).

Table K-1. EPA-derived Trophic Transfer Factor (TTF) values for freshwater aquatic invertebrates.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

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

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

Table K-2. EPA-derived Trophic Transfer Factor (*TTF*) values for freshwater fish.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79

Common name	Scientific name	AE	IR	k_e	TTF
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

The *TTF* values from these lists could be used exclusively, or in conjunction with *TTF* values obtained from other sources (see below). Note that these tables do not represent an exhaustive list of all *TTF* values that may be required to calculate a site-specific water concentration value. If this list does not include a required *TTF* value, another approach could be considered to obtain an appropriate value.

1.2.3.2 Deriving *TTF* values from existing data

If model users cannot obtain one or more required *TTF* values from Tables K-1 and/or K-2, species-specific *TTF* values could be derived using existing data. One approach for deriving species-specific *TTF* values is to use the physiological coefficients representing food ingestion rate (*IR)*, selenium efflux rate (k_e), and selenium assimilation efficiency (*AE*) to calculate a *TTF* value using Equation K-4 (Equation 3 from the main text, Reinfelder et al. 1998**)** given as:

$$TTF = \frac{AE \times IR}{k_e} \qquad \textit{(Equation K-4)}$$

Where:

TTF = species-specific trophic transfer factor

AE = species-specific assimilation efficiency (%)

IR = species-specific ingestion rate (g/g-d)

k_e = species-specific efflux rate constant (/d)

The physiological coefficients *IR*, *AE* and are species-specific values. Values for AE and k_e can only be derived from laboratory studies. Values for IR may be derived from laboratory studies or obtained from published literature. After the three physiological coefficients are obtained, a *TTF* value can be calculated using Equation K-4.

Another way to derive species-specific *TTF* values is to empirically assess the relationship between the selenium concentration in the tissue of organisms and the selenium concentration in the food they consume using paired measurements from field studies. Species-specific *TTF* values can be derived from such measurements by calculating ratios, using regression techniques, or other scientifically defensible methods.

Model users could choose to use the same approach EPA used to calculate species-specific *TTF* values. EPA derived *TTF* values using a combination median and regression approach. EPA defined the *TTF* value for any trophic level as:

$$TTF^{TLn} = \frac{C_{tissue}^{TLn}}{C_{food}^{TLn}} \qquad \textit{(Equation K-5)}$$

Where:

TTF^{TLn} = The trophic transfer factor of a given trophic level,

C_{tissue}^{TLn} = The selenium concentration (mg/kg dw) in the tissues of the consumer organism,

C_{food}^{TLn} = The selenium concentration (mg/kg dw) in the consumer organism's food.

EPA used the median of the ratios given in Equation K-5 as the species-specific *TTF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear

regression analysis. EPA considered the relationship acceptable if a linear regression of tissue selenium concentration on food selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope (i.e., selenium concentrations in the consumer increases with increasing selenium in food).

1.2.3.3 Deriving *TTF* values by conducting additional studies

Additional studies could be conducted to obtain the data needed to derive *TTF* values for specific needs, or to revise existing *TTF* values, if the existing *TTF* values do not appear to be appropriate for a particular aquatic system.

1.2.3.4 Extrapolating *TTF* values from existing values

If one or more necessary *TTF* values are not available, and the information needed to derive a species-specific *TTF* value is not available or impractical to obtain, model users could consider extrapolating a new *TTF* value from other known *TTF* values. One possible method to extrapolate a *TTF* value is to sequentially consider higher taxonomic classifications until one or more of the organisms with a known *TTF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *TTF* values, the average *TTF* from the matching table entries could be used. The use of taxonomic hierarchies in this way utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993).

EPA used such an extrapolation approach to derive some of the *TTF* values necessary to develop the water column criterion elements. For example, the *TTF* value for *Chrosomus eos* (northern redbelly dace) was not available. *TTF* values were also not available for other species in the genus *Chrosomus*, but *TTF* values were available for species in the family Cyprinidae, including *Rhinichthys atratulus* (blacknose dace), *Cyprinus carpio* (common carp), *Semotilus atromaculatus* (creek chub), *Pimephales promelas* (fathead minnow), *Cyprinella lutrensis* (red shiner), *Richardsonius balteatus* (redside shiner), and *Notropis stramineus* (sand shiner). Because Cyprinidae is the lowest taxonomic classification where *Chrosomus eos* matches one or more species with an available *TTF* value, EPA used the median *TTF* value of blacknose dace, common carp, creek chub, fathead minnow, red shiner, redside shiner, and sand shiner as the *TTF* value for northern redbelly dace.

1.2.4 Determine the Appropriate *EF* Value

The selenium enrichment function (*EF*) value represents the bioavailability of selenium at the base of the aquatic food web. The base of the aquatic food web includes phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma, 2010). EPA refers to this mixture of living and non-living entities as particulate material. The parameter *EF* varies more widely across aquatic systems than any other parameter, and is influenced by

the source and form of selenium, water residence time, the biogeochemical characteristics of the waterbody, and the type of particulate matter collected. Because *EF* can vary greatly across waterbodies, this parameter has the greatest potential to introduce uncertainty in the translation from an egg-ovary selenium concentration to a water column concentration. For this reason, use *EF* values derived from site-specific data is recommended whenever possible in applying the model. One of the following four procedures could be used to derive *EF* values, or other scientifically defensible methods could be used.

1.2.4.1 Deriving a site-specific *EF* value from field measurements

Equation 12 from the main text defines the parameter *EF* as the ratio of the concentration of selenium in particulate material to the concentration of selenium dissolved in water given as:

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \textit{(Equation K-6)}$$

Where:

$C_{particulate}$ = Concentration of selenium in particulate material (µg/g)

C_{water} = Concentration of selenium dissolved in water (µg/L)

EF = Enrichment Function (L/g)

To calculate a site-specific *EF* value, EPA first calculates the ratio of each individual particulate measurement and its associated water measurement (if more than one water measurement is available for any given particulate measurement, the median water measurement is used). If more than one ratio for any given category of particulate material is available (e.g., more than one ratio of algae to water), EPA takes the median of the ratios. EPA then calculates the geometric mean of the median ratios for each category of particular material as the site *EF* value. EPA only uses sediment measurements if there are at least one measurement from either algae or detritus.

Deriving a site-specific *EF* value in this manner is a relatively straightforward procedure. However, consideration of data that appropriately accounts for the spatial and temporal variability of an aquatic system would be useful in the development of any sampling plan. Aquatic system characteristics such as dimension, volume, shape, residence time, velocity, and growing season are a few important factors that should be considered in designing a sampling plan that will adequately account for variability. State and Federal agencies (USGS, ACOE) as well as watershed groups may be useful sources of information that can help characterize the temporal and spatial variability at a particular aquatic system. When developing the selenium criterion, EPA observed a relatively lower correlation between the selenium concentration in water and abiotic (benthic sediments) particulate samples compared to the same analysis between water and biotic (algae and detritus) particulate samples, resulting in EPA's decision

that calculation of any site-specific *EF* values include information from at least one type of biotic particulate indeveloping its criteiron. Prioritization of sampling of biotic particulate material over abiotic samples should be considered. Regaridng selenium measurements from abiotic particulate material, consideration of utilizing at least one type of biotic particulate material when deriving the *EF* value of an aquatic system is recommended.

Site-specific *EF* values using particulate and water samples that are as spatially and temporally coincident as possible would be considered the most robust. Although EPA's analysis of particulate and water samples from a sample population of aquatic systems found that samples taken within one year of each other, based on data availability, were appropriate in deriving the national criterion (Figure 3.5 in the main document), a site-specific EF value would ideally involve collecting particulate and water samples at the same location and time to ensure their representativeness of sirte-specific conditions. One simple and effective sampling and analysis scenario would be to collect water samples or a combination of particulate and water samples, separate the particulate material from the water in each sample by filtering, measure the concentration of selenium in the separated water and particulate material, compute the ratio of the two measurements from each sample, and then calculate the mean or median of all the ratios.

Selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as lakes, reservoirs, oxbows, and wetlands) and with fine particulate sediments high in organic carbon. A well-planned sampling protocol was developed in association with the development of a site-specific water-column criterion for selenium in the San Francisco Bay Delta[2]. States and tribes may also want to consult Doblin et al. (2006) for specific particulate sampling methods. EPA's National Rivers and Streams Assessment[3] also provides methods for quantitative periphyton sampling that commonly represents the base of many aquatic food webs. Analytical methods to measure selenium in particulate material and in water are discussed in Appendix L.

1.2.4.2 Deriving an appropriate *EF* value from existing data

If suitable and sufficient site-specific measurements of selenium in particulate material and water are already available, the model user may be able to use that data to derive an appropriate *EF* value. However, it would be important to ensure that the data represents current conditions, were collected and analyzed using scientifically sound sampling and analytical techniques, and proper quality assurance and quality control protocols were implemented.

1.2.4.3 Extrapolating from *EF* values of similar waters

[2] https://www3.epa.gov/region9/water/ctr/selenium-modeling_admin-report.pdf

[3] https://www.nemi.gov/methods/method_summary/12558/ (EPA-841-B-07-009) and https://www.nemi.gov/methods/method_summary/12565/ (EPA-841-B-12-009)

In circumstances where a site-specific, field-derived *EF* value is not available or practical to develop, an *EF* value from one or more aquatic systems with similar hydrological, geochemical, and biological characteristics could be used to estimate *EF*. However, there is a possibility of introducing significant uncertainty when using *EF* values extrapolated from other aquatic systems. More information on this topic is contained in Appendix H of this document.

1.2.5 Determine the Appropriate *CF* Value

1.2.5.1 Selecting the appropriate *CF* value from the list of values that were used to derive EPA's recommended water criteria concentration values

The parameter *CF* represents the species-specific proportion of selenium in eggs or ovaries relative to the average concentration of selenium in all body tissues. EPA derived species-specific *CF* values for 20 species of fish from studies that measured selenium concentrations in both eggs and/or ovaries and in whole body and/or muscle. These *CF* values can be found in Appendix B and are reproduced below (Table K-3).

Table K-3. Selenium Whole Body to Egg-Ovary Conversion Factors (*CF*).

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Species				
Bluegill	2.13			2.13
Bluehead sucker	1.82			1.82
Brook trout		1.09	1.27	1.38
Brown trout	1.45			1.45
Creek chub	1.99			1.99
Common carp	1.92			1.92
Cutthroat trout	1.96			1.96
Desert pupfish	1.20			1.20
Dolly Varden		1.26	1.27	1.61
Fathead minnow	1.40			1.40
Flannelmouth sucker	1.41			1.41
Green sunfish	1.45			1.45
Mountain whitefish		5.80	1.27	7.39
Northern pike		1.88	1.27	2.39
Rainbow trout		1.92	1.27	2.44

Common name	Median ratio (Cegg-ovary/ Cwhole-body)	Median ratio (Cegg-ovary/ Cmuscle)	Muscle to whole-body correction factor	Final CF values
Razorback sucker		2.31	1.34	3.11
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sturgeon		1.33	1.27	1.69
White sucker	1.38			1.38
Genus				
Catostomus				1.41
Gila				2.07
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				1.96
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				1.95
Salmonidae				1.71
Order				
Cyprinodontiformes				1.20
Perciformes				1.45
Class				
Actinopterygii				1.45

The data and methods used to derive the *CF* in this table are described in Appendix B.

1.2.5.2 Deriving a *CF* value from existing data

The parameter *CF* is mathematically expressed as Equation K-7 (Equation 16 in the main text):

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}} \qquad \textit{(Equation K-7)}$$

Where:

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

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

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

If suitable and sufficient data are available, a model user could derive a species-specific *CF* value using the same numerical methods described above to calculate the parameter *EF*. The median of the ratios given in Equation K-7 could be used as the species-specific *CF* value, but only if an empirical relationship between the paired measurements could be confirmed by linear regression analysis. IN deriving the national criterion, EPA considered it to be acceptable if a linear regression of egg-ovary selenium concentration on whole body selenium concentration resulted in both a statistically significant fit ($P < 0.05$) and a positive slope. Other scientifically defensible methods could be used. Regardless of the method used, the user should ensure that the data used to derive *CF* values were collected using adequate quality assurance and quality control protocols.

1.2.5.3 Deriving a *CF* value by conducting additional studies

Additional studies could be performed to obtain data needed to derive *CF* values for specific needs or to revise existing *CF* values if there is reason to believe doing so may increase the accuracy of the resulting water concentration value. Analytical methods to measure selenium in tissue are discussed in Appendix L. Where appropriate, additional data could be obtained as part of a NPDES permit application by invoking authority under CWA section 308 (or comparable state or tribal authority) to require NPDES-regulated facilities to collect information necessary to develop permit limits**.**

1.2.5.4 Extrapolating the CF value from the list of values that were used to derive EPA's recommended water criteria concentration values

If one or more necessary *CF* values are not available, and the information needed to derive a species-specific *CF* value is not available or impractical to obtain, a model user could could consider extrapolating a new *CF* value from other known *CF* values. One possible method to extrapolate a *CF* value is to use the same taxonomic approach EPA uses for *TTF* values that are not available for specific

species (Section 1.2.3.4). Sequentially consider higher taxonomic classifications could be considered until one or more of the fish species with a known *CF* value matches the taxon being considered. If the lowest matching taxon is common to more than one of the available *CF* values, the average *CF* value from the matching table entries could be used.

1.2.6 Translate the Selenium Egg-Ovary Criterion Element into a Site-Specific Water Concentration Value using Equation K-1

Model users could derive a site-specific water concentration value from the egg-ovary criterion element value using Equation K-1 with appropriate values of *CF*, $TTF^{composite}$ (derived from the product of the individual *TTF* values from each trophic level) and *EF*. Note that NPDES permitting regulations at 40 CFR § 122.45(c) requires that a Water Quality-Based Effluent Limit (WQBEL) for metals be expressed as total recoverable metal, unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Equation K-1 assumes selenium concentrations dissolved in water. While states and tribes may express ambient water quality criteria in water quality standards as dissolved selenium, an additional step would be necessary to convert the dissolved selenium concentration to a total recoverable selenium concentration for the purpose of NPDES permitting. Guidance for converting expression of metal concentrations in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996).

1.3 Managing Uncertainty using the Mechanistic Modeling Approach

Uncertainty in the translation of the egg-ovary criterion element to a water column value using the mechanistic bioaccumulation modeling approach (Equation K-1) can arise from several sources. These include:

- Measurement error when deriving input parameters,
- Inaccurate food web models due to misidentification and/or incorrect proportions of prey organisms,
- Inaccurate or inappropriate *EF*, *TTF*, and/or *CF* values,
- Biological variability,
- Unaccounted factors affecting bioaccumulation (e.g. selenium speciation), and
- Other unknown factors.

The most influential step in selenium bioaccumulation occurs at the base of aquatic food webs (Chapman et al. 2010). The parameter *EF* characterizes this step by quantifying the partitioning of

selenium between the dissolved and particulate state. *EF* can vary by at least two orders of magnitude across aquatic systems (Presser and Luoma 2010). The greatest reduction in uncertainty could be achieved when translating a fish tissue concentration of selenium to a water column concentration using Equation K-1 by using temporally and spatially coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity to accurately characterize *EF*.

Presser (2013) provides several recommendation to reduce uncertainty in an ecosystem scale approach to deriving a site-specific selenium water column criterion in a coal mining impacted area of West Virginia. Suggested actions to reduce uncertainty include:

- Obtaining temporally matched pairs of selenium measurements in dissolved and particulate material across a broad range of sites to ensure the samples accurately characterize the aquatic system and to assess sample variability;
- Characterizing particulate material across seasons to better define the base of the food web;
- Evaluating aquatic systems variables such as residence time, watershed dilution, and physical habitat attributes on as fine a scale as possible;
- Refining model assumptions to accurately characterize dietary preferences and composition of fish, and develop additional *TTF* values if necessary;
- Identify and target fish species particularly sensitive to selenium;
- Consider temporal changes in the bioaccumulation potential of the aquatic system and changes in selenium sensitivity over the life cycle of fish; and
- Consider variability in hydrology and selenium discharges.

The suitability of selected equation parameters could be determined by obtaining fish tissue and water column measurements of selenium from small-scale field studies, use of equation K-1 to estimate one measurements using the other, and comparison of the estimated concentration with the actual concentration (see Section 6.2.1 of the main document for a description of EPA's validation approach).

1.4 Example Calculations

Below are six hypothetical examples that demonstrate how to translate the egg-ovary FCV to a site-specific water concentration criterion using Equation K-1. These examples encompass a variety of hypothetical aquatic systems with various fish species and food webs. For these hypothetical examples, species-specific *TTF* values were taken from Tables K-1 and K-2, and *CF* values were taken from Table K-3. To calculate EF in these examples, the EPA used a hypothetical water concentration of 5 µg/L and the hypothetical particulate concentrations of 4.25 µg/g and 8.75 µg/g in lotic and lentic aquatic systems, respectively.

1.4.1 Example 1

Bluegill (*Lepomis macrochirus*) in a river that consume mostly amphipods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for amphipods (TTF^{TL2})	1.22
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$\mathrm{EF} = \frac{\mathrm{C_{particulate}}}{\mathrm{C_{water}}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$
$$= 1.03 \times 1.22$$
$$= 1.26$$

$$C_{water} = \frac{15.1}{1.26 \times 0.85 \times 2.13}$$

= 6.62 µg/L

1.4.2 Example 2

Fathead minnow (*Pimephales promelas*) in a river that consume mostly copepods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods (TTF^{TL2})	1.41
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

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

$= 1.57 \times 1.41$

$= 2.21$

$$C_{water} = \frac{15.1}{2.21 \times 0.85 \times 1.40}$$

= 5.74 µg/L

1.4.3 Example 3

Bluegill (*Lepomis macrochirus*) in a lake that consume mostly aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	8.75
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for aquatic insects (median of Odonates, Water boatman, Midges, and Mayflies) (TTF^{TL2})	2.14
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{8.75}{5.00}$$

= 1.75 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

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

= 1.03 x 2.14

= 2.20

$$C_{water} = \frac{15.1}{2.20 \times 1.75 \times 2.13}$$

= 1.84 µg/L

1.4.4 Example 4

Fathead minnow (*Pimephales promelas*) in a river that consume approximately ⅔ copepods and ⅓ aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods and aquatic insects (TTF^{TL2}) Copepods = 1.41 Average of all aquatic insects = 2.14 $TTF^{TL2} = \sum_{i=1}^{n}(TTF_i \times w_i)$ $= (1.41 \times ⅔) + (2.14 \times ⅓)$ $= 1.65$	1.65
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

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

= 1.57 × 1.65

= 2.59

$$C_{water} = \frac{15.1}{2.59 \times 0.85 \times 1.40}$$

= 4.90 µg/L

1.5.5 Example 5

Flathead chub (*Platygobio gracilis*) in a river with a diet of approximately 80% aquatic insects and 20% algae:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of flathead chub: Lowest matching taxon is the family Cyprinidae. Therefore, the TTF value of Cyprinidae is used (TTF^{TL3})	1.20
Trophic transfer factor for insects (TTF^{TL2}) Average of all aquatic insects = 2.14	2.14
Egg-ovary to whole-body conversion factor for flathead chub (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	1.95
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times w_2]$$

Where:
w_1 = Proportion of fathead chub diet from insects; and
w_2 = Proportion of fathead chub diet from algae

$$TTF^{comb} = [1.20 \times 2.14 \times 0.8] + [1.20 \times 0.2]$$
= 2.29

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{2.29 \times 0.85 \times 1.95}$$

= 3.98 µg/L

1.5.6 Example 6

Largemouth bass (*Micropterus salmoides*) in a large river that consume mostly Western mosquitofish (*Gambusia affinis*) that consume approximately ¾ insects and ¼ crustaceans:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of largemouth bass (TTF^{TL4})	1.39
Trophic transfer factor of Western mosquitofish (TTF^{TL3})	1.21
Trophic transfer factor for insects and crustaceans (TTF^{TL2}) Median all Insects – 2.14 Median all Crustaceans – 1.41 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i^{TL2} w_i\right)$ = (2.14 x 0.75) + (1.41 x 0.25) = 1.96	1.96
Egg-ovary to whole-body conversion factor for largemouth bass (species-specific value not available, so median CF for genus Micropterus is used) (CF)	1.42
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$
$= 1.39 \times 1.21 \times 1.96$
$= 3.30$

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{3.30 \times 0.85 \times 1.42}$$

= 3.79 µg/L

2.0 TRANSLATING THE CONCENTRATION OF SELENIUM IN TISSUE TO A CONCENTRATION IN WATER USING BIOACCUMULATION FACTORS (BAF)

2.1 Summary of the BAF Approach

A bioaccumulation factor (BAF) is the ratio (in milligrams/kilogram per milligrams/liter, or liters per kilogram) of the concentration of a chemical in the tissue of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). BAFs are used to relate chemical concentrations in aquatic organisms to concentrations in the ambient media of aquatic ecosystems where both the organism and its food are exposed and the ratio does not change substantially over time. The BAF is expressed mathematically as:

$$BAF = \frac{C_{tissue}}{C_{water}} \qquad \textit{(Equation K-8)}$$

Where:

BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)
C_{tissue}	=	concentration of chemical in fish tissue (mg/kg)
C_{water}	=	ambient concentration of chemical in water (mg/L)

The site-specific BAF can then be applied to the tissue criterion to solve for a target site-specific water column criterion (C_{target}):

$$C_{target} \times \frac{C_{egg-ovary\ criterion}}{BAF} \qquad \textit{(Equation K-9)}$$

Where:

C_{target}	=	site-specific water criterion concentration (mg/L)
$C_{egg\text{-}ovary\ criterion}$	=	national egg-ovary tissue criterion (15.1 mg Se/kg dw)
BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)

To translate a fish tissue criterion to a water concentration value, a site-specific, field-measured BAF for the waterbody could be developed, and then a water concentration criterion could be calculated using Equation K-9. Detailed information about how to derive a site-specific, field-measured BAF is provided in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume 3: Development of Site-specific Bioaccumulation*

Factors (U.S. EPA 2009). Although this guidance was developed for deriving human health criteria, the methodological approach is also applicable to the derivation of aquatic life criteria. The following example illustrates the calculation of a site specific water column criterion using the BAF approach.

2.1.1 Example: Derivation of a site specific water column criterion for a waterbody impacted by selenium

Available data for a hypothetical site indicate that the average egg/ovary tissue concentration of selenium for the bluegill (*Lepomis macrochirus*) is 22 mg/kg (dw). This concentration exceeds the USEPA proposed egg/ovary criterion of 15.1 mg/kg (dw). The ambient selenium water column concentration at that hypothetical site is 4.0 µg/L. The following calculation shows how to derive a target water column that would achieve a site-specific criterion using the bioaccumulation factor (BAF) approach.

Site specific selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.1
Ambient selenium water column concentration (µg/L)	4.0
Target water column concentration (µg/L)	X

Set up proportional equation to solve for allowable water column concentration:

$$\frac{Site\ specific\ egg/ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Site\ specific\ water\ concentration\ (\frac{\mu g\ Se}{L})} = \frac{Criterion\ egg\ ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Solve for the target water concentration that will achieve a site-specific criterion:

$$\frac{22.0\ (\frac{mg\ Se}{kg\ dw})}{4.0\ (\frac{\mu g\ Se}{L})} = \frac{15.1\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Target water concentration = 2.75 µg/L.

2.2 Managing Uncertainty using the BAF Approach

Uncertainty can be introduced when using the BAF approach to derive a water concentration value from a fish tissue criterion concentration. Inaccurate water concentration values can result when BAFs are derived from water and fish tissue concentration measurements that are obtained from sources that do not closely represent site characteristics, or from field data collected from large-scale sites that encompass multiple water bodies or ecosystems. Most of this uncertainty results from differences in the

bioavailability of selenium between the study sites where measurements are made to derive the BAF, and the site(s) to which the BAF is used to derive needed water concentration values.

Because of uncertainties associated with the BAF approach, EPA does not recommend developing BAFs from data extrapolated from different sites or across large spatial scales. EPA's Framework for Metals Risk Assessment (U.S. EPA 2007) outlines key principles about metals and describes how they should be considered in conducting human health and ecological risk assessments due the the effects of water chemistry on bioavilability of such chemicals. The current science does not support the use of a single, generic threshold BAF value as an indicator of metal bioaccumulation. The use of BAFs are appropriate only for site-specific applications where sufficient measurements have been taken from the site of interest and there is little or no extrapolation of BAF values across differing exposure conditions and species.

The preferred approach for using a BAF to implement the selenium fish tissue criterion is to calculate a site-specific, field-measured BAF from data gathered at the site of interest, and to apply that BAF to that site. A site-specific, field-measured BAF is a direct measure of bioaccumulation in an aquatic system because the data are collected from the aquatic ecosystem itself and thus reflects real-world exposure through all relevant exposure routes. A site-specific, field-measured BAF also reflects biotic and abiotic factors that influence the bioavailability, biomagnification, metabolism, and biogeochemical cycling of selenium that might affect bioaccumulation in the aquatic organism or its food web. Appropriately developed site-specific, field-measured BAFs are appropriate for all bioaccumulative chemicals, regardless of the extent of chemical metabolism in biota from a site (U.S. EPA 2000).

Although a site-specific, field-measured BAF is a direct measure of bioaccumulation, its predictive power depends on a number of important factors being properly addressed in the design of the field sampling effort. For example, sampling in areas with relatively long water residence times should be a priority because selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as wetlands, oxbows, and estuaries) and with fine particulate sediments high in organic carbon. In addition, migratory species should generally not be used because their exposure to selenium could reflect selenium concentrations in areas other than where the fish were caught. Fish may also need to be sampled and BAF values recalculated if selenium levels significantly change over time because BAFs are known to be affected by the ambient concentration of the metals in the aquatic environment (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). States and tribes should refer to *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume* (U.S. EPA 2009) for guidance on appropriate methods for developing a site-specific, field-derive BAF.

The advantage of using the BAF approach is its relative simplicity, especially when the data necessary to derive the BAF is already available. Furthermore, the BAF approach is completely empirical and does not require any specific knowledge about the physical, chemical, or biological characteristics of the waterbody. The relationship between the concentration of selenium in fish tissue and water is directly determined by direct measurements in these media. This may be advantageous when there are uncertainties with how to collect a particulate sample that is representative of the base of the food web, or dilution concerns (e.g., sandy streams with little surface area for algae sampling and high potential for sand contamination of a benthic sediment sample).

Limitations of the BAF approach should be considered before deciding if this method is appropriate for translating the selenium FCV to a water concentration value. One disadvantage of the BAF approach is the considerable effort and resources necessary to collect sufficient data to establish the relationship between tissue and water concentrations. Resource use increases as the spatial scale and complexity of the aquatic system increases. Furthermore, the BAF approach does not allow extrapolation across species, space, and large time scales because the site-specific factors that might influence bioaccumulation are integrated within the tissue concentration measurements and thus cannot be individually adjusted to extrapolate to other conditions. Thus, site-specific, field-measured BAFs only provide an accounting of the uptake and accumulation of selenium for an organism at a specific site and point in time. This is more important in lotic habitats, since the kinetics of selenium bioaccumulation may be very different at a site upstream or downstream from the site of interest.

As noted previously, NPDES permitting regulations at 40 CFR § 122.45(c) require WQBELs for metals be expressed as total recoverable metal unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Guidance for converting expression of metals in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996). Whether or not a water concentration value derived from a site-specific, field-derived BAF requires conversion from dissolved to total recoverable selenium depends on how the BAF is developed. Generally, conversion would not be necessary if the BAF is derived from water concentration values that measure total selenium; however, conversion would be necessary if the BAF was derived from water concentration values that measured dissolved selenium. Table K-4 compares some of the principle characteristics of the mechanistic bioaccumulation modeling approach or the BAF approach for translating the selenium FCV to a water concentration.

3.0 COMPARISON OF MECHANISTIC BIOACCUMULATION MODELING AND BAF APPROACHES

Data from Saiki et al. (1993) are used here to illustrate an example comparison of the two translation approaches, the mechanistic bioaccumulation modeling approach and the bioaccumulation factor (BAF) model approach. Definitive selenium measurements for all ecosystem compartments (e.g., water, algae, etc.) are available for two species, bluegill and largemouth bass, at four sites. Food web pathways were calculated using results of gut content analysis. Although Saiki et al. (1993) satisfies the minimum requirements for a site specific translation, it represents a sparse dataset, with only two measurements per ecosystem compartment, one for the spring and fall of 1987, respectively. For purposes of this exercise, samples from the same site collected at different time periods will be treated as replicate data; however, EPA recommends using larger sample sizes collected during the same time period when calculating a site specific criterion.

Selenium data used to calculate site specific water criteria are included in Table K-4. Median concentrations and coefficients of variation for each ecosystem compartment at each site are included in Table K-5. Because at most only two concentrations were available for each ecosystem, site median are equal to site averages. Site specific translations for both approaches will be calculated using median selenium concentrations.

Table K-4. Selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw.

Site	Date	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	Fall 1987	3	7.40	22	4.6	8.9	5.2	2.4	6.4	6.8
Mud Slough at Gun Club Road	Spring 1987	9	1.60	7.9	3.3	7.2	4.4	5.4	5	6.9
Salt Slough at the San Luis National Wildlife Refuge	Fall 1987	3	0.38	8.9	3.4	5.4	3.1	4.5	4.5	4.7
Salt Slough at the San Luis National Wildlife Refuge	Spring 1987	13	2.40	7.9	3.7	6.9	3.2	4.4	4.3	4
San Joaquin R. above Hills Ferry Road	Fall 1987	3	1.20	6.6	3.8	6	1.7	2.6	3.3	2.2
San Joaquin R. above Hills Ferry Road	Spring 1987	11	1.30	3.4	2.8	4.1	1.9	4.3	2.7	2.4
San Joaquin R. at Durham Ferry State Recreation Area	Fall 1987	1	0.39	1.2	1.5	1.5	0.77	1.6	2	1.8
San Joaquin R. at Durham Ferry State Recreation Area	Spring 1987		0.50	1.3	1.1	1.6	1.3	1.8	1.9	1.7

Table K-5. Median selenium concentrations in ecosystem compartments for four sites described in Saiki et al. (1993).
For purposes of this exercise, spring and fall samples measured at the same site are treated as replicates. Water concentrations expressed as µg/L, all other concentrations expressed as mg/kg dw. Coefficients of determination included in parentheses.

Site	Water	Algae	Detritus	Amphipod	Chironomid	Crayfish	Zooplankton	Bluegill	Largemouth Bass
Mud Slough at Gun Club Road	6.0 (0.71)	4.50 (0.91)	14.95 (0.67)	3.95 (0.23)	8.05 (0.15)	4.80 (0.12)	3.90 (0.54)	5.70 (0.17)	6.85 (0.01)
Salt Slough at the San Luis National Wildlife Refuge	8.0 (0.88)	1.39 (1.03)	8.40 (0.08)	3.55 (0.06)	6.15 (0.17)	3.15 (0.02)	4.45 (0.02)	4.40 (0.03)	4.35 (0.11)
San Joaquin R. above Hills Ferry Road	7.0 (0.81)	1.25 (0.06)	5.00 (0.45)	3.30 (0.21)	5.05 (0.27)	1.80 (0.08)	3.45 (0.35)	3.00 (0.14)	2.30 (0.06)
San Joaquin R. at Durham Ferry State Recreation Area	1.0 (na)	0.45 (0.17)	1.25 (0.06)	1.30 (0.22)	1.55 (0.05)	1.04 (0.36)	1.70 (0.08)	1.95 (0.04)	1.75 (0.04)

3.1 Translation using the BAF Approach

Site specific BAFs were calculated for bluegill and largemouth bass at each of the four sites (Table K-6). A site-specific water criterion was calculated for each species at the four sites using equation K-8, which is equivalent to the BAF example shown in the previous section. The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$BAF = \frac{C_{tissue}}{C_{water}} = \frac{4.4\ \mu g/g}{8\ \mu g/L} = 0.55\ L/g$$

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{BAF} = \frac{8.5\ mg/kg}{0.55\ L/g} = 15.5\ \mu g/L$$

The whole body tissue criterion of 8.5 mg/kg is used here because whole body fish tissue selenium measurements were made. If site specific egg ovary fish tissue had been measured, then the egg ovary tissue criterion of 15.1 mg/kg would have been used.

Table K-6. Site and species specific translated water concentrations using the BAF translation approach.

		Bluegill:			**Largemouth Bass:**		
Site	**Water (µg/L)**	**WB Se (mg/kg)**	**BAF (L/g)**	**Water SSC[a] (µg/L)**	**WB Se (mg/kg)**	**BAF (L/g)**	**Water SSC[a] (µg/L)**
Mud Slough at Gun Club Road	6.0	5.70	0.95	8.95	6.85	1.14	7.45
Salt Slough at the San Luis National Wildlife Refuge	8.0	4.40	0.55	15.45	4.35	0.54	15.63
San Joaquin R. above Hills Ferry Road	7.0	3.00	0.43	19.83	2.30	0.33	25.87
San Joaquin R. at Durham Ferry State Recreation Area	1.0	1.95	1.95	4.36	1.75	1.75	4.86

a – Site specific criterion based on BAF for respective species.

At each site, the lowest translated water criterion for all species is used as the site specific criterion. At site "Mud Slough at Gun Club Road," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the BAF approach range from 4.4 to 19.8 µg/L Table K-6).

3.2 Translation using the Mechanistic Bioaccumulation Modeling Approach

The first step in the bioaccumulation modeling approach is the calculation of site specific enrichment factors (*EF*s). Because both algae and detritus selenium concentrations were available, the first step was the calculation of separate *EF*s for algae and detritus at each site, following the procedures described in section 1.2.4.1. Algal and detrital *EF*s, respectively, were calculated using the median of all Se concentrations in algae (or detritus) at a site by the median of all Se concentrations in water at the same site. After calculating separate algal and detrital *EF*s, the final *EF* at each site was calculated as the geometric mean of the algal and detrital *EF* at a given site. Algal, detrital, and site *EF*s are shown in Table K-7.

Table K-7. Se concentrations in water, algae, detritus, and site specific *EF*s.

Site	Water (µg/L)	Algae (mg/kg)	Detritus (mg/kg)	EF (L/g)
Mud Slough at Gun Club Road	6.0	4.50	14.95	1.37
Salt Slough at the San Luis National Wildlife Refuge	8.0	1.39	8.40	0.43
San Joaquin R. above Hills Ferry Road	7.0	1.25	5.00	0.36
San Joaquin R. at Durham Ferry State Recreation Area	1.0	0.45	1.25	0.75

As an example, the EF calculation for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$EF_{algae} = \frac{C_{algae}}{water}\ ;\ EF_{detritus} = \frac{C_{detritus}}{water}$$

$$EF_{site} = \sqrt{\left(EF_{algae} \times EF_{detritus}\right)}$$

$$EF_{algae} = \frac{1.39\ mg/kg}{8.0\ \mu g/L};\ EF_{detritus} = \frac{8.4\ mg/kg}{8.0\ \mu g/L}$$

$$EF_{site} = \sqrt{(0.17 \times 1.05)}$$

$$EF_{site} = 0.43\ L/g$$

The second step in the bioaccumulation modeling approach is the calculation of site specific composite trophic transfer factors ($TTF^{composite}$). Based on gut content analysis, bluegill diets consisted of

47% amphipods, 23% chironomids, and 30% zooplankton, while largemouth bass diets consisted of 73% bluegill and 27% crayfish.

The composite TTF for bluegill was calculated using the following equation:

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times TTF^{TL2} \times w_2] + [TTF^{TL3} \times TTF^{TL2} \times w_3]$$

Where:

W_1 = proportion of diet from amphipods,

W_2 = proportion of diet from chironomids, and

W_3 = proportion of diet from zooplankton.

For each of the 3 species in the bluegill diet, site specific TTF^{TL3} and TTF^{TL2} were calculated separately. Using median concentrations from Table K-5, $TTF^{composite}$ were calculated for each of the sites and are included in Table K-8.

Table K-8. Trophic transfer factors (TTFs) for bluegill and bluegill prey.

	TL2 TTFs:			**TL3 TTFs:**			**$TTF^{composite}$:**
Site	**Amphipod**	**Chironomid**	**Zooplankton**	**BG-Amph**	**BG-Chiro**	**BG-Zoo**	**Bluegill**
Mud Slough at Gun Club Road	0.41	0.83	0.40	1.44	0.71	1.46	0.59
Salt Slough at the San Luis National Wildlife Refuge	0.73	1.26	0.91	1.24	0.72	0.99	0.90
San Joaquin R. above Hills Ferry Road	1.06	1.62	1.10	0.91	0.59	0.87	0.96
San Joaquin R. at Durham Ferry State Recreation Area	1.53	1.83	2.01	1.50	1.26	1.15	2.30

As an example, the bluegill $TTF^{composite}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

$$TTF^{composite} = [1.24 \times 0.73 \times 0.47] + [0.72 \times 1.26 \times 0.23] + [0.99 \times 0.91 \times 0.30]$$

$$TTF^{composite} = 0.90$$

The composite TTF for largemouth bass was calculated using the following equation:

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

Where:

W_1 = proportion of diet from bluegill, and

W_2 = proportion of diet from crayfish

For the proportion of the largemouth bass diet consisting of bluegill, TTF^{TL3} x TTF^{TL2} was equal to the $TTF^{composite}$ for bluegill. As was the case for bluegill, site specific TTFs were calculated for each species, and are included in Table K-9.

Table K-9. Trophic transfer factors (TTFs) for largemouth bass and largemouth bass prey.

	Crayfish dietary fraction:		Bluegill dietary fraction:		$TTF^{composite}$:
Site	**Crayfish**	**LMB-Cray**	**Bluegill[a]**	**LMB-BG**	**LMB**
Mud Slough at Gun Club Road	0.49	1.43	0.59	0.70	0.49
Salt Slough at the San Luis National Wildlife Refuge	0.64	1.38	0.90	0.89	0.82
San Joaquin R. above Hills Ferry Road	0.58	1.28	0.96	0.74	0.71
San Joaquin R. at Durham Ferry State Recreation Area	1.22	1.69	2.30	2.06	4.03

a – $TTF^{composite}$ for bluegill.

As an example, the largemouth bass $TTF^{combined}$ for site "Salt Slough at the San Luis National Wildlife Refuge" is shown below.

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

$$TTF^{composite} = [0.89 \times 0.90 \times 0.73] + [1.38 \times 0.64 \times 0.27]$$

$$TTF^{composite} = 0.82$$

After calculating site and species specific EF and $TTF^{combined}$, site specific water criterion concentrations were calculated using a modified version of equation K-1, shown below.

$$C_{water\ criterion} = \frac{C_{tissue\ criterion}}{EF\ x\ TTF^{composite}}$$

The site specific criterion calculation for bluegill at site "Salt Slough at the San Luis National Wildlife Refuge" is included below as an example.

$$C_{water\ criterion} = \frac{8.5\ mg/kg}{0.43\ L/g\ x\ 0.90} = 22.1\ \mu g/L$$

Because the selenium in fish tissue at these sites were measured as whole body concentrations, the whole body criterion of 8.5 µg/L is used, and an egg-ovary to whole body conversion factor is not required. As with the BAF approach, the lowest translated water criterion for all species is used as the site specific criterion. At site "San Joaquin R. at Durham Ferry State Recreation Area," the site specific criterion is based on the translated concentration for largemouth bass, and at the other 3 sites, the site specific criterion is based on the translated concentration for bluegill. Site specific water concentrations calculated using the mechanistic bioaccumulation modeling approach are more variable than concentrations calculated using the BAF approach, and range from 2.8 to 33.3 µg/L Table K-10). At all sites using both methods, the translated site specific water concentration criteria were higher than the measured water concentrations.

Table K-10. Site and species specific translated water concentrations using the mechanistic bioaccumulation modeling approach.

		Bluegill:			**Largemouth Bass:**		
Site	**EF (L/g)**	**WB Se (mg/kg)**	**TTF**	**Water SSC (µg/L)**	**WB Se (mg/kg)**	**TTF**	**Water SSC (µg/L)**
Mud Slough at Gun Club Road	1.37	5.70	0.59	10.61	6.85	0.49	12.65
Salt Slough at the San Luis National Wildlife Refuge	0.43	4.40	0.90	22.14	4.35	0.82	24.18
San Joaquin R. above Hills Ferry Road	0.36	3.00	0.96	24.79	2.30	0.71	33.31
San Joaquin R. at Durham Ferry State Recreation Area	0.75	1.95	2.30	4.95	1.75	4.03	2.83

3.3 Summary Comparison of the Mechanistic Bioaccumulation and BAF Approaches

A comparison of the mechanistic bioaccumulation and BAF approaches is included in Table K-11.

Table K-11. Comparison of mechanistic bioaccumulation and BAF approaches.

Mechanistic bioaccumulation modeling	Bioaccumulation Factor (BAF)
Knowledge of the aquatic system needed	No information on aquatic system needed
Choice of input parameters at discretion of state or tribe	No input parameters to choose
Species-specific	Species-specific
Can be applied at different sites if site *EF* can be estimated.	Site-specific
Fish tissue sampling not required for translation	Fish tissue and water sampling required

APPENDIX L: ANALYTICAL METHODS FOR MEASURING SELENIUM

The Clean Water Act (CWA) establishes an EPA approval process for certain analytical methods used in the National Pollutant Discharge Elimination System (NPDES) program and for section 401 certifications. EPA has several approved methods for measuring selenium in water under 40 CFR § 136. EPA generally requires the use of EPA-approved methods for the NPDES program and for CWA section 401 certifications issued by states and tribes (40 CFR § 136.1). However, since there are no EPA approved methods for the analysis of selenium in fish tissue, states and tribes may use analytical methods not approved by EPA to evaluate the attainment of water quality standards or to develop or implement Total Maximum Daily Loads provided that these methods are scientifically sound (40 CFR 122.21(g)(7)).

Implementation of a water quality standard for selenium may require the ability to detect and measure the concentration of selenium in effluent, ambient water, tissue, and other media that is below the detection limit or limit of quantitation that some analytical methods can provide. States and tribes should choose an analytical method that is sufficiently sensitive to implement its water quality standard for selenium. Below are descriptions of some of the methods available for measuring selenium concentrations with sufficient sensitivity to implement EPA's recommended selenium criterion. Complete descriptions of analytical methods appropriate for analyzing selenium in different media can be found in the National Environmental Methods Index at http://www.nemi.gov.

1.0 General Considerations when Measuring Concentrations of Selenium

The oxidation states of selenium dissolved in surface water are usually selenate (+6), selenite (+4), and organo-selenium (-2). The presence of selenium in different oxidation states complicates some analytical methods (Presser and Ohlendorf 1987). EPA recommends using standard reference samples to check for the percentage recovery of each species of selenium (selenate, selenite and organo-selenium) during initial testing of selenium methodologies for quality control and assurance.

If water samples are not filtered, particulate species such as elemental selenium and particulate organo-selenium will also be measured. In addition, federal regulations at 40 CFR §122.45(c) generally requires considering total recoverable metals when establishing effluent limits and reporting requirements.

2.0 Analytical Methods Recommended for Measuring Selenium in Water

EPA has several approved analytical methods under 40 CFR § 136 specifically for measuring total selenium in water. These regulations state that measurements for NPDES permit applications and permittee reporting should be made using analytical methods approved by EPA. Because EPA has

approved methods for analyzing selenium in water, these methods must be used for NPDES permits (40 CFR § 122.21(g)(7), 122.41(j), 136.1, 136.3, and 136.6).

A complete list of EPA-approved analytical methods for selenium can be found at: http://www.epa.gov/waterscience/methods/method/. Three EPA-approved methods that may be sufficiently sensitive[4] for the purposes of implementing a selenium water quality criterion are listed below (Table L-1).

Table L-1. Suggested EPA-Approved Methods for Selenium in Water

Method	Technique	Method detection limit
American Public Health Standard Method 3114 B (2009) or 3114 C (2009)	Hydride generation atomic absorption spectrometry (HG-AAS)	2 µg/L
EPA Method 200.8, Rev 5.4 (1998)	Inductively coupled plasma mass spectrometry (ICP-MS)	7.9 µg/L
EPA Method 200.9, Rev.2.2 (1994)	Stabilized temperature graphite furnace atomic absorption (STGF-AA)	0.6 µg/L

2.1 American Public Health Standard Method 3114 B

For measuring selenium in water, American Public Health Standard Method 3114 B uses the HG-AAS technique. Method 3114 B has a method detection limit (MDL) of 2 µg/L. Samples for dissolved analytes should be filtered on-site through 0.45-micron capsule filters certified free of trace-element contamination or other appropriate filtering equipment (Wilde et al. 1999). Dissolved samples should be preserved with high purity hydrochloric acid or nitric acid to a pH less than 2.

For measuring total selenium, samples should not be filtered. In addition, all selenium in the sample should be in the form of selenite (+4). Thus, the following pre-treatment steps to convert all selenium in the sample to selenite are critical when using the HG-AAS method:

1. Boiling with persulfate to oxidize and digest organic material.
2. Boiling with hydrochloric acid to reduce selenate species to selenite.
3. Reduction by sodium borohydride to hydrogen selenide in the quartz tube of the AAS.

[4]For more information on choosing a sufficiently sensitive method, see the memorandum *Analytical Methods for Mercury in National Pollutant Discharge Elimination System (NPDES) Permits* from James A. Hanlon, Director of the Office of Wastewater Management, dated August 23, 2007, available at http://www.epa.gov/npdes/pubs/mercurymemo_analyticalmethods.pdf.

Optimal conversion conditions are essential for accurate results because too mild a reduction could lead to incomplete reduction of selenate and too rigorous a reduction could lead to plating out of elemental selenium (Cutter 1987, 1983; Presser and Barnes 1984, 1985).

Method 3114 B has the advantage that it is a fully validated method, is commonly used by many laboratories, is relatively inexpensive, is less susceptible to background interference (Cutter 1987, 1983; Presser and Barnes 1984, 1985), and has sufficient sensitivity to accurately measure what can be expected in many lotic aquatic systems. However, this method may not be sufficiently sensitive for some lentic aquatic systems where relatively lower selenium concentrations may need to be measured. If no selenium is detected in a lentic system using this method, EPA recommends using a more sensitive analytical method.

2.2 EPA Method 200.8

EPA method 200.8 has a MDL of 7.9 µg/L using the ICP-MS analytical technique. This method has the advantage that no pre-treatment steps are necessary. However, this method may not be sufficiently sensitive in many applications of the selenium criterion (Lamothe et al. 1999). If no selenium is detected using this method, EPA recommends monitoring with a more sensitive method.

2.3 EPA Method 200.9

Method 200.9 has a MDL of 0.6 µg/L using the STGF-AA analytical technique. This method has the advantage that it can detect selenium at very low concentrations. However, graphite furnace techniques require careful matrix matching.

Of these three EPA approved methods, Method 3114B using the HG-AAS technique is the most cost-effective, with sufficient sensitivity and relatively low risk of interference in most cases. EPA Method 200.8 may be used where appropriate, such as when selenium concentrations in effluent are known to be higher than 7.9 µg/L. EPA Method 200.9 may be used if a very low MDL is needed. Some additional methods not approved by EPA that states and tribes might consider are:

- Collision/Reaction Cell Inductively Coupled Plasma Mass Spectroscopy (cICP-MS) (Garbarino et al. 2005) - A relatively new technique that is a sensitive and selective detector for metal analysis. However, isobaric interference can cause problems for quantitative determination as well as identification based on the analyte pattern. Collision cells, reaction cells or other interfaces reducing sample matrix effects that might otherwise interfere in the mass selective determinative step are allowed in CWA analyses provided the method performance specifications relevant to ICP-MS measurements are met

- Fluorometric Analysis - a wet chemistry technique using diaminonapthalene. This method also achieves acceptable precision and accuracy on standard reference samples (Olson 1969; Olson et al. 1975; American Public Health Association Standard Method 3500, on-line version).

Methods for measuring different species of selenium dissolved in water are also available. These methods determine the species of dissolved selenium present in a sample through differential digestion and hydride generation atomic adsorption spectrophotometry (Cutter 1978, 1983; Presser and Barnes, 1984; 1985; May et al. 2007). Selenite can be measured in samples with no pre-treatment. Selenate plus selenite can be measured in samples subjected to boiling with hydrochloric acid. Subtraction of the measured selenite fraction from the measured combined fraction would yield a measure of the selenate fraction. If a sample is analyzed to measure total dissolved selenium as described above, then measurements of the combined fraction can be subtracted to yield measurements of the dissolved organo-selenium fraction.

3.0 ANALYTICAL METHODS AVAILABLE FOR MEASURING SELENIUM IN FISH TISSUE

EPA does not have approved methods under 40 CFR § 136 for measuring selenium in fish tissue. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in fish tissue if the samples are made soluble. Tissue samples are homogenized and digested prior to analysis using strong acid or dry-ashing digestion as described below. A review of sample digestion techniques has been published (Ihnat 1992). Standard reference materials, analytical duplicates, and matrix spike samples are recommended to determine the applicability of a selected digestion procedure.

3.1 Strong acid digestion

Solid samples can be subjected to strong acid digestion to break down mineral and organic matrices. Samples are typically dried and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Note that some strong acid digestion methods may not be suitable for fish tissue. Strong acid digestion methods are categorized by the type of material or amount of organic material present (e.g., solid waste; biological tissue, plants, soil, sediment, rock, coal) and degrees of tissue solubilization needed (extraction, leachate, or complete destruction). Methods differ in acid mixture and degree and type of heating (EPA Method 3050B, Revision 2, 1996; EPA Method 200.2,

Revision 2.8, 1994; Briggs and Crock, 1986; Taggart, 2002, chapters I, J, and K). High boiling acids (perchloric and sulfuric) may lead to a loss of selenium if solutions are heated to dryness.

3.2 Dry-ashing digestion

Dry-ashing digestion is applicable to biological samples (Brumbaugh and Walther, 1989; May et al., 2007). Biological samples are normally lyophilized (freeze-dried) and homogenized before digestion. Determination of percent moisture may be part of the drying procedure. Dried solid samples are:

1. Boiled in nitric acid for solubilization and oxidation
2. Ashed at 500º C with magnesium nitrate to complete oxidation and decompose remaining organic material
3. Heated with hydrochloric acid to dissolve the ash and reduce selenium to the selenite (+4) state required for detection by HG-AAS.

3.3 Analytical methods available for measuring selenium in particulate material

There are no 40 CFR § 136 methods for analyzing selenium in particulate material. However, states and tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or reports.

The techniques described above for analyzing selenium in water (HG-AAS, ICP-MS, and STGF-AA) can be used to measure selenium in particulate material after the sample has been separated from the water and pre-treated using the same methods used for fish tissue. In order to obtain a particulate material sample, a water column sample should be filtered to separate the particulate material and bed sediment. Various techniques for collection of suspended particulate material using filtration are available from the EPA (e.g. Method 1669) and the U.S. Geological Survey (Moulton et al. 2002; USGS, Britton and Greeson 1987). These techniques include:

- EPA Method 1669 (1996) includes filtration through a 0.45 µm capsule filter at the field site.
- USGS protocols for collection of phytoplankton and seston in rivers and streams as part of their National Water Quality Assessment Program for watershed and habitat assessment (http://water.usgs.gov/nawqa/protocols.html).
- Textbooks such as *Limnological Analyses* address sampling of lakes using traditional techniques including phytoplankton nets. (Wetzel and Likens 1991).
- Sampling of suspended material from estuaries where particulates are a substantial part of the ecosystem is described in Doblin et al. (2005) as part of their work on the San Francisco Bay-Delta Estuary.
- Separating suspended sediment using high-speed centrifugation and decantation when the concentration of particulate material is relatively low (Horowitz et al. 1989).

APPENDIX M: ABBREVIATIONS

REFERENCE AND SITE ABBREVIATIONS

Reference	Site		Species	
Bi:	22	Miller's Lake, Wellington CO	FM	Fathead minnow
Birkner 1978	27	Sweitzer Lake, Delta CO	FM	Fathead minnow
	23	Twin Buttes Reservoir, Laramie WY	FM	Fathead minnow
	20	East Allen Reservoir, Medicine Bow WY	ID	Iowa darter
	7	Galett Lake, Laramie WY	ID	Iowa darter
	22	Miller's Lake, Wellington CO	ID	Iowa darter
	23	Twin Buttes Reservoir, Laramie WY	ID	Iowa darter
	30	Larimer Highway 9 Pond, Fort Collins CO	NPK	Northern plains killfish
	3	Meeboer Lake, Laramie WY	NPK	Northern plains killfish
	27	Sweitzer Lake, Delta CO	NPK	Northern plains killfish
	23	Twin Buttes Reservoir, Laramie WY	NPK	Northern plains killfish
Bu91:	4	Uncompahgre River at Colona	BhS	Bluehead sucker
Butler et al. 1991	4	Uncompahgre River at Colona	BnT	Brown trout
	4	Uncompahgre River at Colona	FS	Flannelmouth sucker
	4	Uncompahgre River at Colona	MS	Mottled sculpin
	4	Uncompahgre River at Colona	RT	Rainbow trout
	4	Uncompahgre River at Colona	WS	White sucker
Bu93:	SP2	Spring Creek at La Boca	BhS	Bluehead sucker
Butler et al. 1993	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BT	Brown trout
	SP2	Spring Creek at La Boca	BT	Brown trout
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	BB	Black bullhead
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	ChC	Channel catfish
	N2	Navajo Reservoir, Piedra R. Arm, near La Boca	CC	Common carp

Reference	Site		Species	
	SP2	Spring Creek at La Boca	FM	Fathead minnow
	SP2	Spring Creek at La Boca	SD	Speckled dace
	SP2	Spring Creek at La Boca	WS	White sucker
Bu95:	ME2	McElmo Cr., downstream from Alkali Canyon	BhS	Bluehead sucker
Butler et al. 1995	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BhS	Bluehead sucker
	NW	Navajo Wash near Towaoc	BhS	Bluehead sucker
	SJ1	San Juan R. at Four Corners	BhS	Bluehead sucker
	SJ3	San Juan R. at Mexican Hat Utah	BhS	Bluehead sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	BB	Black bullhead
	SJ1	San Juan R. at Four Corners	ChC	Channel catfish
	SJ3	San Juan R. at Mexican Hat Utah	ChC	Channel catfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	CC	Common carp
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	CC	Common carp
	SJ1	San Juan R. at Four Corners	CC	Common carp
	SJ3	San Juan R. at Mexican Hat Utah	CC	Common carp
	HD2	Hartman Draw near mouth, at Cortez	FM	Fathead minnow
	ME1	McElmo Cr. at Hwy. 160, near Cortez	FM	Fathead minnow
	ME2	McElmo Cr., downstream from Alkali Canyon	FM	Fathead minnow
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FM	Fathead minnow
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FM	Fathead minnow
	WC	Woods Canyon near Yellow Jacket	FM	Fathead minnow
	SJ1	San Juan R. at Four Corners	FS	Flannelmouth sucker
	HD2	Hartman Draw near mouth, at Cortez	FS	Flannelmouth sucker
	ME2	McElmo Cr., downstream from Alkali Canyon	FS	Flannelmouth sucker
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	FS	Flannelmouth sucker
	SJ3	San Juan R. at Mexican Hat Utah	FS	Flannelmouth sucker
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	GnS	Green sunfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	RSh	Red shiner
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	RSh	Red shiner

Reference	Site		Species	
	SJ1	San Juan R. at Four Corners	RSh	Red shiner
	ME1	McElmo Cr. at Hwy. 160, near Cortez	SD	Speckled dace
	ME2	McElmo Cr., downstream from Alkali Canyon	SD	Speckled dace
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	SD	Speckled dace
	NW	Navajo Wash near Towaoc	SD	Speckled dace
	SJ1	San Juan R. at Four Corners	SD	Speckled dace
	HD2	Hartman Draw near mouth, at Cortez	Su	Sucker
Bu97:	MUD2	Mud Cr. at Hwy. 32, near Cortez	BhS	Bluehead sucker
Butler et al. 1997	MNP2	Large pond south of G Road, southern Mancos Valley	FM	Fathead minnow
	MUD2	Mud Cr. at Hwy. 32, near Cortez	FM	Fathead minnow
	WCP	Pond on Woods Canyon at 15 Road	FM	Fathead minnow
	CH1	Cahone Canyon at Hwy. 666	GnS	Green sunfish
	MUD2	Mud Cr. at Hwy. 32, near Cortez	GnS	Green sunfish
	MNP3	Pond downstream from site MNP2, southern Mancos Valley	SB	Smallmouth bass
Ca:	DC	Deerlick Creek	RT	Rainbow trout
Casey **2005**	LC	Luscar Creek	RT	Rainbow trout
Fo:	CC-1A	Crow Creek – 1A	BnT	Brown trout
Formation 2012	CC-3A	Crow Creek – 3A	BnT	Brown trout
	CC-150	Crow Creek – 150	BnT	Brown trout
	CC-350	Crow Creek – 350	BnT	Brown trout
	CC-75	Crow Creek – 75	BnT	Brown trout
	DC	Deer Creek	BnT	Brown trout
	HS	Hoopes Spring	BnT	Brown trout
	HS-3	Hoopes Spring – 3	BnT	Brown trout
	LSV-2C	Sage Creek – 2C	BnT	Brown trout
	LSV-4	Sage Creek – 4	BnT	Brown trout
	SFTC	South Fork Tincup Creek	BnT	Brown trout

Reference	Site		Species	
	CC-1A	Crow Creek – 1A	Sc	Sculpin
	CC-3A	Crow Creek – 3A	Sc	Sculpin
	CC-150	Crow Creek – 150	Sc	Sculpin
	CC-350	Crow Creek – 350	Sc	Sculpin
	CC-75	Crow Creek – 75	Sc	Sculpin
	DC	Deer Creek	Sc	Sculpin
	HS	Hoopes Spring	Sc	Sculpin
	HS-3	Hoopes Spring – 3	Sc	Sculpin
	LSV-2C	Sage Creek – 2C	Sc	Sculpin
	LSV-4	Sage Creek – 4	Sc	Sculpin
	SFTC	South Fork Tincup Creek	Sc	Sculpin
Gr:	17	Arapahoe Wetlands Pond	FM	Fathead minnow
Grasso et al. 1995	17	Arapahoe Wetlands Pond	WS	White sucker
HB:	LEMC	Lower East Mill Creek	CT	Cutthroat trout
Hamilton and				
Buhl 2004				
Le:	BA	Badin Lake	BB	Black bullhead
Lemly 1985	BE	Belews Lake	BB	Black bullhead
	HR	High Rock Lake	BB	Black bullhead
	BA	Badin Lake	CC	Common carp
	BE	Belews Lake	CC	Common carp
	HR	High Rock Lake	CC	Common carp
	BA	Badin Lake	FM	Fathead minnow
	BE	Belews Lake	FM	Fathead minnow
	HR	High Rock Lake	FM	Fathead minnow
	BA	Badin Lake	GnS	Green sunfish
	BE	Belews Lake	GnS	Green sunfish
	HR	High Rock Lake	GnS	Green sunfish

Reference	Site		Species	
	BA	Badin Lake	WM	Western mosquitofish
	BE	Belews Lake	WM	Western mosquitofish
	HR	High Rock Lake	WM	Western mosquitofish
	BA	Badin Lake	RSh	Red shiner
	BE	Belews Lake	RSh	Red shiner
	HR	High Rock Lake	RSh	Red shiner
Sa87:	KP11	Kesterson Pond 11	WM	Western mosquitofish
Saiki and	KP2	Kesterson Pond 2	WM	Western mosquitofish
Lowe 1987	KP8	Kesterson Pond 8	WM	Western mosquitofish
	SLD	San Luis Drain	WM	Western mosquitofish
	VP26	Volta Pond 26	WM	Western mosquitofish
	VW	Volta Wasteway	WM	Western mosquitofish
Sa93:	GT4	Salt Slough at San Luis Wildlife Refuge	Bg	Bluegill
Saiki et al. 1993	GT5	Mud Slough at San Luis Wildlife Refuge	Bg	Bluegill
	SJR2	San Joaquin R. above Hills Ferry Rd.	Bg	Bluegill
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	Bg	Bluegill
	GT4	Salt Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	GT5	Mud Slough at San Luis Wildlife Refuge	LMB	Largemouth bass
	SJR2	San Joaquin R. above Hills Ferry Rd.	LMB	Largemouth bass
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	LMB	Largemouth bass
	GT4	Salt Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	GT5	Mud Slough at San Luis Wildlife Refuge	WM	Western mosquitofish
	SJR2	San Joaquin R. above Hills Ferry Rd.	WM	Western mosquitofish
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	WM	Western mosquitofish
St:	M4720	Marsh 4720	BB	Black bullhead
Stephens et al. 1988	M4720	Marsh 4720	CC	Common carp

APPENDIX N: COMPARISON OF APPROACHES FOR CALCULATING SELENIUM TISSUE CONVERSION FACTORS

1.0 COMPARISON OF THE MEDIAN RATIO AND REGRESSION APPROACHES

Regression analysis and the application of median ratios are two approaches that can be used to quantify the relationship between two variables, such as the concentration of selenium within two tissue types. When concentrations in the two tissues are plotted, each point represents the ratio of one tissue type to another. A regression analysis calculates the line that best fits those tissue concentrations, which is characterized by both a slope and a y-intercept. In contrast, the median ratio is a single value representing the 50^{th} centile of all ratios. Conversion factors (CFs) are presently calculated as the median ratio of two tissue types. The use of median ratios grew out of the goal of patterning the translation procedure after the Luoma and Presser selenium bioaccumulation model, where field derived factors representing the transfer of selenium from one ecosystem compartment to another were represented as single values calculated using constrained (y-intercept passes through the origin) regression. Median ratios were implemented to produce a single value that was operationally similar to a constrained regression slope, but that was free from the issues associated with constrained regression, particularly cases where the y-intercept was notably different from zero, which would result in slopes that were highly divergent from slopes derived using conventional regression. Both median ratios and conventional regression (with or without log transformation) are far superior to constrained (no y-intercept) regression. The following discussion will compare median ratios and conventional linear regression.

A median is a measure of central tendency that is free from all parametric assumptions associated with linear regression. As the 50^{th} centile of all y/x ratios, it is independent of the effects of outliers or the overall distribution of ratios. As implemented in the criterion document, median ratios were assumed to be representative of the linear relationship between the concentration in tissue y to the concentration in tissue x. This assumption was tested with a pre-screening procedure using conventional linear regression. If the regression model had a positive slope and was statistically significant at $P<0.05$, then the relationship was assumed to be positive and linear, and a median ratio was used as representative of that linear relationship.

A log-log regression includes both a slope and a y-intercept. Because they apply to log space, these parameters mean something different than similar parameters in arithmetic space. Linear relationships in log space translate back to power functions in arithmetic space. That is, the log space straight line function:

$$\log(EO) = m \cdot \log(WB) + b \qquad (1)$$

translates to:

$$EO = a \cdot WB^{m} \qquad (2)$$

where the coefficient $a=10^{b}$. The log-log plot intercept b represents the value of log EO when WB=1 mg/kg (that is when log(WB)=0).

The key point when comparing log-log regression to the median ratio approach is that when log-log slope m=1, then Equation 2 reduces to a simple direct proportion EO = a·WB in arithmetic space**.** Figure N-1 illustrates the behavior of CF (that is, the ratio EO/WB), depending on whether the log-log slope m of the plot of log(EO) versus log(WB) has a value 1, >1, or <1.

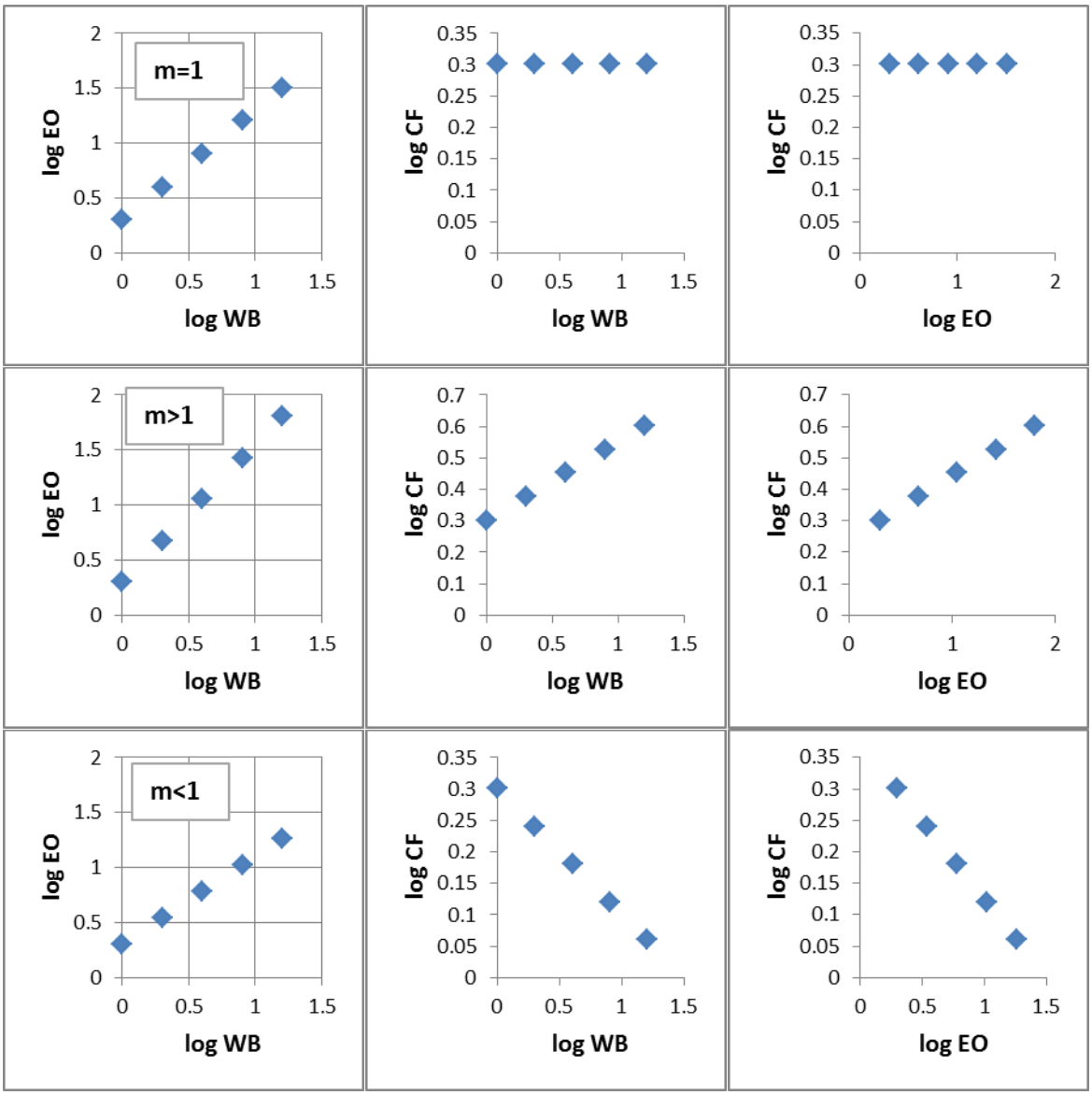


Figure N-1. Idealized illustration (sans scatter) of the effect of log-log slope, m, on whether CF is stable, increases, or decreases with concentration (whether measured as WB in column 2 or EO in column 3).

Top row: m=1. Second row: m=1.25. Bottom row: m=0.8. In all cases, CF=EO/WB, but the three rows were not designed to yield the same median CF. Were the five idealized data points replaced by a large number of well-behaved real-world data points, the straight lines would tend to be replaced by oval clouds of points having the illustrated slopes.

When the log-log slope m≈1, CF does not change with concentration. In that case, CF is the simple proportionality constant as assumed in all previous versions of the criterion document. When m is noticeably different from 1, CF changes with concentration, and we would solve for its value at the EO criterion concentration. If the EO criterion concentration is not near the median EO value in the graphed data, then the regression-calculated CF value may differ somewhat from the median CF.

While both a median ratio and a linear regression account for all of the plotted values within a particular relationship, the regression model is derived from the specific locations of every data point, whereas the median is derived independent of the specific distribution of the data points. In this way, a regression contains more information about the entire data distribution, and as such, is more affected by deviations from linearity. This second point can be an advantage or a disadvantage, depending on the data distribution. For some CF relationships in the database, there is evidence of slight non-proportionality, in which the y/x ratios at higher concentrations are different than the y/x ratios at lower concentrations. In these instances, a log transformation of the tissue concentrations will serve to better linearize the overall relationship, so that the resulting regression model will better capture the y/x relationship across the full concentration range. The median ratio of these models will be the same regardless of whether or not the data are transformed. However, because the use of median ratios is based on the assumption of proportionality, CFs calculated using regression of log transformed values will provide slightly more accurate representations of the relationship across the full concentration range than a median ratio, for those datasets that show some evidence of non-proportionality. An exception would be a case where the midpoint of the data distribution, where the median ratio is more likely to be located, is similar to the criterion concentration. In these instances the median ratio would be expected to be similar the regression based CF regardless of slope. Finally, for those datasets that do not show this effect, selection of either the median ratio or the regression based CF approach are both equally valid approaches.

Another source of uncertainty can occur for species with a CF derived from a narrow concentration range that does not encompass the criterion concentration. In these instances, the slope of the regression model may not be representative of the slope had there been concentrations bracketing the criterion. Similarly, the median of the concentration ratios within this small range may not be representative of the median ratio if there had been concentrations bracketing the criterion. However, it may be preferable, or “safer”, to use a non-parametric median rather than the result of an extrapolated regression equation, particularly when the regression is based on few data points (no matter how good r^2 is).

To conclude, CFs calculated from median ratios have the advantages of simplicity, being easier to explain and implement, and they are “safer” in the sense that they are not affected by outliers or the distribution of variance across the data range. CFs calculated from log regression include more

information about the entire data distribution, but can be sensitive to outliers. CFs calculated from the two approaches can diverge in cases where the data range does not encompass the criterion concentration, particularly in cases those where the log transformed slope is also much greater or less than one. Overall, the median ratio and log regression approaches generate similar CFs for this dataset, and have little effect on the translated water criterion elements.

In general, as indicated by the idealized data in Exhibit A, the median and the TLS regression-predicted CF will be similar under either of the following conditions: (a) log-log EO/WB slope near 1.0, or (b) criterion near the middle of the observed data range of tissue concentrations for the species. They are likely to differ from each other when both of the following conditions simultaneously occur: (c) log-log slope distant from 1.0, and (d) criterion distant from the center of the data range.

2.0 COMPARISON OF THE ORDINARY LEAST SQUARES AND TOTAL LEAST SQUARES REGRESSION APPROACHES

The calculation of conversion factors using linear regression following log transformation addresses the issues associated with non-proportional relationships between Se in different tissues, and is the approach recommended by several public commenters. Conventional ordinary least squares (OLS) regression results can vary depending on which tissue type is assigned to the x and y axis, respectively. This is because OLS regression assumes that the variable on the y axis is dependent on the variable on the x axis, and the resulting regression is the line that minimizes the sum of the squared distances between observed y-values and predicted y-values. OLS regression assumes that the values on the x-axis have no uncertainty. For datasets such as the paired tissue concentrations used to calculate CF, there is no dependency between the selenium concentrations in one tissue type to another tissue type, and concentrations in both tissue types are equally uncertain. Because of this, we could assign either tissue type to either axis, and the resulting CF would be slightly different. By convention, we assign egg-ovary to the y-axis when comparing it to whole-body or muscle, and we assign muscle to the y-axis when comparing it to whole-body, because these are the ratios used in the translation equations. While CFs using median ratios are not affected by axis assignment, CFs using OLS are, for reasons described above.

An alternative regression approach that corrects this issue is total least squares (TLS) regression. TLS regression is preferable to OLS regression in cases where there is error associated with each of the variables, and there is no dependency of one variable on the other. With TLS, the regression is the line that minimizes the sum of the squared distances between observed predicted x- and y-values, and produced the same result regardless of which variable is assigned to which axis. Curves drawn by eye tend to mimic TLS, not OLS. Without thinking about it, the person drawing the line naturally attempts to

minimize both vertical and horizontal errors. However, a significant disadvantage of TLS regression is that Excel has no built-in function to perform it, and many readers will be unfamiliar with it.

Table N-1 shows the effect of the different calculation procedures (median ratio, log OLS regression - xyOLS, log OLS regression with reversed axes - yxOLS, and log TLS regression) on all directly measured CFs. Median ratio CFs tend to diverge from regression based CFs for datasets where log-log slopes are markedly different than 1 and the criterion is not near the center of the observed concentrations. CFs calculated from TLS regression nearly always fall between CFs calculated from OLS regression with and without the axes reversed, and are not affected by axis order.

	Directly calculated conversion factors for each tissue ratio, by method											
	EO/WB				M/WB				EO/M			
Species	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS	Ratio	xyOLS	yxOLS	TLS
bluegill	2.13	1.90	2.04	1.98	1.32	1.36	1.37	1.36	1.38	1.11	1.24	1.18
bluehead sucker	1.82	1.41	1.50	1.45	1.23	1.70	1.67	1.59	1.48	0.82	0.91	0.85
brook trout									1.09	0.96		0.99
brown trout	1.45	1.53	1.77	1.74								
common carp	1.92	1.62	1.63	1.62	1.61	1.36	1.41	1.45	1.14	1.06	1.18	1.14
creek chub	1.99	2.05	2.01	2.03								
cutthroat trout	1.96	1.37	1.67	1.48					1.81	1.97	1.83	1.89
desert pupfish	1.20	1.14	1.14	1.14								
dolly varden									1.26	1.64	1.52	1.59
fathead minnow	1.40	1.71	1.56	1.64								
flannelmouth sucker	1.41	1.14	1.84	1.49	1.46	1.94	1.89	1.85	1.08	0.51	1.06	0.69
green sunfish	1.45	1.35	1.45	1.40	1.23	1.28	1.32	1.24	1.21	1.08	1.17	1.12
mountain whitefish									5.80	10.47	4.98	7.35
northern pike									1.88	1.65	1.78	1.70
rainbow trout									1.92	1.82	1.88	1.82
razorback sucker									2.31	1.93	1.89	1.90
roundtail chub	2.07	2.22	2.26	2.24	1.05	1.08	1.10	1.05	2.04	1.99	2.10	2.06
smallmouth bass	1.42	1.31	1.68	1.52	1.23	1.88	1.97	1.68	1.19	0.67	0.88	0.72
white sturgeon									1.33	0.97	1.07	1.01
white sucker	1.38	1.02	1.25	1.12	1.34	1.43	1.54	1.45	1.00	0.59	0.84	0.67

Table N-1. Comparison of all directly calculated conversion factors by method.
Methods include median ratio (Ratio), log ordinary least squares (xyOLS), log ordinary least squares with axes reversed (yxOLS), and log total least squares (logTLS). Regression based CFs were calculated at the egg ovary criterion concentration of 15.1 mg/kg. Muscle to whole body (M/WB) CFs were calculated at the muscle concentration at the egg-ovary criterion.

The following examples illustrate the differences between OLS and TLS regressions, and the effect of axis assignment on CF.

2.1 Example 1 – Flannelmouth Sucker (Egg-ovary/Whole-body)

CF by approach: (1.41 –median ratio, 1.13 – log OLS, 1.86 – log OLS with reversed axes, 1.48 – log TLS)

Model comparison 1a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log (Egg-ovary) = (0.7966 x (log Whole-body)) + 0.2857

log (Whole-Body) = (log (16 mg/kg) – 0.2857)/0.7966 = 1.153

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.13

TLS: log (Egg-ovary) = (0.9877 x (log Whole-body)) + 0.1843

log (Whole-Body) = (log (16 mg/kg) – 0.1843)/0.9877 = 1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

In Figure N-2, the fitted regression lines do not appear particularly divergent; however, these points cover a relatively narrow (and low), concentration range. At the criterion concentration (log E/O = 1.204), the predictions lines are more divergent, resulting in the differences between the CFs. Also, note that the TLS slope is close to 1. The resulting TLS-derived CF is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in a CF for the OLS model that is notably different than the median ratio CF (1.13 vs 1.41).

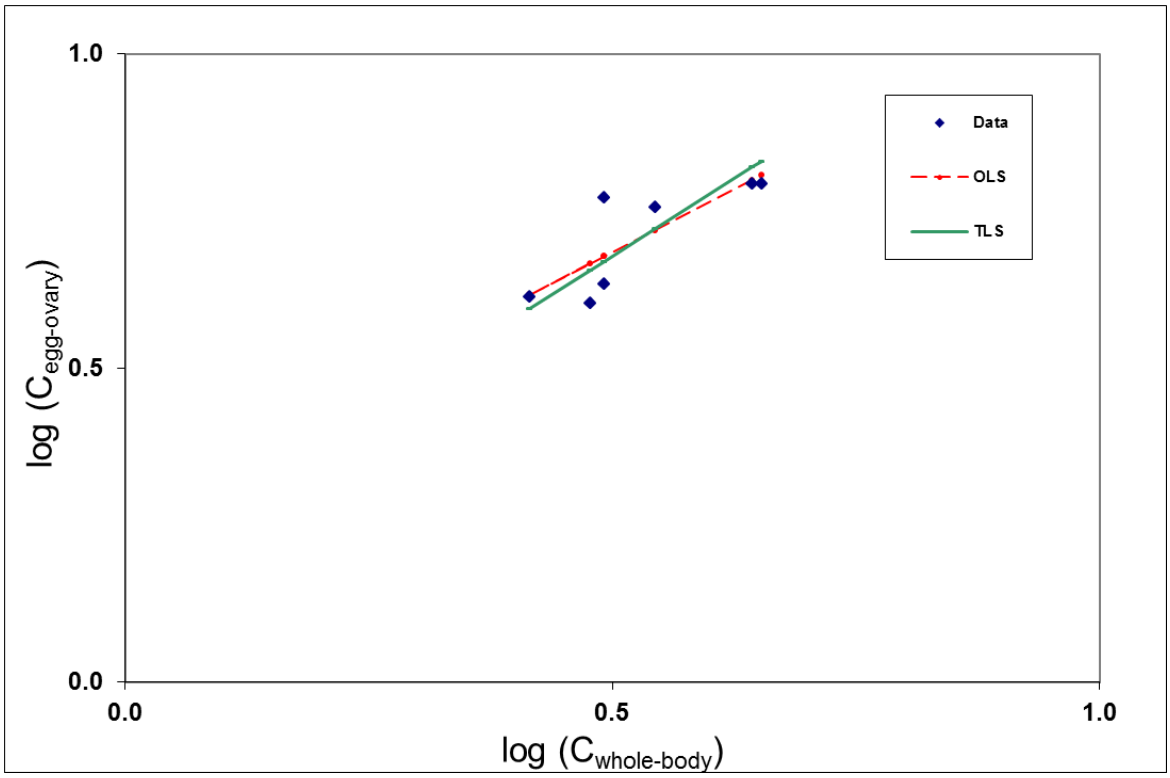


Figure N-2. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 1b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.8126 x (log Egg-ovary)) - 0.0450 = 0.9335

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.86

TLS: log(Whole-body) = (1.012 x (log Egg-ovary)) - 0.1866 =1.033

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.48

At first glance, Figure N-3 appears very similar to Figure N-2. However, note that the axes are reversed, and because we are now solving for y (whole body concentration at egg-ovary criterion concentration), the shallower slope of the reverse OLS figure results a whole body concentration at the egg-ovary criterion lower than in the upper figures, which in turn results in a larger CF. Also, note that the TLS model is a mirror image of the model in Figure N-3, and as such has the same calculated CF. As above, the TLS slope is close to 1, with a TLS-derived CF that is similar to the median ratio CF (1.48 vs 1.41). In contrast, the OLS slope is lower than 1, resulting in an OLS-derived CF that is notably different than the median ratio CF (1.86 vs 1.41).

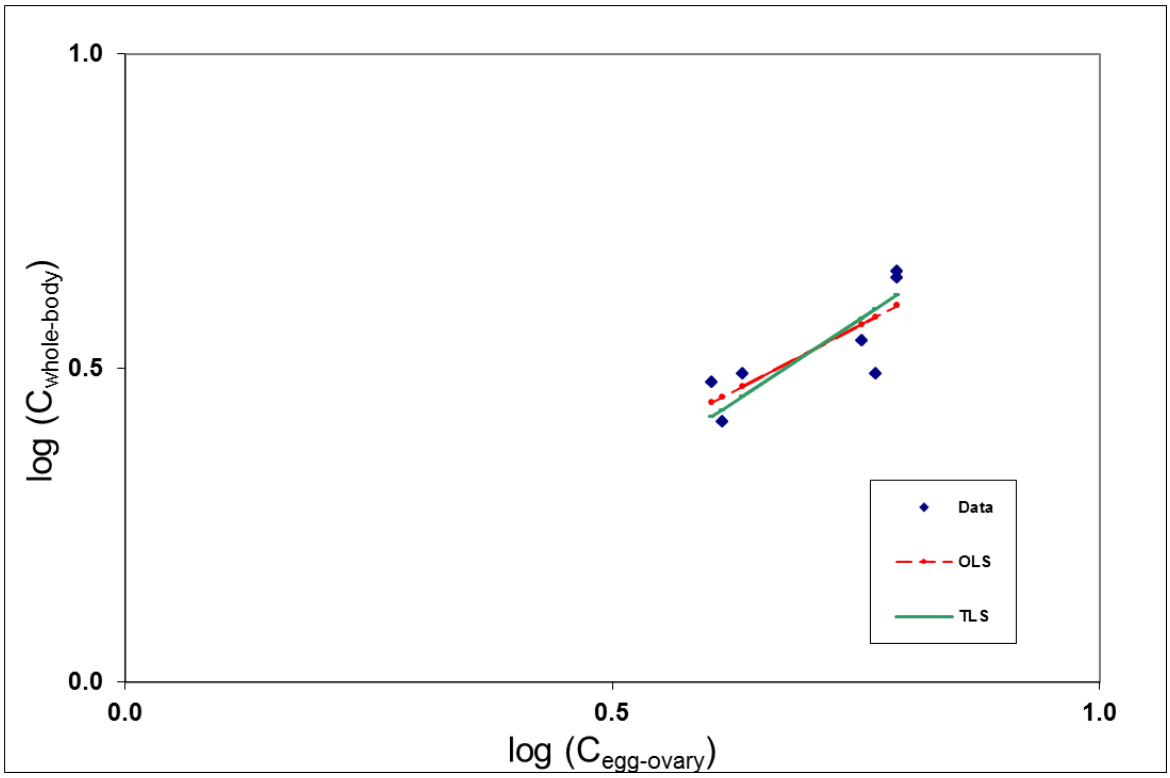


Figure N-3. OLS vs. TLS regression model fits for flannelmouth sucker.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

2.2 Example 2 – Bluegill (Egg-ovary/Whole-body)

CF by approach: (2.13 –median ratio, 1.90 – log OLS, 2.07 – log OLS with reversed axes, 2.01 – log TLS)

Model comparison 2a - Regression model results and calculation of CF (Egg-ovary y-axis; Whole-body x-axis):

OLS: log(Egg-ovary) = (1.061 x (log Whole-body)) + 0.2227

log (Whole-Body) = (log (16 mg/kg) – 0.2227)/1.061 = 0.9250

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 1.90

TLS: log(Egg-ovary) = (1.240 x (log Whole-body)) + 0.0.0861

log (Whole-Body) = (log (16 mg/kg) – 0.0861)/1.240 = 0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly larger calculated CF (Figure N-4). Even though the slopes are larger than 1, the data range encompasses the criterion concentration, which is close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

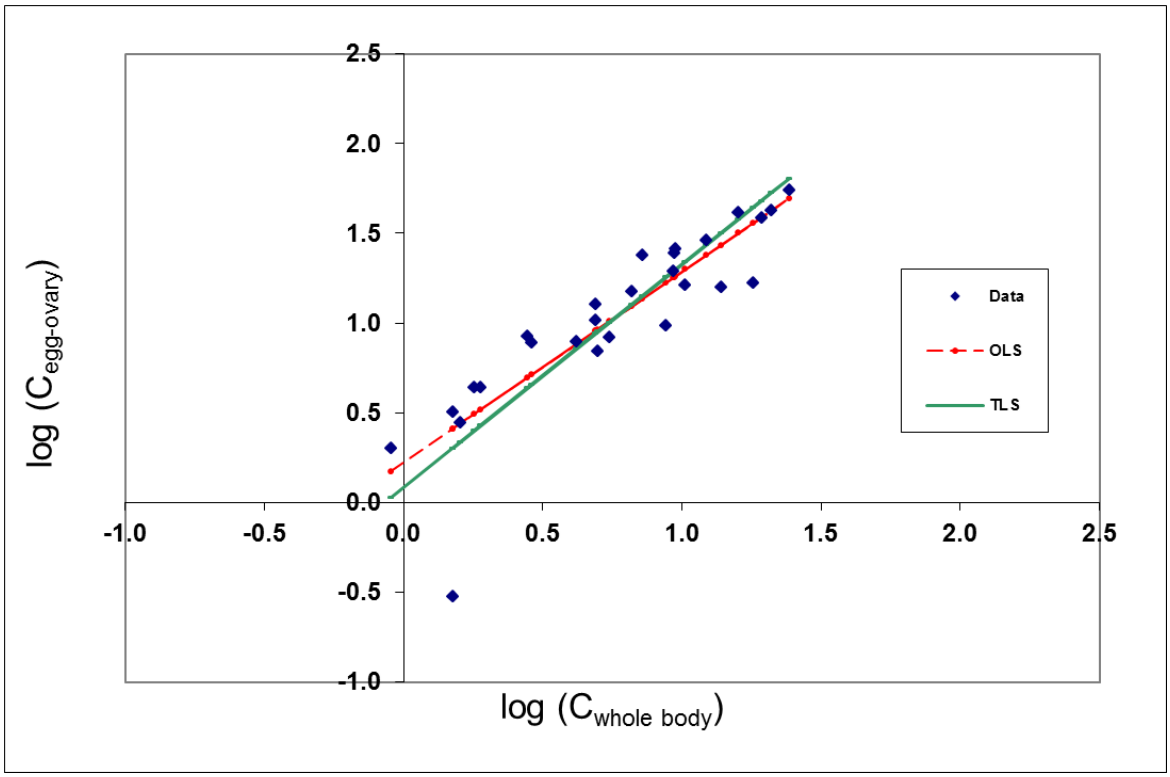


Figure N-4. OLS vs. TLS regression model fits for bluegill. Egg-ovary concentrations are on the y-axis and whole-body concentrations are on the x-axis.

Model comparison 2b - Regression model results and calculation of CF (Egg-ovary x-axis; Whole-body y-axis):

OLS: log(Whole-body) = (0.7269 x (log Egg-ovary)) + 0.0129 = 0.8883

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.07

TLS: log(Whole-body) = (0.8066 x (log Egg-ovary)) - 0.0695 =0.9018

CF at egg-ovary criterion = 10^((log Egg-ovary) – (log Whole-body)) = 2.01

Compared to the OLS regression line, the slope of the TLS regression line is slightly steeper, resulting in a slightly smaller calculated CF (Figure N-5). Even though the slopes are less than 1, the data range encompasses the criterion concentration, which is also close to the middle of the data distribution. As a result, the regression based CFs are similar overall to the median ratio CF.

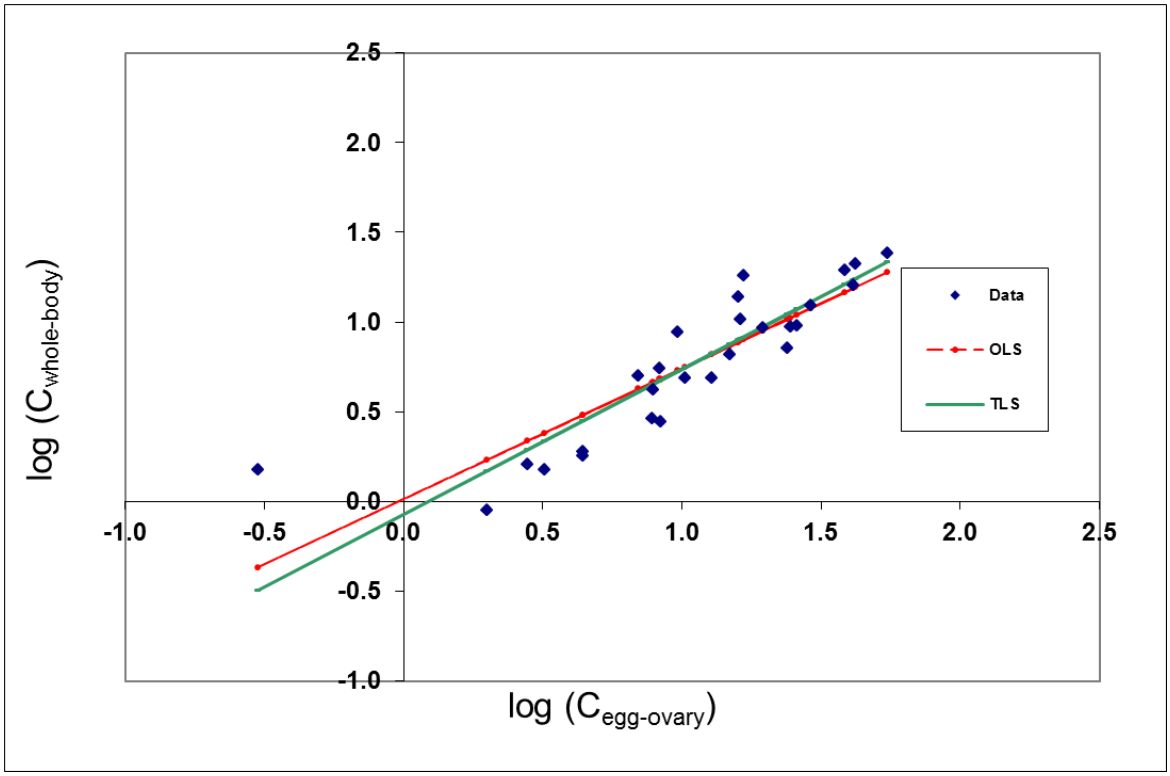


Figure N-5. OLS vs. TLS regression model fits for bluegill.
Egg-ovary concentrations are on the x-axis and whole-body concentrations are on the y-axis.

The effect of the CFs calculated by the different approaches has a minor effect on the final translated water criterion elements. Compared to the median ratio method, translated water criterion element concentrations are slightly higher using CFs calculated from the log OLS regression methods, CFs calculated from the reverse axis log OLS are slightly lower. Lentic translated water criterion element

concentrations are the same using CFs from median ratios and log TLS regression methods, while lotic concentrations calculated from log TLS CFs are slightly lower compared to those calculated using median ratio CFs (**Table N-2**).

Table N-2. Translated water concentration criterion element criterion concentrations by CF calculation method.

Method	Lentic (µg/L)	Lotic (µg/L)
Median Ratio	1.5	3.1
log OLS regression	1.6	3.3
inverse log OLS regression	1.4	2.9
log TLS regression	1.5	2.9

3.0 COMPARISON OF MEDIAN- AND REGRESSION-BASED CONVERSION FACTORS TO CALCULATE CHRONIC VALUES FOR MUSCLE AND WHOLE BODY TISSUES

Besides being used in the translation of the egg-ovary (EO) tissue criterion to water, conversion factors (CF) were also used to convert egg-ovary (EO) chronic values (CV) to muscle or whole body tissue concentrations. These conversions were done when the data from a reproductive toxicity study did not have muscle or whole body selenium concentrations or if the latter tissue data was not usable to determine a chronic value. Directly calculated CVs using either muscle or whole body selenium measurements from a study was preferred over converted CVs in the determination of the final chronic value (= criterion).

Table N-3 provides a comparison of median-based and regression-based CFs when they are used to convert an EO selenium concentration to muscle or whole body. Regression-based CFs used total least squares (TLS) regression for the reasons stated above. The table lists each taxon in the reproductive toxicity data set and presents CVs that are either directly calculated or converted from the EO CV using either the median or TLS CF. Generally, the median-based and TLS-based CFs were similar for both tissue types and this similarity resulted in similar criteria (bottom row). The muscle criterion for the data set that contained directly calculated CVs and converted CVs was similar whether median or TLS CFs were used, 11.3 and 10.6, respectively. The whole body criterion was also similar using these two approaches, 8.5 and 9.6, respectively. The median-based CFs were selected based on reasons stated in the previous section.

Table N-3. Comparison of muscle and whole body chronic values when calculated directly and converted from egg-ovary concentrations using median- and TLS regression-based conversion factors.

Taxon	EO CV	Muscle chronic values (CV) and conversion factors (CF)						Whole body chronic values (CV) and conversion factors (CF)					
		Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV	Direct + Median	Direct + TLS	Median CF	Median CV	TLS CF	TLS CV
Salvelinus	56.22	44.48	35.36	1.26	44.48	1.59	35.36	34.92	24.34	1.61	34.92	2.31	24.34
Esox	34	21.70[d]	21.70[d]	1.88	18.13	1.70	20.00	14.23	13.77	2.39	14.23	2.47	13.77
Cyprinodon	27	28.72	34.18	0.94	28.72	0.79	34.18	22.50	23.68	1.20	22.50	1.14	23.68
O. mykiss	24.5	12.79	13.46	1.92	12.79	1.82	13.46	10.04	9.28	2.44	10.04	2.64	9.28
O. clarkii, Rudolph	24.7	16.6[d]	16.6[d]	1.81	13.65	1.89	13.07	12.60	16.69	1.96	12.60	1.48	16.69
O. clarkii, Nautilus	27.7	15.30	14.66	1.81	15.30	1.89	14.66	14.13	18.72	1.96	14.13	1.48	18.72
Oncorhynchus	25.31	14.28	14.49	NA	13.59	NA	13.65	11.58	12.81	NA	11.58	NA	12.81
Micropterus	26.3	22.16	36.53	1.19	22.16	0.72	36.53	18.52	17.30	1.42	18.52	1.52	17.30
L. macrochirus, Coyle	26.3	19.13	22.29	1.38	NA	1.18	NA	8.6[d]	8.6[d]	NA	NA	NA	NA
L. macrochirus, Doroshov	22.6	15.7[d]	15.7[d]	NA	NA	NA	NA	10.61	11.41	2.13	NA	1.98	11.41
L. macrochirus, Hermanutz	14.7	13.4[d]	13.4[d]	NA	NA	NA	NA	10.6[d]	10.6[d]	NA	NA	NA	NA
Lepomis	20.60	15.91	16.74	1.38	14.98	1.18	17.45	9.890	10.13	2.13	9.656	1.98	10.40
Salmo	21	18.50	17.50	1.14	18.50	1.20	17.50	13.2[d]	13.2[d]	1.45	14.48	1.74	12.07
Acipenser	15.6	11.9[d]	11.9[d]	1.33	11.73	1.01	15.45	9.209	10.68	1.69	9.209	1.46	10.68
Criterion	**15.10**	**11.34**	**11.57**	NA	**10.99**	NA	**13.35**	**8.538**	**9.567**	NA	**8.189**	NA	**9.879**

[d] directly calculated from muscle or whole body selenium concentrations

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A Methodology for Ecosystem-Scale Modeling of Selenium

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ABSTRACT

The main route of exposure for selenium (Se) is dietary, yet regulations lack biologically based protocols for evaluations of risk. We propose here an ecosystem-scale model that conceptualizes and quantifies the variables that determine how Se is processed from water through diet to predators. This approach uses biogeochemical and physiological factors from laboratory and field studies and considers loading, speciation, transformation to particulate material, bioavailability, bioaccumulation in invertebrates, and trophic transfer to predators. Validation of the model is through data sets from 29 historic and recent field case studies of Se-exposed sites. The model links Se concentrations across media (water, particulate, tissue of different food web species). It can be used to forecast toxicity under different management or regulatory proposals or as a methodology for translating a fish-tissue (or other predator tissue) Se concentration guideline to a dissolved Se concentration. The model illustrates some critical aspects of implementing a tissue criterion: 1) the choice of fish species determines the food web through which Se should be modeled, 2) the choice of food web is critical because the particulate material to prey kinetics of bioaccumulation differs widely among invertebrates, 3) the characterization of the type and phase of particulate material is important to quantifying Se exposure to prey through the base of the food web, and 4) the metric describing partitioning between particulate material and dissolved Se concentrations allows determination of a site-specific dissolved Se concentration that would be responsible for that fish body burden in the specific environment. The linked approach illustrates that environmentally safe dissolved Se concentrations will differ among ecosystems depending on the ecological pathways and biogeochemical conditions in that system. Uncertainties and model sensitivities can be directly illustrated by varying exposure scenarios based on site-specific knowledge. The model can also be used to facilitate site-specific regulation and to present generic comparisons to illustrate limitations imposed by ecosystem setting and inhabitants. Used optimally, the model provides a tool for framing a site-specific ecological problem or occurrence of Se exposure, quantify exposure within that ecosystem, and narrow uncertainties about how to protect it by understanding the specifics of the underlying system ecology, biogeochemistry, and hydrology. Integr Environ Assess Manag 2010;6:685–710. © 2010 SETAC

Keywords: Selenium Food web Bioaccumulation Site-specific ecological exposure Ecosystem-scale

Original Research

INTRODUCTION

Effects from Se toxicity have proven dramatic because of extirpations (i.e., local extinctions) of fish populations and occurrences of deformities of aquatic birds in impacted habitats (Skorupa 1998; Chapman et al. 2010). The large geologic extent of Se sources is connected to the environment by anthropogenic activities that include power generation, oil refining, mining, and irrigation drainage (Presser, Piper, et al. 2004). Toxicity arises when dissolved Se is transformed to organic Se after uptake by bacteria, algae, fungi, and plants (i.e., synthesis of Se-containing amino acids de novo) and then passed through food webs. Biochemical pathways, unable to distinguish Se from S, substitute excess Se into proteins and alter their structure and function (Stadtman 1974). The impact of these reactions is recorded most importantly during hatching of eggs or development of young life stages. Thus, the reproductive consequences of maternal transfer are the most direct and sensitive predictors of the effects of Se (Heinz 1996).

Each step in this sequence of processes is relatively well known, but the existing protocols for quantifying the linkage between Se concentrations in the environment and effects on animals have orders of magnitude of uncertainties. Conventional methodologies relate dissolved or water-column Se concentrations and tissue Se concentrations through simple ratios (i.e., bioconcentration factor, BCF; bioaccumulation factor, BAF), regressions, or probability distribution functions (DuBowy 1989; Peterson and Nebeker 1992; McGeer et al. 2003; Toll et al. 2005; Brix et al. 2005; DeForest et al. 2007). None of these approaches adequately accounts for each of the important processes that connect Se concentrations in water to the bioavailability, bioaccumulation, and toxicity of Se.

In this paper, we present an ecosystem-scale methodology that reduces uncertainty by systematically quantifying each of the influential processes that links source inputs of Se to toxicity. In particular, we emphasize a methodology for relating dissolved Se to bioaccumulated Se. The methodology allows us to 1) model Se exposure with greater certainty than previously achieved through traditional approaches that skip steps, 2) explain or predict Se toxicity (or lack of toxicity) in site-specific circumstances, and 3) translate proposed Se guidelines among media under different management or regulatory scenarios.

Important components of the methodology are 1) empirically determined environmental partitioning factors between water and particulate material that quantify the effects of dissolved speciation and phase transformation, 2) concentrations of Se in living and nonliving particulates at the base

All Supplemental Data may be found in the online version of this article.

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of the food web that determine Se bioavailability to invertebrates, 3) Se biodynamic food web transfer factors that quantify the physiological potential for bioaccumulation from particulate matter to consumer organisms and prey to their predators, and 4) critical tissue values that relate bioaccumulated Se concentrations to toxicity in predators. We compile data from 1) laboratory experiments that measured physiological biodynamic parameters for the dietary pathways of invertebrates and fish, and 2) field studies that simultaneously collected particulate, prey, and predator Se concentrations to develop species-specific trophic transfer factors. Additionally, we compiled data from field studies that simultaneously collected dissolved and particulate Se concentrations to evaluate partitioning into the base of the food web. Alternative approaches for modeling of aquatic birds are illustrated because biodynamic data for wildlife are limited. We show that enough data exist, or can be derived site specifically, to address food web transfer in many types of ecosystems. Finally, we test the predictions derived from the ecosystem-scale methodology against observations from nature and compare the outcomes of alternative exposure choices to assess implications for ecosystem management and protection.

Regulatory aspects

Persistent toxicants such as Hg and xenobiotic organic substances are among the most hazardous of contaminants because they efficiently bioaccumulate or biomagnify in food webs and put fish, wildlife, and humans at risk (Thomann 1989; Gobas 1993). Early in the history of pollution by these types of chemicals, a measure of bioaccumulative potential (or trophic transfer potential) was deemed necessary ''because acute toxicity is low (water pathway) and, once chronic effects appear, corrective actions such as terminating the addition of chemical to an ecosystem may not take hold soon enough to alleviate the situation before irreparable damage has been done'' (Neely et al. 1974). Selenium shares many attributes with bioaccumulative chemicals when toxicity is determined from diet, not dissolved exposure (Sappington 2002). Classification of Se as a hazard equivalent to other bioaccumulative chemicals has been contentious (Luoma and Presser 2009).

Regulating agencies such as the US Environmental Protection Agency (USEPA) have recognized that development of water quality Se criteria for the protection of aquatic life and wildlife require consideration of exposures other than solely dissolved Se to understand and assess environmental protection with certainty (USEPA 1998; US Fish and Wildlife Service [USFWS] and National Marine Fisheries Service [NMFS] 1998, amended 2000; Reiley et al. 2003). As of 2010, the USEPA has under consideration a national fish-tissue criterion and other state-, region-, or site-specific approaches for managing Se contamination (USEPA 1997, amended 2000, 2004). In general, this type of criterion would help fill the need to connect effects from a dietary exposure pathway into a regulatory framework. However, such regulations do not yet reflect the current state of knowledge concerning the transfer of Se through ecosystems (Sappington 2002; Reiley et al. 2003), nor do they formalize the knowledge necessary to understand the basis of protective criteria for Se. Furthermore, implementation of a fish-tissue criterion would require translation to a dissolved Se concentration to satisfy other regulatory requirements, such as permit and load limits. An important purpose of this paper is to demonstrate how a step-by-step ecosystem-scale methodology can address these problems and facilitate translation across steps to harmonize regulation.

Overview of modeling approach

A conceptual model (Figure 1) illustrates the linked factors (Table 1) that determine the effects of Se in ecosystems. Figure 1 also shows the data needed (e.g., Se speciation) for optimally modeling or fully understanding these linkages. The first 8 variables (source loads to health effects; Table 1) are considered systematically in developing and implementing an ecosystem-scale methodology. Predator life cycle (constraining the model in time and space) and demographics are listed as components of a comprehensive site-specific assessment but are not covered in detail in this paper. Emphasis in this paper is on protection of fish and birds, but similar modeling techniques could be used to evaluate amphibians and mammals.

The organizing principle for the methodology is the progressive solution of a set of simple equations or models, each of which quantifies a process important in Se exposure (Figure 2). Environmental partitioning between dissolved and particulate phases (K_d) is used here to characterize operationally the uptake and transformation (commonly termed bioconcentration) of dissolved Se into the base of the food web (Figure 2). K_d is environment specific (i.e., dependent on site hydrology, dissolved speciation, and type of particulate material) and is the ratio of the particulate material Se concentration (in dry weight, dw) to the dissolved Se concentration observed at any instant. The base of the food web, as sampled in the environment and characterized by K_d, can include phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment, or attached vascular plants. For simplicity, in our discussion we define this mixture of living and nonliving entities as particulate material. Dissolved or total Se can be specified in the derivation of K_d for modeling to accommodate use of existing data sets, but this substitution is a possible source of variability. Consideration of the amount of suspended particulate material and its contribution to the total Se measurement gives an indication of the difference incurred by this substitution. In our discussions, we refer to a generalized water-column Se concentration, but the preferred parameter to measure and model would be dissolved Se.

Kinetic bioaccumulation models (i.e., biodynamic models; Luoma and Fisher 1997; Luoma and Rainbow 2005) account for the now well-established principle that Se bioaccumulates in food webs principally through dietary exposure. Tissue Se attributable to dissolved exposure makes up less than 5% of overall tissue Se in almost all circumstances (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). Biodynamic modeling (Figure 2) further shows that the extent of Se bioaccumulation (the concentration achieved by the organism) is driven by physiological processes that are specific to each species (Reinfelder et al. 1998; Baines et al. 2002; Wang 2002; Stewart et al. 2004). Experimental protocols for measuring parameters such as assimilation efficiency (AE), ingestion rate (IR), and the rate constant that describes Se excretion or loss from the animal (k_e) are

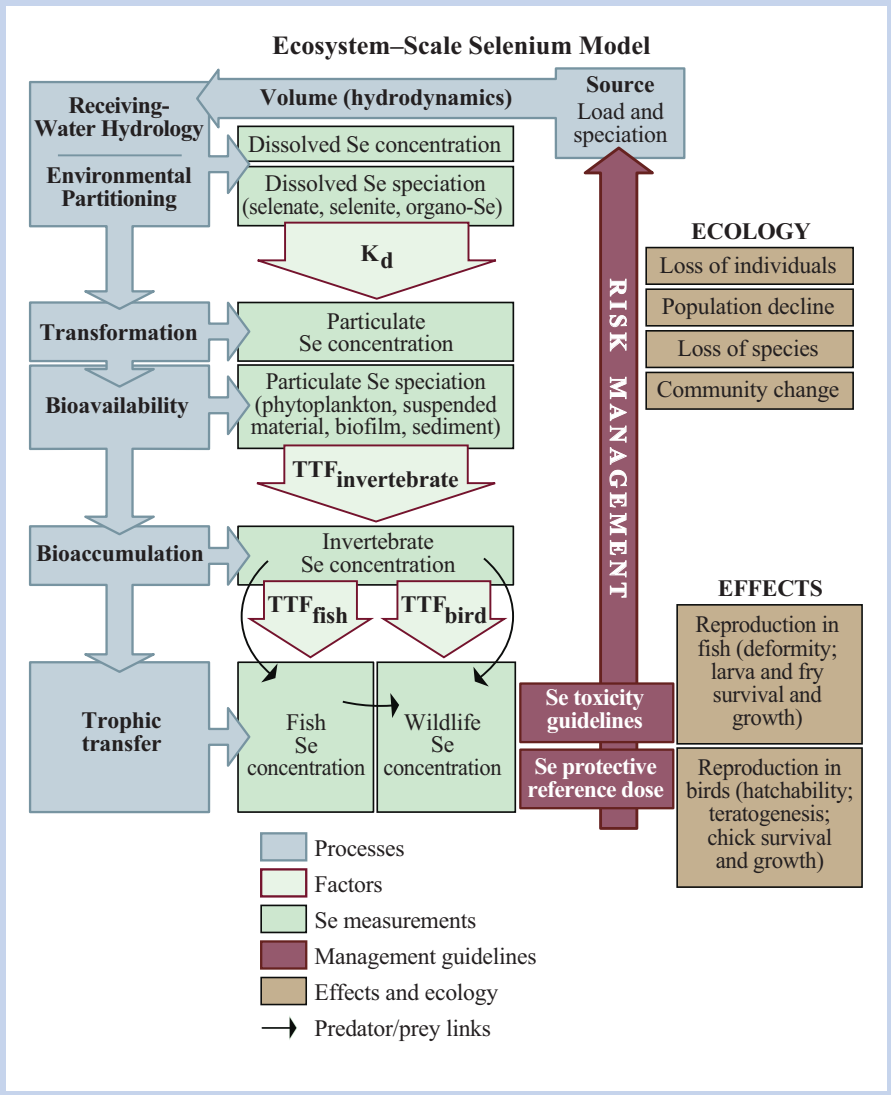

Figure 1. Ecosystem-scale Se model. The model conceptualizes processes and parameters important for quantifying and understanding the effects of Se in the environment. The model can be applied to forecast exposure and to evaluate the implications of management or regulatory choices. K_d = empirically determined environmental partitioning factor between water and particulate material; TTF = biodynamic food web transfer factor between an animal and its food.

now well developed (Wang et al. 1996; Luoma and Rainbow 2005).

Biodynamic models have the further advantage of providing a basis for deriving a simplified measure of the linkage between trophic levels: trophic transfer factors (TTFs; Figure 2; Reinfelder et al. 1998). TTFs are species-specific and link particulate, invertebrate, and predator Se concentrations (e.g., TTF_{clam} or $TTF_{sturgeon}$). They can be derived from laboratory experiments or from field data. $TTF_{invertebrate}$ and $TTF_{predator}$ differ from traditional BAFs in that they are the ratio of the Se concentration in each animal to the Se concentration in its food (Figure 2), whereas BAFs almost always are implemented as the Se concentration in an animal to the Se concentration in the water of its environment. Biodynamic model calculations and ratios derived here employ dw for media (particulate material and tissue). Variability or uncertainty in processes such as AEs or IRs can be directly accounted for in sensitivity analysis as shown for Se by Wang et al. (1996). This is accomplished by considering the range in the experimental observations for the specific animal in the model. Field-derived factors require some knowledge of feeding habits and depend upon available data for that species. Laboratory and field factors for a species can be compared and refined to improve levels of certainty in modeling. Hence, both physiological TTFs derived from kinetic experiments for a species and ecological TTFs derived either from data for a species across different field sites (global) or from one site (site-specific) are of value in modeling and understanding an ecosystem.

By modeling different exposure scenarios, it is possible to differentiate consumer species and food webs in terms of bioaccumulative potential, an important step in reducing uncertainties in predicting ecological risks (Stewart et al. 2004). To translate exposure into toxicity, we employ results from dietary toxicity studies in predators that correlate the two. There has been considerable discussion about choices of protective levels for fish and wildlife (Skorupa 1998; DeForest et al. 1999; Hamilton 2004; Lemly 2002; Adams et al. 2003; Ohlendorf 2003). Nevertheless, tissue guidelines are being proposed to be nationally promulgated by USEPA,

Table 1. Variables considered for ecosystem-scale modeling of Se

Variable	Ecosystem-scale modeling
Source load	Coal fly ash disposal, agriculture drainage, oil refinery effluent, phosphate and coal mining waste leachate, mining discharge
Dissolved speciation	Selenate, selenite, organo-Se
Receiving-water partitioning and/or transformation environment	Wetland and/or marsh, pond, backwater and/or oxbow, stream, river, estuary, ocean, freshwater or saltwater
Particulate speciation	Elemental Se, adsorbed selenite and/or selenate, organo-Se
Bioavailability	Sediment, detritus, phytoplankton, periphyton, biofilm
Invertebrate specific bioaccumulation	Species-specific physiological parameters (ingestion rate, assimilation efficiency, efflux rate, growth), field derived factors
Trophic transfer to fish or aquatic birds[a]	Species-specific physiological parameters (ingestion rate, assimilation efficiency, efflux rate, growth), field derived factors, dose–response curves
Health effect endpoints	Reproduction, teratogenesis, decreased growth, decreased survival (especially in winter), disease (immunosuppression), sublethal (chronic effects)
Predator life cycle	Species-specific energetics (body weight and ingestion rate), life stage (breeding, larval, adult), distribution (resident, mobile, migratory), timing (route, duration), feeding behavior (prey availability and preference, foraging pattern, background intake)
Demographics	Loss of individuals (threatened or endangered species), population reduction, community change, loss of species

[a]Modeling can be extended to terrestrial birds, amphibians, and mammals.

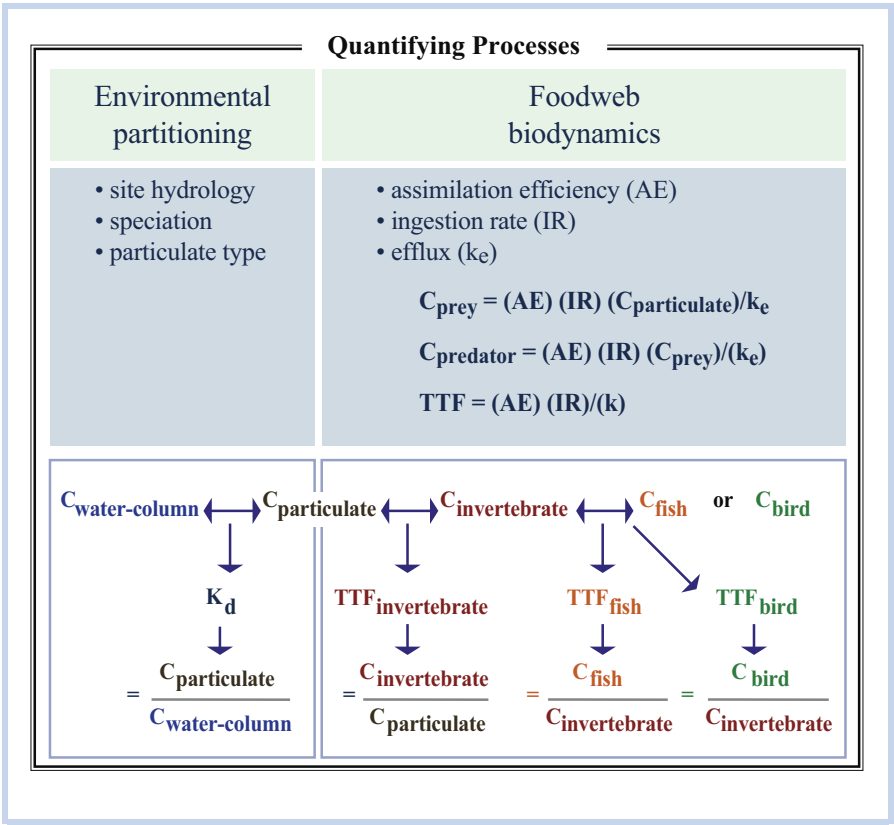

Figure 2. Critical factors for linked steps in ecosystem-scale Se modeling. Environmental partitioning and biodynamic physiological parameters quantify dietary pathways in nature. For modeling, the physiological parameters are combined into a TTF, which characterizes the bioaccumulation potential for each specific particulate–invertebrate pair or prey–predator pair.

recommended by USFWS as part of Endangered Species Act consultation, or stipulated for watershed or regional regulation based on a review of existing toxicity literature (USFWS and NMFS 1998, amended 2000; USEPA 2004). Steps in wildlife criteria development (e.g., ingestion models that use rate of consumption, body weight, and reference dose to calculate a dietary limitation or wildlife criterion) are also delineated here to illustrate that our approach is compatible with a more traditional regulatory approach to the protection of wildlife.

Another use of the model is in understanding the environmental concentrations and conditions that would result in a predetermined Se concentration in the tissues of a predator. Assuming that the tissue guideline is generic for all fish or birds (for example), the choice of the predator species in which to assess that concentration is still important because it determines the $TTF_{invertebrate}$. That specific predator's feeding habits drive the choice of invertebrate, for which a species-specific TTF is used to calculate particulate Se concentrations. A K_d feasible for that ecosystem (or a range of K_ds) is then used to determine the allowable water-column Se concentration, which is ultimately the concentration in that specific type of environment and food web that would result in the specified Se concentration in the predator (i.e., the applied criterion). Thus the allowable water-column concentration can differ among environments, an outcome that reflects the realities of nature. This biologically explicit approach also forces consideration of the desired uses and benefits in a watershed (i.e., which species of birds and fish are the most threatened by Se and/or are the most important to protect).

In the absence of detailed knowledge of the watershed, choices can be made based on rudimentary knowledge about prey and predator pairs; however, the more rudimentary the choices, the greater the uncertainty. Thus, implicitly, the modeling approach creates incentives to understand ecosystems better for which enough is at stake to invest in data collection. Explicitly, it points toward critical choices for data collection. As the knowledge necessary for a full conceptual ecosystem-scale model for Se is developed at a selected site, uncertainties about effects of Se are progressively narrowed. A strength of this approach is that Se bioaccumulation and trophic transfer predicted by the methodology (Figure 1) can be used to validate or estimate uncertainties through comparison of predicted invertebrate, fish, and bird Se concentrations with independent observations of those concentrations from field studies.

MODEL COMPONENTS

Sources of selenium

Knowledge of a dissolved Se concentration in a water body is a crucial first step in understanding the potential for adverse ecological effects. Documenting how different sources and processes contribute to that concentration is also essential (Figure 1). Potential sources of Se in the environment have been well described elsewhere (Seiler et al. 2003; Presser, Piper, et al. 2004). In brief, organics-rich marine sedimentary rocks, especially black shales, petroleum source rocks, and phosphorites, are major sources of Se. In terms of Se as a commodity, Se's source is in igneous Cu deposits. The interface of aquatic systems with waste products or overburden from coal, phosphate, and metals mining; oil refining; fossil fuel combustion; and irrigation in arid regions can deliver Se to the environment on a large scale.

Each of the above sources typically can release Se with a different speciation. Selenate is often dominant in agricultural drainage, mountaintop coal mining and/or valley fill leachate, and Cu mining discharge (Presser and Ohlendorf 1987; Naftz et al. 2009; West Virginia Department of Environmental Protection 2009). Selenite is frequently found in oil refinery and fly-ash-disposal effluents (Bowie et al. 1996; Cutter and Cutter 2004). Combinations of selenite and organo-Se are common in pond-treated agricultural drainage (Amweg et al. 2003) and the oceans (Cutter and Bruland 1984). Speciation in phosphate mining overburden leachate in streams depends on season and flow conditions: selenate during maximum flow, selenite and organo-Se during minimum flow (Presser, Hardy, et al. 2004).

Hydrologic environment

How inputs (source loads) of Se interact in a specific hydrologic environment determines receiving-water Se concentrations (Figure 1). Comprehensive hydrodynamic models can be used to represent Se transport and smaller scale effects such as elevated concentrations near sources of inflow or detailed distribution within receiving waters. Models have been used successfully to describe Se concentrations in complex environments by incorporating basic physical and geochemical processes involved in determining how load and volume interact (Meseck and Cutter 2006; Diaz et al. 2008; Naftz et al. 2009). Simpler approaches can be used to estimate regional scale effects. For example, Se concentrations in San Francisco Bay were estimated by quantifying mass inputs, broadly differentiating seasonal flow regimes, and characterizing source signatures to understand the overall response of the ecosystem to several sources of Se contamination (Presser and Luoma 2006). Regional scale estimates agreed with observations from the use of this abbreviated approach.

Modeling of interactions of Se loading and hydrodynamics initiates the ecosystem-scale approach by developing an understanding of dissolved Se concentrations in a given environment (Figure 1). However, complex physical modeling is not sufficient to determine the ultimate effects of Se in an ecosystem (Wrench and Measures 1982), nor is a detailed understanding of physical processes or dissolved Se distributions adequate to unravel questions about Se effects or its regulation compared with understanding and incorporating phase transformation, biological reactions, and the influences of ecology into modeling.

Partitioning and transformation environments: Speciation and bioavailability

Phase transformation reactions from dissolved to particulate Se are of toxicological significance because particulate Se is the primary form by which Se enters food webs (Figure 1) (Cutter and Bruland 1984; Oremland et al. 1989; Luoma et al. 1992). The different biogeochemical transformation reactions also result in different forms of Se in particulate material: organo-Se, elemental Se, or adsorbed Se. The resulting particulate Se speciation, in turn, affects the bioavailability of Se to invertebrates depending on how an invertebrate samples the complex water, sediment, and particulate milieu that composes its environment.

Given this sequence (Figure 1), the first requirement for reducing the uncertainty in tying dissolved Se to effects on

predators is quantification of the linkage between dissolved Se and Se concentrations in particulate material at the base of the food web. In a data-rich environment, biogeochemical models might be able to capture at least some of the processes that drive phase transformation (see, e.g., Meseck and Cutter 2006), but even sophisticated models, to some extent, rely in their development on empirical observations of partitioning between dissolved and particulate Se.

With the present state of knowledge, it is feasible to use field observations to quantify the relationship between particulate material and dissolved Se as expressed by

$$K_d = C_{particulate} \div C_{water-column}. \quad (1)$$

This operationally defined ratio is an instantaneous observation in which $C_{particulate}$ is the particulate material Se concentration in μg/kg dw and $C_{water\text{-}column}$ is the water-column Se concentration in μg/L. Use of a partitioning descriptor can be controversial because K_d formally implies an equilibrium constant. Indeed, thermodynamic equilibrium does not govern Se distributions in the environment (Cutter and Bruland 1984; Oremland et al. 1989), and partitioning coefficients for Se are known to be highly variable (McGeer et al. 2003; Brix et al. 2005), but K_d can be a useful construct if it is recognized that the instantaneous ratio is not intended to differentiate processes or to be predictive beyond the specific circumstance in which it is determined. The sole intention is to describe the particulate to water ratio at the moment when the sample is taken.

Experience shows that repeated observations of this operational K_d can narrow uncertainties about local conditions. However, K_d will vary widely among hydrologic environments (i.e., in parts of a watershed such as wetlands, streams, or estuaries) and potentially among seasons. Consideration of the characteristics of the environment such as speciation, residence time, and/or particle type can also be used to narrow this potential variability, but K_d remains a large source of uncertainty in the model if translation to a water-column Se concentration is required. Initiation of modeling with a particulate Se concentration (see below under *Validation*) eliminates this step and the associated uncertainty and points to the importance of particulate phases in determining Se toxicity. If required, performing calculations with several alternative, but plausible, site-specific choices for K_d can elucidate and constrain the uncertainty around the introduction of K_d.

Speciation. Dissolved Se can exist as selenate, selenite, or organo-Se (+6, SeO_4^{-2}; +4, SeO_3^{-2}, −2, Se-II, respectively). The dissolved species that are present will influence the type of phase transformation reaction that creates particulate Se. Examples of types of reactions include 1) uptake by plants and phytoplankton of selenate, selenite, or dissolved organo-Se and reduction to particulate organo-Se by assimilatory reduction (see, e.g., Sandholm et al. 1973; Riedel et al. 1996; Wang and Dei 1999; Fournier et al. 2006); 2) sequestration of selenate into sediments as particulate elemental Se by dissimilatory biogeochemical reduction (e.g., Oremland et al. 1989); 3) adsorption as coprecipitated selenate or selenite through reactions with particle surfaces; and 4) recycling of particulate phases back into water as detritus after organisms die and decay (see, e.g., Reinfelder and Fisher 1991; Velinsky and Cutter 1991; Zhang and Moore 1996). Selenate is the least reactive of the 3 forms of Se, and its uptake by plants is slow. If all other conditions are the same, K_d will increase as selenite and dissolved organo-Se concentrations increase (even if that increase is small). Experimental data support this conclusion. Calculations using data from laboratory microcosms and experimental ponds show speciation-specific K_ds of 140 to 493 when selenate is the dominant form, 720 to 2800 when an elevated proportion of selenite exists, and 12 197 to 36 300 for 100% dissolved seleno-methionine uptake into at least some algae or periphyton (Besser et al. 1989; Kiffney and Knight 1990; Graham et al. 1992).

Residence time. The conditions in the receiving-water environment are also important to phase transformation. When selenate is the only form of Se and residence times are short (e.g., streams and rivers), the limited reactivity of selenate means that partitioning of Se into particulate material tends to be low. Similarly, dissimilatory reduction does not seem efficient unless water residence times are extended. Longer water residence times, in sloughs, lakes, wetlands, and estuaries, for example, seem to allow greater uptake by plants, algae, and microorganisms. This is accompanied by greater recycling of selenite and organo-Se back into solution, further accelerating uptake (Bowie et al. 1996; Lemly 2002; Meseck and Cutter 2006). Neither selenite nor organo-Se is easily reoxidized to selenate, because the reaction takes hundreds of years (Cutter and Bruland 1984). Therefore, the net outcome in a watershed that flows through wetland areas or estuaries is a gradual build-up of selenite and organo-Se in water and higher partitioning into particulate material (Lemly 1999; Presser and Luoma 2009). Environments downstream in a watershed can also have higher concentrations of selenite and organo-Se, and higher K_ds, reflecting the cumulative contributions of upstream recycling in a hydrologic system.

Differences in Se bioaccumulation have been described between lentic (stream) and lotic (lake) environments (Hamilton and Palace 2001; Brix et al. 2005; Orr et al. 2006). This could at least partially reflect the observations described above: if other conditions are similar, environments with longer residence times, such as lakes, tend to have greater recycling, a higher ratio of particulate and/or dissolved Se, and higher concentrations of Se entering the food web. Exceptions also occur, however. For example, flow period or season might be a consideration even within individual segments of a watershed.

Particle type. The K_d can also be influenced by the type of material in the sediment. For example, field data for Luscar Creek in Alberta, Canada, show a hierarchy of Se concentrations: 2.4 μg/g in sediment, 3.2 μg/g biofilm, and 5.5 μg/g for filamentous algae (Casey 2005). Using these concentrations with a field-measured water-column Se concentration of 10.7 μg/L yields K_ds of 224, 299, and 514, respectively, with an average K_d of 346. Similarly, field data for a slough tributary to the San Joaquin River, California, USA, show a hierarchy of particulate Se concentrations: 0.47 μg/g in sediment, 2.4 μg/g in algae, and 7.9 μg/g in detritus (Saiki et al. 1993). Using these concentrations with a field measured water-column Se concentration of 13 μg/L yields K_ds of 36, 185, and 608, respectively, with an average K_d of 276. In these instances, the influence of particle type is not as great as that of speciation and residence time.

Calculation of K_d. Knowing the range of K_ds in nature for a specific category of site (e.g., ponds, rivers, estuaries) allows some generalization about the potential range of particulate Se concentrations that could occur at a site under different modeled receiving-water conditions. We compiled data from 52 field studies in which both water-column Se concentrations and particulate Se concentrations were determined and calculated K_ds (Supplemental Data Table A). The K_ds across the complete variety of ecosystems vary by as much as 2 orders of magnitude (100–10 000) and measure up to 40 000 (Table 2). Even higher K_ds have been measured in experimental studies using cultured phyoplankton (Reinfelder and Fisher 1991; Baines and Fisher 2001).

There is, however, some consistency among types of environments. Most rivers and creeks show K_ds of greater than 100 and less than 300 (Table 2). For example, K_ds for the Fording River, British Columbia, Canada, and San Joaquin River are 122 and 146, respectively. Lakes and reservoirs are mainly greater than 300, with many being in the 500 to 2000 range. The K_ds for Salton Sea, California, USA, and the Great Salt Lake, Utah, USA, are 1196 and 1759 respectively. The K_ds for Hyco Reservoir, North Carolina, USA, and Belews Lake, North Carolina, USA, based on data from the 1980s are approximately 3000. Those K_ds greater than 5000 are usually associated with estuary and ocean conditions (e.g., seaward San Francisco Bay and Newport Bay, California, >10 000). Exceptions from this categorization of streams included a set of streams in southeastern Idaho receiving runoff from phosphate mining waste characterized by a majority of selenite plus organo-Se under certain flow conditions (Presser, Hardy, et al. 2004). The overall K_d average for these streams is 1708, with the range among individual streams showing considerable variability (494–3000). These data were for partitioning into mainly attached algae.

Modeling and data requirements. Data collected in site-specific field situations for particulate phases can include benthic or suspended phytoplankton, microbial biomass, detritus, biofilms, and nonliving organic materials associated with fine-grained (<100 μm) surficial sediment (Luoma et al. 1992). For modeling, if the data are available, averaging concentrations of Se in sediment, detritus, biofilm, and algae to define K_d may help to take into account partitioning in different media and best represent the dynamic conditions present in an aquatic system. At a minimum, interpretation and modeling of particulate Se concentration data should take into consideration the nature of the particulate material. In that regard, collection of one consistent type of material that can be compared among locations is an option. Bed sediments are the least desirable choice for calculating K_d, especially if the sediments vary from sand to fine-grained among the samples. In general, sandy sediments dilute concentrations with a high mass of inorganic material and may yield K_ds that are anomalously low (Luoma and Rainbow 2008).

Bioaccumulation: Invertebrates

Biodynamics and kinetic trophic transfer factors. A key aspect of Se risk is bioaccumulation (i.e., internal exposure) in prey and predators (Figure 1; Luoma and Rainbow 2005). Bioaccumulation of Se is modeled here through a biodynamic quantification of the processes that lead to bioaccumulation. These pathway-specific bioaccumulation models (e.g., the dynamic multipathway bioaccumulation [DYMBAM] model) quantify Se tissues concentrations through consideration of 1) the form and concentration of Se in food (i.e., particulate material) and water, and 2) the physiology (AE, IR, k_e, and growth) of invertebrates (Luoma et al. 1992; Wang et al. 1996; Schlekat et al. 2002a) as expressed by

$$C_{species}/dt = [(k_u)(C_w) + (AE)(IR)(C_f)] - (k_e + k_g)(C_{species}), \quad (2)$$

where $C_{species}$ is the contaminant concentration in the animal (μg/g dw); t is the time of exposure (d), k_u is the uptake rate constant from the dissolved phase ($L \cdot g^{-1} d^{-1}$), C_w is the contaminant concentration in the dissolved phase (μg/L), AE is the assimilation efficiency from ingested particles (%) or the proportion of ingested Se that is taken up into tissues, IR is the ingestion rate of particles ($g \cdot g^{-1} d^{-1}$), C_f is the contaminant concentration in ingested material (μg/g dw), k_e is the efflux rate constant (/d), and k_g is the growth rate constant (/d). The differential equation describing these processes can be solved to determine metal concentrations at steady state as

$$C_{species} = [(k_u)(C_w) + (AE)(IR)(C_f)] \div [k_e + k_g]. \quad (3)$$

The physiological components of the model are species-specific, and each can be determined experimentally for any given species (see, e.g., Luoma et al. 1992; Wang et al. 1996). The mathematics state that bioaccumulation in an organism results from a combination of gross influx rate as balanced by the gross efflux rate (i.e., biodynamics). Gross efflux is an instantaneous function of the concentration in tissues and the rate constants of loss. Gross influx can come from water or from food. The uptake rate from each is a function of the concentration of Se in that phase.

Biodynamic experiments (Figure 2) mimic dietary pathways in nature by using radiolabeled dissolved selenite to radiolabel food (i.e., particulate material) that is then fed to invertebrates (Luoma and Fisher 1997). A large body of evidence shows that uptake rates of dissolved Se are almost always sufficiently slow in invertebrates that uptake from the dissolved phase is irrelevant compared with uptake from particulate sources such as phytoplankton, detritus, or sedimentary material (Fowler and Benayoun 1976; Luoma et al. 1992; Wang and Fisher 1999; Wang 2002; Schlekat et al. 2004; Lee et al. 2006). For example, the calculated tissue component attributable to dissolved selenite uptake using experimentally determined physiological parameters for the large copepods *Tortanus* sp. and *Acartia* sp. is 1.7% and for the clam *Corbula amurensis* is 1.3% (Schlekat et al. 2002b, 2004; Lee et al. 2006). Thus, a simplification to exposure from only food is justified. The rate constant of growth is significant only when it is comparable in magnitude to the rate constant of Se loss from the organism. Consideration of the complications of growth can usually be eliminated if the model is restricted to a long-term, averaged accumulation in adult animals (Wang et al. 1996).

In the absence of rapid growth, a simplified, resolved exposure equation for invertebrates is

$$C_{invertebrate} = [(AE)(IR)(C_{particulate})] \div [k_e]. \quad (4)$$

To simplify modeling, these physiological parameters can be combined to calculate a $TTF_{invertebrate}$, which characterizes

Table 2. Calculated K_ds based on field studies (supporting data and references for each site are shown in Supplemental Data Table A)

K_d	Ecosystem
107	San Diego Creek, California
110	Alamo River, California
122	Fording River, British Columbia (sediment)
146	San Joaquin River, California
>200	
255	San Diego Creek, constructed pond, California
256	New River, California
269	Tulare Basin, evaporation ponds, California (range 109–500)
272	Upper Newport Bay, California (range 101–776)
276	Mud Slough, California
340	Benton Lake (pool 2), Montana
346	Luscar Creek, Alberta, Canada (range 220–514)
355	Kesterson Reservoir (SLD/pond 2), California (range 200–500)
359	Salt Slough, California
494	Sage Creek, Idaho
≥500	
500	Benton Lake, Montana, pool 5
512	Benton Lake, Montana, pool 1 channel
591	Elk River, British Columbia
611	Lower Great Lakes, Lake Ontario
625	East Allen Reservoir, Wyoming
657	Crow Creek at Toner, Idaho
667	Meeboer Lake, Wyoming
750	Diamond Lake, Wyoming
762	Chevron Marsh (constructed), California (range 214–1241)
767	Miller's Lake, Colorado
784	San Diego Creek constructed marsh, California
818	Mac Mesa Reservoir, Colorado
968	Sweitzer Lake, Colorado
968	Desert Reservoir, Colorado
>1000	
1104	Mud River at Spurlock, West Virginia
1196	Salton Sea, California
1224	Twin Buttes Reservoir, Wyoming
1312	Galett Lake, Wyoming
1341	Angus Creek, Idaho
1388	Lower Great Lakes, Hamilton Harbor
1436	Tulare Basin, evaporation ponds, California
1498	Big Canyon Wash (sites 1 and 2), California
1579	Cobb Lake, Colorado
1619	Timber Lake, Colorado
1717	Larimer Hwy. 9 pond, Colorado
1759	Great Salt Lake, Utah
1800	Upper Mud River Reservoir at Palermo, West Virginia
1818	Crow Creek above Sage Creek, Idaho
1941	Wellington State Pond, Colorado
1943	Thompson Creek, Idaho
>2000	
2143	Highline Reservoir, Colorado
2250	Deer Creek, Idaho
2798	Belews Lake, North Carolina
2902	Kesterson Reservoir (pond 8), California
>3000	
3044	Hyco Reservoir, North Carolina
3150	Big Canyon Wash (site 3), California
3556	Kesterson Reservoir (pond 11), California
4000	Delaware River (tidal freshwater), Delaware
>5000	
6500	Great Marsh, Delaware
7800	San Francisco Bay (1998–1999) (range 3198–26 912)
9456	Salton Sea estuary, Alamo River
12 000	Salton Sea estuary, Whitewater River
13 800	Seaward San Francisco Bay (1998–1999) (range 8136–26 912)
15 000	Xiamen Bay, Fujian Province, China
17 400	Salton Sea estuary, New River, California
18 900	Lower Newport Bay, California (range 6933–42 715)
21 500	San Francisco Bay (1986; 1995–1996) (range 3000–40 000)

the potential for each invertebrate species to bioaccumulate Se. $TTF_{invertebrate}$ is defined as

$$TTF_{invertebrate} = (AE)\,(IR) \div k_e. \quad (5)$$

For clams and mussels, AEs as low as 20% have been found for sediments containing elemental Se (Luoma et al. 1992; Roditi and Fisher 1999; Lee et al. 2006). Assimilation efficiencies of about 40% are typical for experiments in which mussels are exposed to Se adsorbed to particulate materials (see, e.g., Wang and Fisher 1996). However, both elemental and adsorbed Se are probably minor components of the food of most organisms. Assimilation of Se is more efficient when animals ingest living food or detritus, both of which are dominated by organo-Se. From these materials, AEs vary from 55 to 86% among species, with smaller differences among living food types such as different species of algae (see, e.g., Reinfelder et al. 1997; Roditi and Fisher 1999; Schlekat et al. 2004; Lee et al. 2006). If data on particulate speciation are available (see, e.g., Doblin et al. 2006; Meseck and Cutter 2006), then a composite AE may be employed. In this case, the AE for each form of the particulate Se is applied to its fraction of the total Se in sediments. However, particulate speciation data are rarely available. Because most particulate feeders seek organic material in their food, AEs of >50% are probably the best generic representation of assimilation efficiency in nature. Use of species-specific data may result in a more precise value, but validation studies suggest that use of a generic AE, determined for the species of interest with an average-type food, does not add great uncertainty to the calculations (see, e.g., Luoma et al. 1992; Luoma and Rainbow 2005).

Schlekat et al. (2004) determined physiological parameters for the copepods *Tortanus* sp. and *Acartia* sp. of AE = 52% and $k_e = 0.155$. They assumed an IR = 42% from the literature. If the copepods consume diatoms containing 0.5 μg/g Se, then bioaccumulated Se at steady state is

$$\begin{aligned} C_{copepod} &= (0.52)(0.42)(0.5\,\mu g/g) \div 0.155 \\ &= 0.72\,\mu g/g. \end{aligned} \quad (6)$$

Combining the physiological parameters gives a $TTF_{copepod}$ of 1.4. In contrast, Lee et al. (2006) determined physiological parameters for the bivalve C. *amurensis* of AE = 45%, IR = 25%, $k_e = 0.025$. If C. *amurensis* consumed phytoplankton containing 0.5 μg/g Se, then bioaccumulated Se at steady state is

$$C_{clam} = (0.45)(0.25)(0.5\mu g/g) \div 0.025 = 2.36\mu g/g, \quad (7)$$

and the TTF_{clam} is 4.5. The difference in Se concentrations between the copepod and the clam is primarily driven by the slower rate constant of loss in the bivalve compared with the copepod (i.e., 0.155 vs. 0.025). In both cases, Se concentrations increased from one trophic level to the next (TTF >1), but much more so in the bivalve.

Uncertainties about generic constants are least if species-specific and site-specific information is available for 1) assimilation efficiencies of different types of particulate matter, 2) concentrations of Se in particulate phases (such as suspended particulate material), and 3) proportions of different foods likely to be eaten by that species. Then, a concentration of Se in food can be calculated that takes into account site-specific bioavailability of particulate material to invertebrates. The generalized equation is

$$\begin{aligned} C_{particulate} = {} & (AE)(C_{particulate\,a})(\text{sediment fraction}) \\ & + (AE)(C_{particulate\,b})(\text{detritus fraction}) \\ & + (AE)(C_{particulate\,c})(\text{algae fraction}). \end{aligned} \quad (8)$$

Hypothetically, let us assume that particulate material is composed of 20% sediment, 40% detritus, and 40% algae and that Se particulate concentrations are 0.5 μg/g in sediment, 2.0 μg/g in detritus, and 4.0 μg/g in algae. From the literature, reasonable assimilation efficiencies for these phases are 15% for sediment, 35% for detritus, 60% for algae. Consequently, the particulate Se concentration for use in modeling is

$$\begin{aligned} &0.02\mu g/g \text{ from sediment} + 0.28\mu g/g \text{ from detritus} \\ &\quad + 0.96\mu g/g \text{ from algae} = 1.3\mu g/g. \end{aligned} \quad (9)$$

We compiled physiological parameters for invertebrates available in the literature in which AE, IR, and k_e data were determined for an identified test species (Supplemental Data Table B). Sufficient species-specific data, although mainly from marine species, are available from kinetic experiments to calculate $TTF_{invertebrate}$ for a number of species from different feeding guilds. These are enough data at least to begin to model important food webs. A summary of the available laboratory data for the marine environment used for modeling shows that TTFs for invertebrates vary from 0.6 for amphipods to 23 for barnacles (Table 3). The vast majority of TTFs are >1. The TTFs vary 38-fold among species, but increasing Se concentrations from the base of the food web into invertebrates is the rule, rather than the exception, for the available data. This 38-fold variability is propagated up food webs by subsequent trophic transfer steps. The result is that some predators are exposed to much higher Se concentrations than other predators.

Field-derived trophic transfer factors. The kinetic experiments cited above focused mainly on marine species; the freshwater invertebrate kinetic database is weak. However, many field studies are conducted at freshwater sites. When laboratory data are not available, a field $TTF_{invertebrate}$ can be defined from matched data sets (in dw or converted to dw) of particulate and invertebrate Se concentrations as

$$TTF_{invertebrate} = C_{invertebrate} \div C_{particulate}. \quad (10)$$

We calculated freshwater TTFs from field studies documented in the literature (Supplemental Data Table C) and summarized the TTFs by species of invertebrate for modeling (Table 3). We narrowed uncertainties inherent in the field-data approach by constraining the compilation to real-time data that have clearly defined particulate phases and food webs. Either 1) field averages of multiple matched data sets (Se concentrations in particulate material and invertebrates that is time-specific) from sites with similar food webs or 2) regressions of particulate to invertebrate Se concentrations for a series of individual sites with similar food webs were used. Nevertheless, the field TTFs are likely to be more uncertain than the laboratory-derived TTFs. The availability of additional field observed TTFs surely will be improved upon in the future.

Table 3. Summary of selected TTFs for invertebrates, fish, and birds used in modeling and validation (TTFs are derived from data and references shown in Supplemental Data Tables A, B, and C)

Invertebrate	TTF
Amphipod (marine) (*Leptocheirus plumulosus*)	0.6
Amphipod (freshwater) *(Hyalella azteca, Gammarus fasciatus, Corophium* spp.)	0.9
Mysid (marine) (*Neomysis mercedis*)	1.3
Euphausiid (marine) (*Meganyctiphanes norvegica*)	1.3
Copepod (marine) (*Acartia tonsa, Temora longicornis, Tortanus* sp., *Oithona, Limnoithona*)	1.35
Zooplankton (freshwater composite)	1.5
Crayfish (*Procambarus clarki*, Astacidae, *Orconectes* sp.)	1.6
Brine fly (*Ephydra gracilis*)	1.65
Daphnia (Daphnia magna)	1.9
Oyster (*Crassostrea virginica*)	2.05
Corixid (*Cenocorixa* sp.)	2.14
Cranefly (Tipulidae)	2.3
Brine shrimp (young) (*Artemia franciscana*)	2.4
Stonefly (Perlodidae/Perlidae, Chloroperlidae)	2.6
Damselfly (Coenagrionidae)	2.6
Mayfly (Baetidae, Heptageniidae, Ephemerellidae)	2.7
Chironomid (*Chironomus* sp.)	2.7
Clam (*Corbicula fluminea*)	2.8
Aquatic insect (average)[a]	2.8
Caddisfly (Rhyacophilidae, Hydropsychidae)	3.2
Aquatic insect composite	3.2
Brine shrimp (adult)	4.2
Clam (*Macoma balthica*)	4.5
Mussel (*Dreissena polymorpha*)	6.0
Clam (*Corbula amurensis*)	6.25
Mussel (*Mytilus edulis*)	6.3
Clam (*Puditapes philippinarum*)	11.8
Barnacle (*Elminius modestus*)	15.8
Barnacle (*Balanus amphitrite*)	20.3
Clam (*Mercenaria mercenaria*)	23
Fish (whole-body or muscle)	
Leopard shark (*Triakis semifasciata*)	0.52
Gilthead sea bream (*Sparus auratus*)	0.6
Brook trout (*Salvelinus fontinalis*)	0.77
Smooth toadfish (*Tetractenos glaber*)	0.8
Chinese mudskipper (*Periophthalmus cantonensis*)	0.84
Striped bass (juvenile) (*Morone saxatilis*)	0.89
Sucker (*Catostomus* sp.) (Utah and mountain suckers are common in Idaho)	0.97
Rainbow trout (*Oncorhynchus mykiss*)	0.98
Fathead minnow (larval and adult) (*Pimephales promelas*)	1.0
Largemouth bass (*Micropterus salmoides*)	1.0
Cutthroat trout (*Oncorhynchus clarkii*)	1.0
Bluegill (*Lepomis macrochirus*)	1.06
Mangrove snapper (*Lutjanus argentimaculatus*)	1.1
European sea bass (*Dicentrarchus labrax*)	1.1
Chub (*Gila* sp.) (Utah chub is common in Idaho)	1.2
Yellowfin goby (*Acanthogobius flavimanus*)	1.2
Western mosquitofish (*Gambusia affinis*)	1.25
White sturgeon (*Acipenser transmontanus*)	1.3
Brown trout (*Salmo trutta*)	1.3
Mountain whitefish (*Prosopium williamsoni*)	1.3
Sailfin molly (*Poecilia latipinna*)	1.4
Mottled sculpin (*Cottus bairdi*)	1.4
Longnose dace (*Rhinichthys cataractae*)	1.5
Redside shiner (*Richardsonius balteatus*)	1.5
Starry flounder (*Platichthys stellatus*)	1.6
Bird (egg)	
Mallard (*Anas platyrhynchos*)	1.8

[a]Mean of mayfly, caddisfly, crane fly, stonefly, damselfly, corixid, and chironomid.

Freshwater invertebrate TTFs compiled for modeling range from 0.9 for amphipods to 6.0 for zebra mussels (Table 3). Invertebrate TTFs fall into several broad categories in terms of bioaccumulative potential that include means of $\leq$1 for amphipods, 1.3 to 1.9 for crustaceans, 2.8 for aquatic insects, and $\geq$2.8 to 6.0 for clams and mussels. To illustrate the level of uncertainty for one group of organisms, the value for $TTF_{aquatic\ insect}$ used in modeling (2.8) can be compared with several sets of data for insects that include mayfly, caddisfly, cranefly, stonefly, damselfly, corixid, and chironomid (TTF range 2.3–3.2; Supplemental Data Table C and Table 3; Birkner 1978; Saiki et al. 1993; Casey 2005; Harding et al. 2005). Few species-specific comparisons of physiologically derived TTFs with comprehensively derived field TTFs are available (Supplemental Data Tables B and C). However, the range of values for freshwater invertebrates is remarkably

similar to that for marine invertebrates determined in the laboratory, as are the values for comparable taxa (Table 3).

TTFs are species-specific because of the influence of the physiology of the animal. They may vary to some extent as a function of the concentration in food, or if AE or IR vary (Besser et al. 1993; Luoma and Rainbow 2005). For modeling here, TTFs from laboratory studies are calculated using a chosen set of physiological or kinetic parameters, usually a mean from the range of experimental data, presented for a specific species. TTFs from field studies are calculated from averages or regressions for specific particulate material–prey pairs. These approaches lead to consideration of a single TTF to quantify trophic transfer from particulate material to invertebrate for each species. If enough data are available to develop diet–tissue concentration regressions specific to inhabitants of a watershed, then use of those regressions would provide more detailed TTFs than single determinations. Additionally, in nature, if it is assumed that organisms regulate a constant minimum concentration of Se, then the observed TTF will increase when the concentration in food is insufficient to maintain the regulated concentration. Data sets from which TTFs are derived for use in modeling here were collected from sites exposed to Se contamination and identified as problematic because of Se bioaccumulation. As noted previously, the relatively small variation of TTF within taxonomically similar animals is evidence that these potential sources of uncertainty may be classified as small (less than 2-fold; see Landrum et al. 1992).

Trophic transfer: Fish

Biodynamics and kinetic trophic transfer factors. Biodynamics can also be applied to fish that feed on invertebrates (Figures 1 and 2). Laboratory test systems extend water–particulate–invertebrate food webs by feeding radiolabeled invertebrates to fish (Reinfelder and Fisher 1994; Baines et al. 2002; Xu and Wang 2002). The mechanistic equations for modeling of Se bioaccumulation in fish tissue are the same as for invertebrates, if whole body concentrations in fish are the endpoint. The choice of C_f (i.e., the contaminant concentration in the ingested food) for fish should reflect the preferred foods of the specific species. Thus, modeling is specific for each fish species in terms of both physiology and food choices.

Uptake of selenite from solution contributes even less to bioaccumulation in fish than it does in invertebrates. For example, the calculated tissue component attributable to dissolved selenite using experimentally determined physiological parameters for mangrove snapper (*Lutjanus argentimaculatus*) is <0.16% (Xu and Wang 2002).

In the absence of rapid growth, the exposure equation for a fish that eats aquatic insects, for example, simplifies to

$$C_{fish} = [(AE)(IR)(C_{invertebrate})] \div [k_e]. \quad (11)$$

A TTF_{fish} characterizes the potential for each fish species to bioaccumulate Se and is defined as

$$TTF_{fish} = (AE)\,(IR) \div k_e. \quad (12)$$

Complete species-specific information (i.e., AE, IR, k_e) from kinetic experiments is available for few fish species (Supplemental Data Table D). To expand the limited kinetic database for fish species, entries that contain some measured values and some assumed parameters (e.g., 5% ingestion rate, 50% assimilation efficiency) are included. For modeling, we compiled TTF_{fish} by combining these physiological parameters for each fish species for which some experimental data are available (Table 3).

Selenium concentration in whole-body fish is calculated in modeling because that type of data is experimentally available, routinely collected, and proposed for Se regulation. Transfer to fish ovaries or egg tissue is more meaningful in terms of a direct connection to reproductive endpoints, but available data are scant (North America Metals Council 2008). Additional conversion factors could be derived to link to ovary or egg Se concentrations (Lemly 2002).

Xu and Wang (2002) determined physiological parameters for mangrove snapper (AE = 69%, IR = 5%, $k_e = 0.027$). To calculate a TTF_{fish}, if a snapper consumes brine shrimp larvae with an Se concentration of 5 μg/g, then the calculated snapper tissue Se concentration is

$$C_{snapper} = (0.69)(0.05)(5\mu g/g) \div 0.027 = 5.6\mu g/g. \quad (13)$$

Some increase in snapper Se concentration is shown in this example, insofar as the $TTF_{snapper}$ is 1.1. For comparison, Baines et al. (2002) determined physiological parameters for juvenile striped bass (*Morone saxatilis*; AE = 42%, IR = 17%, $k_e = 8\%$). If a bass consumes brine shrimp with an Se concentration of 5.0 μg/g, the calculated bass tissue Se concentration is

$$C_{striped\,bass} = (0.42)(0.17)(5.0\mu g/g) \div 0.08 = 4.46\mu g/g. \quad (14)$$

The $TTF_{striped\ bass}$ is 0.89, signifying efficient food web transfer but an accumulated body burden slightly less than that occurring in the invertebrate diet.

Field-derived trophic transfer factors. Given the paucity of experimental kinetic data for fish, we reviewed field data to obtain species-specific TTFs relevant to freshwater and marine fish (Supplemental Data Table D). A field derived species-specific TTF_{fish} is defined as

$$TTF_{fish} = C_{fish} \div C_{invertebrate}, \quad (15)$$

where $C_{invertebrate}$ is for a known prey species, C_{fish} is reported as muscle or whole-body tissue, and both Se concentrations are reported in dw. The calculations were constrained as described above for field-derived $TTF_{invertebrate}$ by using real-time data and those studies that have clearly defined food webs (i.e., matched data sets of invertebrate and fish Se concentrations in dw). Derived freshwater TTF_{fish} are summarized by species for modeling (Table 3). For example, a species-specific $TTF_{white\ sturgeon}$ of 1.3 was calculated from field studies of San Francisco Bay using matched data sets for clams and sturgeon. Species-specific TTFs of 1.04 and 0.91 (mean 0.98) were calculated for rainbow trout from field studies in southeast Idaho, USA, and Alberta, Canada, using matched data sets for aquatic insects (mainly mayflies) and trout (Supplemental Data Table D and Table 3). The range of TTFs derived for fish from laboratory experiments and field data is remarkably similar, with a mean TTF of 1.1 for 25 fish species. TTFs for all fish species fall within a relatively narrow range (0.5–1.6, or less than a 4-fold variation) compared with those among

invertebrate species (38-fold variation; Table 3). Consequently, variability in bioaccumulated Se among fish species and among food webs is driven more by a fish species' dietary choice of invertebrate and the bioaccumulation kinetics of that invertebrate than by differences in dietary transfer to the fish itself.

Most fish, of course, eat a mixed diet, with tendencies toward certain types of foods. Modeling of Se bioaccumulation can represent a diet that includes a mixed proportion of prey in the diet through use of the equation

$$\begin{aligned} C_{fish} = (TTF_{fish})[&(C_{invertebrate\,a})(\text{prey fraction}) \\ &+ (C_{invertebrate\,b})(\text{prey fraction}) \\ &+ (C_{invertebrate\,c})(\text{prey fraction})]. \end{aligned} \quad (16)$$

For example, using a hypothetical, but typical, TTF_{fish} of 1.1, a mixed invertebrate diet of 50% amphipods at 1.8 μg/g, 25% daphnids at 3.8 μg/g, and 25% chironomids at 5.6 μg/g, the equation yields

$$\begin{aligned} 1.1[&(1.8\mu g/g)(50\%) + (3.8\mu g/g)(25\%) \\ &+ (5.6\mu g/g)(25\%)] \\ &= 3.6\mu g/g. \end{aligned} \quad (17)$$

This Se concentration is in contrast to a concentration of 6.2 μg/g if a single component diet of chironomids is considered.

Modeling of fish tissue can also represent stepwise or sequential bioaccumulation from particulate material through invertebrate to fish by combining the equations

$$\begin{aligned} C_{invertebrate} &= (TTF_{invertebrate})(C_{particulate}) \text{ and } C_{fish} \\ &= TTF_{fish}(C_{invertebrate}). \end{aligned} \quad (18)$$

to give

$$C_{fish} = (TTF_{invertebrate})(C_{particulate})(TTF_{fish}). \quad (19)$$

For example, if a stream contains a particulate Se concentration of 2 μg/g and is inhabited by trout (TTF 1.0) that are eating a single invertebrate diet of mayflies (TTF 2.8), then the fish-tissue Se concentration, C_{trout}, derived from the particulate material Se concentration is 5.6 μg/g.

Modeling can also accommodate longer food webs that contain more than one higher-trophic-level consumer (e.g., forage fish being eaten by predatory fish) by incorporating additional TTFs. One equation for this type of example is

$$\begin{aligned} C_{predator\,fish} = (TTF_{invertebrate})(C_{particulate})(TTF_{forage\,fish}) \\ (TTF_{predator\,fish}). \end{aligned} \quad (20)$$

Trophic transfer: Birds

Trophic transfer factors. A link to wildlife, as illustrated here for aquatic-dependent birds, is not as straightforward as in the case for fish (Figure 1). Little information is available for a biodynamic approach to modeling exposure of birds through water and diet. Theoretically, the biodynamic exposure equation for a selected bird species would be similar to that for fish. The equation for calculating a bird tissue Se concentration for a single invertebrate diet is

$$C_{bird} = (AE)(IR)(C_{invertebrate}) \div (k_e). \quad (21)$$

A TTF_{bird} can be defined either as

$$TTF_{bird} = (AE)(IR) \div k_e \quad (22)$$

or

$$TTF_{bird} = C_{bird} \div C_{invertebrate} \quad (23)$$

to give

$$C_{bird} = (TTF_{bird})(C_{invertebrate}). \quad (24)$$

Selenium concentration in bird tissue can be for muscle if desired, but transfer to egg tissue is more meaningful in terms of a direct connection to reproductive endpoints.

Modeling of bird tissue can represent stepwise or sequential bioaccumulation from particulate material through invertebrate to bird by combining the equations

$$\begin{aligned} C_{invertebrate} &= (TTF_{invertebrate})(C_{particulate}) \text{ and } C_{bird} \\ &= TTF_{bird}(C_{invertebrate}) \end{aligned} \quad (25)$$

to give

$$C_{bird} = (TTF_{invertebrate})(C_{particulate})(TTF_{bird}). \quad (26)$$

Modeling for bird tissue can also represent Se transfer through longer or more complex food webs (e.g., additional TTFs for invertebrate to fish and fish to birds) by combining the equations

$$\begin{aligned} C_{invertebrate} &= (TTF_{invertebrate})(C_{particulate}); C_{fish} \\ &= TTF_{fish}(C_{invertebrate}) \end{aligned} \quad (27)$$

and

$$C_{bird} = (TTF_{bird})(C_{fish}) \quad (28)$$

to give

$$C_{bird} = (TTF_{invertebrate})(C_{particulate})(TTF_{fish})(TTF_{bird}). \quad (29)$$

Modeling approach. Laboratory data relating dietary Se concentrations to egg Se concentrations are used for modeling and derivation of TTFs of birds. A synthesis of data from controlled feeding of captive mallards (*Anas platyrhynchos*) exposed to known dietary Se concentrations showed the relationship of egg hatchability and egg tissue Se concentration (i.e., a dose–response curve; Ohlendorf 2003). Ohlendorf (2003) conducted logistic regressions on a set of pooled results from different studies to be able to calculate mean Se concentrations that are associated with different percentages of reduction in the hatchability of mallard eggs (e.g., the 10% effect concentration or EC10 is associated with a 10% reduction in hatchability). The range of $TTF_{bird\ egg}$ calculated from the compilation given by Ohlendorf (2003) for mallards is 1.5 to 4.5. Although mallards are believed to be a sensitive species based on reproductive endpoints in the laboratory, chickens and quail were shown to be more sensitive than mallards (Detwiler 2002). An order that reflects the effects of field factors present at Kesterson

Reservoir, California, USA, and is based on the number of dead or deformed embryos or chicks is (coot = grebe) > (stilt = duck = killdeer) > avocet (Ohlendorf 1989; Skorupa 1998).

The model can be run using any chosen $TTF_{bird\ egg}$, but a $TTF_{bird\ egg}$ of 1.8 (near the lower limit from the captive mallard studies) will be assumed here for modeling purposes (Table 3). Generalized species-specific or site-specific, species-specific TTFs for birds may also be derived from field studies, as was suggested for fish, which would take into account variables intrinsic to bird behavior and habitat use. Resident bird species nesting in a contaminated area may be the best choice for such a compilation.

TOXICITY: EFFECTS

Linking modeling to effects requires knowledge of species toxicological sensitivity through 1) effect guidelines for diet or tissue based on chronic Se exposure of predators; 2) toxicity reference values (TRV) specific to target receptor groups, endpoints, exposure routes, and uncertainty levels; or 3) national, state, or local regulatory guidance on diet or tissue Se concentrations. The chosen guideline can link diet, fish tissue, or bird tissue to toxicity.

Several authors give comprehensive compilations of Se guidelines (USDOI 1998; Lemly 2002; Presser and Luoma 2006; Luoma and Rainbow 2008). The controversy over choice of protective levels of Se for fish and birds is intense in part as a result of the steepness of the Se dose–response curves and the use of different models for quantifying those relationships (Skorupa 1998; Lemly 2002; Ohlendorf 2003; Beckon et al. 2008). Specificity in several variables based on experimental conditions when referencing a Se guideline is desirable. These variables include 1) endpoint (e.g., toxicity, reproductive, survival, growth, immunosuppression); 2) life stage (e.g., larvae, fry, adult); 3) form (e.g., selenate, selenite, selenomethionine, selenized yeast); 4) route of transfer (e.g., dietary, maternal); 5) definition of protection (e.g., threshold, toxicity level, criterion, target); and 6) toxicity basis (e.g., EC10). In general, for Se, reproductive endpoints are more sensitive than toxicity and mortality in adult birds and fish (Skorupa 1998; Lemly 2002; Chapman et al. 2010). Within reproductive endpoints, larval survival in fish and hatchability (i.e., embryo survival) in birds are considered the most sensitive endpoints. Effects guidelines that focus on a combination of the most sensitive assessment measures might include, for example, a seleno-methionine diet, parental exposure, and embryonic or larval life stage (Presser and Luoma 2006).

Any criterion, guideline, or target may be used in modeling to predict effects on predators, and, whatever the choice, the model can give its implications. For illustration purposes, we use a single value for each type of effects guideline (dietary = 4.5 μg/g dw, fish whole body = 5 μg/g dw, and bird egg = 8 μg/g dw), while recognizing that debate is still occurring about determining critical tissue values that relate bioaccumulated Se concentrations to toxicity in predators.

VALIDATION AND APPLICATION OF METHODOLOGY

Validation

Validation is necessary to establish sufficient confidence that the predictions from a model can be usefully applied to the environment. Advantages of the ecosystem-scale approach are that some aspects of the model are built from observations from natural systems, and the predictions from the biodynamic model center around bioaccumulated Se in a specific species. Thus, predictions from the model can be unambiguously compared with independent observations of Se concentrations in that same species resident in the environment of interest. The comparison of these 2 independent values illustrates both validity and uncertainty.

We tested the proposed methodology by comparing predictions and observations from 29 locations that were either historically, or are presently, affected by Se (Table 4 and Supplemental Data Tables E and F). The case studies include several types of hydrologic regimes, streams, rivers, ponds, lakes, reservoirs, wetlands, and estuaries, and many species of invertebrates, fish, and birds (see Supplemental Data). Sources of Se and food webs represented at sites used for the validation are also shown in Table 4. All sites are relatively well-known for associated Se contamination, and many are still in remediation or being mitigated because of ecosystem bioaccumulation of Se. In all case studies, reasonable food webs were identified and sufficient high-quality field data were available across media (particulate material, invertebrates, fish and/or bird tissue) and during a constrained time period (i.e., data were temporally and spatially matched; Supplemental Data Tables E and F). In 3 study area investigations (Kesterson Reservoir, McLeod River/Luscar Creek watershed, San Joaquin River), sites identified as reference sites are included to help illustrate the prediction capability of the model at the lower end of the concentration gradient.

The equations used for validation begin with a particulate material Se concentration, and thus do not incorporate the uncertainties associated with dissolved and/or particulate transformations (K_d), which we address below. We progressively calculate 1) invertebrate Se concentrations from particulate material, and 2) fish or bird tissue Se concentrations from the predicted invertebrate Se concentrations. Combining the progressive equations

$$C_{invertebrate} = (C_{particulate})(TTF_{invertebrate}), \quad (30)$$

$$C_{fish} = (C_{invertebrate})(TTF_{fish}), \quad (31)$$

and

$$C_{bird\ egg} = (C_{invertebrate})(TTF_{bird}) \quad (32)$$

yields

$$C_{fish} = (C_{particulate})(TTF_{invertebrate})(TTF_{fish}) \quad (33)$$

and

$$C_{bird\ egg} = (C_{particulate})(TTF_{invertebrate})(TTF_{bird}). \quad (34)$$

Thus, this approach tests whether bioaccumulation at the invertebrate and predator trophic levels can be predicted accurately if particulate Se concentrations are known.

For the predictions of Se concentrations in invertebrates, the observed particulate Se concentration at a site is multiplied by a species-specific TTF for the species of invertebrate in the identified food web (Supplemental Data Table E). The TTFs selected for use in the validation are a subset of those given in Table 3. The case studies allow 101 paired predicted

Table 4. Site locations, associated Se sources, and available prey and predator data for case studies used in model validation (see Supplemental Data Tables E and F for data sets)

Location or watershed	Sources	Available prey data	Available predator data
Belews Lake, North Carolina	Coal fly-ash disposal	Phytoplankton + zooplankton, insect, mollusk, crustacean, annelid	Bluegill, warmouth, redear sunfish, pumpkinseed, largemouth bass
Cienega de Santa Clara, Colorado River Delta	Agricultural drainage	Brine shrimp, crayfish	Sailfin molly, largemouth bass, striped mullet, common carp
Converse County, Wyoming	Uranium mining	Grasshopper	Red-winged blackbird
Elk River and Fording River watersheds, British Columbia, Canada	Coal mining	Insect, composite benthic invertebrate, mayfly, stonefly, caddisfly, cranefly	Cutthroat trout, mountain whitefish, American dipper, spotted sandpiper
Goose Lake, Kendrick Reclamation Project, Wyoming	Agricultural drainage	Composite insect	Eared grebe
Great Salt Lake, California	Copper mining	Brine shrimp, brine fly	American avocet, black-necked stilt, California gull
Hyco Reservoir, North Carolina	Coal fly-ash disposal	Benthic insects	Bluegill
Illco Pond, Kendrick Reclamation Project, Wyoming	Agricultural drainage	Composite insect	Common carp
Imperial National Wildlife Refuge, Lower Colorado River watershed, Arizona and Colorado	Agricultural drainage	Clam, crayfish	Lesser nighthawk, green heron, pied-billed grebe, least bittern
Kesterson National Wildlife Refuge, California	Agricultural drainage	Net plankton, corixid, chironomid, dragonfly, damselfly, beetle, diptera	Western mosquitofish (die-off of other fish species); pied-billed and eared grebe, American coot, mallard, gadwall, cinnamon teal, northern pintail, redhead, ruddy duck, black-necked stilt, American avocet, killdeer, western meadowlark, tri-colored blackbird, cliff and barn swallow
McClean Lake area, Saskatchewan, Canada	Uranium mining	Chironomid, caddisfly, dragonfly, leech, snail	Northern pike, white sucker, stickleback, burbot
McLeod River/Luscar Creek watersheds, Alberta, Canada	Coal mining	Insect	Rainbow, brook, and bull trout, mountain whitefish
Miller's Lake, Colorado	Agricultural drainage	Chironomid, corixid, crayfish	Fathead minnow
Newport Bay, California	Agricultural drainage	Amphipod, bivalve, clam, mussel, isopod, clam, snail	Topsmelt, diamond turbot, deep body anchovy, California halibut, striped mullet, California killifish, shadow, arrow and cheekspot goby, barred and spotted sand bass, staghorn sculpin, black and pile surfperch, American avocet, black-necked stilt, killdeer, clapper rail, pied-billed grebe, least tern, black skimmer
Rasmus Lee Lake, Kendrick Reclamation Project, Wyoming	Agricultural drainage	Composite insect	American avocet
Red Draw Reservoir, Big Spring, Texas	Refinery waste	Chironomid, snail	Inland silverside, sheepshead minnow, gulf killifish

TABLE 4. (*Continued*)

Location or watershed	Sources	Available prey data	Available predator data
Salton Sea, California	Agricultural drainage	Amphipod, corixid, pileworm	Largemouth bass, sargo, redbelly and Mozambique tilapia, Gulf croaker, orangemouth corvina, channel catfish, Caspian tern, white-faced ibis, snowy egret, black skimmer, great egret, black-crowned night heron
San Diego Creek watershed, California	Urban drainage	Zooplankton, corixid, crayfish, clam, snail, backswimmer, chironomid	Western mosquitofish, common carp, American avocet, black-necked stilt, killdeer, pied-billed grebe, American coot, clapper rail
San Francisco Bay–Delta Estuary, California	Oil refinery effluent agricultural drainage	Clam, zooplankton, amphipod, isopod, shrimp	White sturgeon, striped bass, starry flounder, yellowfin goby, leopard shark, Sacramento splittail
San Joaquin River watershed, California	Agricultural drainage	Zooplankton, amphipod, chironomid, crayfish	Bluegill, largemouth bass
Savage River watershed (Blacklick Run), Maryland	Coal stack emissions	Crayfish, mayfly, caddisfly, cranefly, stonefly, dragonfly, dobsonfly	Mottled sculpin, blacknose dace, brook trout
Savannah River (D-area Power Plant), South Carolina	Coal fly-ash disposal	Composite, benthic invertebrates	Lake chubsucker
Sweitzer Lake, Colorado	Agricultural drainage	Damselfly, chironomid, crayfish	Plains killifish
Thompson Creek, Idaho	Molybdenum mining	Composite insect	Slimy sculpin, cutthroat/rainbow trout
Tulare Basin Ponds, California	Agricultural drainage	Brine shrimp, brine fly larvae, corixid, damselfly	American avocet, black-necked stilt, eared grebe
Twin Buttes Reservoir, Wyoming	Agricultural drainage	Plankton, amphipod, corixid, damselfly, chironomid	Plains killifish, Iowa darter, fathead minnow
Uncompahgre River watershed, Colorado	Agricultural drainage	Invertebrates with some insects, crayfish	Bluehead flannelmouth and white sucker, speckled dace, roundtail chub, green sunfish
Upper Blackfoot River watershed, Idaho	Phosphate mining	Insect, composite benthic invertebrate	Cutthroat, brook, and brown trout, mountain whitefish, longnose dace, mottled sculpin, common snipe, American coot, killdeer, eared grebe
Upper Mud Reservoir/Mud River watershed, West Virginia	Coal mining	Dragonfly, crayfish, clam, snail	Bluegill, green sunfish, crappie

and observed data points for invertebrates (Figure 3). The data range across the entire data set probably covers the full extent of Se concentrations that might be expected from the most to the least contaminated environments. The agreement is remarkable, with a calculated correlation coefficient (r^2) for predicted and observed invertebrate Se concentrations of 0.917 ($p < 0.0001$) (Figure 3).

The second correlation compares observed Se concentrations in fish with concentrations predicted from observed particulate concentrations, the previously predicted invertebrate Se concentrations using the most likely food choice of that particular species of fish, and the universal choice of a TTF_{fish} of 1.1 (Supplemental Data Table E). In some cases, when several invertebrate Se concentrations were predicted, an average invertebrate Se concentration was used to predict a fish Se concentration. In cases in which Se concentrations in diet were elevated enough to cause fish die-offs (e.g., Belews Lake, Hyco Reservoir, Kesterson Reservoir, Sweitzer Lake; Skorupa 1998), trophic transfer of Se in fish may be additionally affected by poor feeding efficiency and food avoidance (Hilton et al. 1980; Finley 1985). The case studies allow 46 paired predictions and observations for fish (Figure 4). Again, the agreement is remarkable, with $r^2 = 0.892$ ($p < 0.0001$). These strong regressions show that, if particulate Se concentrations are known and food webs are considered in an ecologically based way, bioaccumulation in the different food webs of an ecosystem can be reliably predicted.

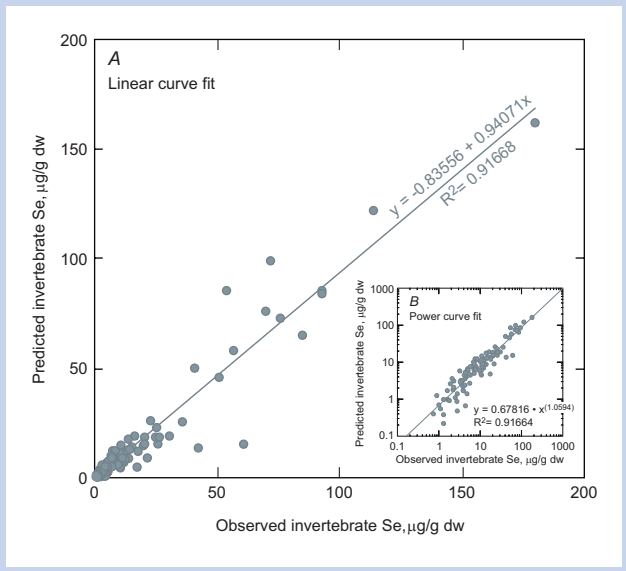

Figure 3. Linear regression and correlation of observed invertebrate Se concentrations from selected field studies and those predicted through ecosystem-scale modeling. Inset shows a power curve fit of data. See Supplemental Data Table E for data and references.

In the same manner, predictions are made of Se concentrations in birds that consume a diet of invertebrates or fish using a TTF_{bird} of 1.8 (Supplemental Data Table F). Because of the severity of exposure at several historical sites (e.g., Kesterson Reservoir, Tulare Basin Ponds, Rasmus Lee Lake, Goose Lake), factors such as food avoidance and poor physical condition might have affected feeding and hence trophic transfer of Se in birds (Ohlendorf et al. 1988; Heinz and Sanderson 1990; Heinz and Fitzgerald 1993; Ohlendorf 1996; Skorupa 1998). At these sites, predicted egg Se concentrations were above observed concentrations. At other sites, predicted bird egg Se concentrations were in the range of observed Se concentrations. The comparison for birds is hampered by the lack of data compared with data for fish, but it is illustrative of a comparable methodology for wildlife. Application of a TTF_{bird} of 1.8 may be useful under certain conditions, but selective regressions of data over a narrow range to represent site-specific conditions or a wildlife criterion methodology (discussed below) may better represent Se transfer at specific sites. This is an area in which greater understanding of the prey-to-predator kinetics in birds is needed.

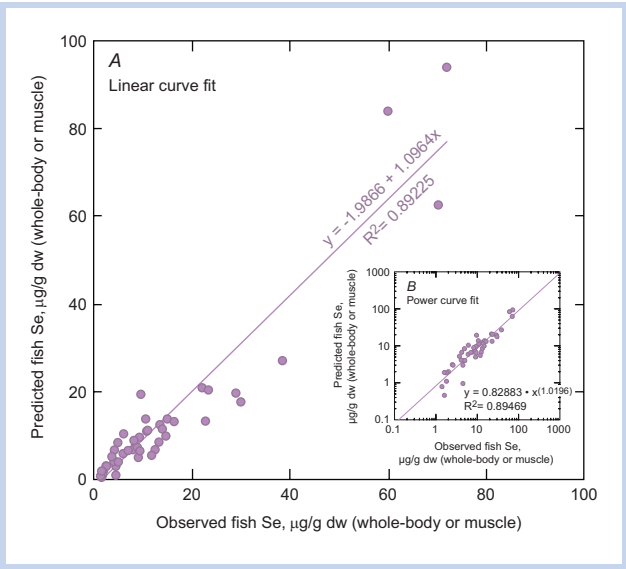

Figure 4. Linear regression and correlation of observed fish Se concentrations from selected field sites and those predicted through ecosystem-scale modeling. Inset shows a power curve fit of data. See Supplemental Data Table E for data and references.

Application

The value of the ecosystem-scale methodology lies in its explanation of how a predator might be accumulating an Se concentration that, for example, exceeds the choice of criterion, guideline, or target concentration in its tissues. The step-by-step approach of the methodology (Figure 1) provides a means of linking water-column Se concentrations to Se bioaccumulation with much more certainty that does the traditional correlation approach. The methodology can also describe implications of different choices of dietary or tissue guidelines. For example, a water-column concentration responsible for an observed bioaccumulated Se concentration can be determined in any specific environment for which some data are available (or a reasonable scenario can be defined). Similarly, it is possible to calculate water-column Se concentrations that might be allowable under a given set of conditions if the environment is to comply with a chosen fish tissue guideline.

Translations to water-column Se concentration and load. The discussions and equations given above address the complexity associated with each major variable listed in Table 1 and quantify the major contributors to Se bioaccumulation within an ecosystem. The complexity of nature is viewed by some as deterring use of such models in simpler applications of effects guidelines. However, even in the absence of site-specific data, simplified choices of model factors can be based on rudimentary knowledge of a watershed and its species-specific food webs, and outputs can be used for the purposes of establishing a perspective on management decisions. For example, one application of the model might be to translate bioaccumulated Se in a predator (observed or established by a model scenario) to the water-column concentration that might be responsible for that body burden, in that specific environment. This could be an instructive exercise for facilitating implementation of a fish tissue or wildlife guideline by allowing visualization of the change in water-column concentration that would be necessary to achieve the tissue guideline.

Several important choices (Table 5) based on information about the watershed or water body must be made to initiate an exercise such as translation.

1. The choice of a predator food web is the basis for derivation of an allowable water-column concentration and allowable load. Several fish species or the most Se-sensitive fish species may be considered as starting points. It should be remembered that sensitivity of a fish species is defined by both potential for exposure (does the fish eat an

Table 5. Steps in ecosystem-scale Se methodology for translation of a tissue Se guideline to a water-column Se concentration for protection of fish

Translation of Fish Tissue Guideline or Criterion to Water-Column Concentration
Develop a conceptual model of food webs in watershed
Choose toxicity guideline for fish in watershed
Choose fish species to be protected in watershed
Choose species-specific TTF_{fish} or use default TTF_{fish} of 1.1
Identify appropriate food web for selected fish species based on species-specific diet
Choose $TTF_{invertebrate}$ for invertebrates in selected food web or use default $TTF_{invertebrate}$ for taxonomic group of invertebrate
Choose K_d indicative of 1) generalized source of Se and receiving water conditions, or 2) site-specific hydrologic type and speciation; or use a default K_d of 1000
Solve equation(s) for allowable water-column concentration for protection of fish
If assume single invertebrate diet · $C_{water} = (C_{fish}) \div (TTF_{fish})(K_d)(TTF_{invertebrate})$
If assume a mixed diet of invertebrates · $C_{water} = (C_{fish}) \div (TTF_{fish})(K_d)[(TTF_{invertebrate\ a})(\text{prey fraction})] + [(TTF_{invertebrate\ b})(\text{prey fraction})] + [(TTF_{invertebrate\ c})(\text{prey fraction})]$
If assume sequential bioaccumulation in longer food webs · $C_{water} = (C_{fish}) \div (TTF_{fish})(K_d)(TTF_{invertebrate\ a})(TTF_{forage\ fish})$ · $C_{water} = (C_{fish}) \div (TTF_{fish})(K_d)(TTF_{TL2\ invertebrate})(TTF_{TL3\ invertebrate})(TTF_{TL3\ fish})$

invertebrate that is a strong bioaccumulator?) and its response in dietary toxicity tests.

2. A TTF must be chosen for invertebrate-to-fish transfer. If a TTF_{fish} specific to the local food web is not available, then a value of 1.1 can be assumed based on the mean value from 25 fish species (Table 3).
3. The choice of a fish species sets the choice of dietary prey; in general, what species of prey does this fish consume?
4. Particulate-to-prey kinetics are incorporated via TTFs for major species of invertebrates, such as those chosen in our validation exercise. These TTFs can then be used to represent a set of common food webs (Table 3).
5. The choice of a value to link water-column concentration to particulate concentration (our K_d) is an exacting challenge. Local data can narrow the range of choices, as long as they are high-quality analytical data. In the absence of a rich data set, the range can be narrowed based on hydrologic and speciation conditions, for example, using the data in Table 2. A K_d of 1000 is a default case that may be an environmentally conservative choice for environments other than reservoirs, estuaries, and the oceans. In any case, it is critical that the assumptions behind the choice of K_d be made explicit, and the potential variability in this crucial factor be recognized. In the absence of well-developed site models, the choice of K_d is usually the greatest source of uncertainty among model parameters.

Once these choices are made, the generalized equation for translation of a fish tissue Se concentration to water-column Se concentration is

$$C_{water} = (C_{fish}) \div (TTF_{fish})(TTF_{invertebrate})K_d, \quad (35)$$

where $(K_d)(C_{water})$ is substituted for $C_{particulate}$ and the equation is solved for C_{water} (Table 5). An analogous equation for translation of a bird egg Se concentration is

$$C_{water} = (C_{bird\,egg}) \div (TTF_{bird})(TTF_{invertebrate})K_d. \quad (36)$$

As an illustration, predators are consuming a diet exclusively composed of one invertebrate species. For example, if the effects guideline is an Se concentration of 5 μg/g in whole-body fish tissue and the selected site is a lake (hypothetical K_d of 1000) inhabited by sunfish (TTF of 1.1) that are eating a diet of mayfly larvae (TTF of 2.8), then the allowable water-column concentration for the lake is

$$C_w = 5\mu g/g \div [1.1 \times 2.8 \times 1000] = 1.6\mu g/L. \quad (37)$$

Under a food web scenario in which a fish with a similar TTF eats *Daphnia* (TTF of 1.9), the allowable Se water-column concentration is

$$C_w = 5\mu g/g \div [1.1 \times 1.9 \times 1000] = 2.4\mu g/L. \quad (38)$$

Table 5 also shows an equation that considers longer food webs. Despite some uncertainty at every biological step and even greater uncertainty with regard to transformation, the predicted allowable values fall across the range of values characteristic of contaminated situations.

Model sensitivity. To test the sensitivity of the predictions to differences in invertebrate species, dissolved concentrations of Se are predicted across a range of invertebrate species [mysid, *Daphnia*, mayfly, clam (*C. amurensis*), and barnacle (*E. modestus*)] using species-specific TTFs (Figure 5). Assumptions are 1) a guideline for whole-body fish tissue of 5 μg/g, 2) a hypothetical K_d of 1000, and 3) a TTF_{fish} of 1.1. The allowable water-column Se concentrations associated with the 5 specific food web exposures that would protect predators under the specified assumptions range from 3.5 μg/L for an invertebrate diet of exclusively mysids to 0.28 μg/L for an invertebrate diet of barnacles.

If 5 μg/g represents a whole-body Se guideline for fish and the TTF_{fish} is relatively constant (i.e., averaging 1.1 among all species of fish for which data were available), then an alternative strategy is a dietary guideline for fish. For the purposes of illustration, we employ a dietary guideline of 4.5 μg/g under these assumptions. Using a paired 8 μg/g bird egg Se guideline and a TTF_{bird} of 1.8 gives 4.4 μg/g for an allowable diet for birds. This similarity in allowable dietary Se concentrations for both fish and birds reinforces the hypothesis that fish and birds are of similar sensitivity in a general sense. Because the dietary guidelines are similar, the graph depicting protective concentrations for fish would apply to the protection of birds (Figure 5). If this were not the case, 2 graphs would be necessary to depict predictive protective Se concentrations for fish and birds. The difference in protection for fish and birds may also diverge in site-specific instances in which detailed predator-specific data are available to determine TTFs across a range of concentrations.

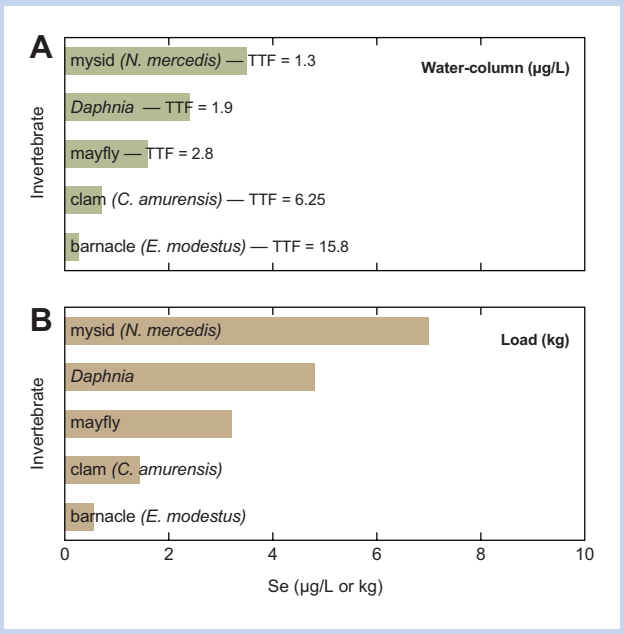

Figure 5. Predicted allowable water-column Se concentrations and Se loads based on choices of invertebrates in food webs. Assumptions are TTF_{fish} of 1.1, whole-body fish guideline of 5.0 µg/g dw, K_d of 1000, and stream flow of 1.2 Mm^3. See Table 3 for TTFs for invertebrates and Equation 39 for consideration of load.

Regulatory considerations such as NPDES permits and TMDLs for 303d listed water bodies put limits on loads. A fundamental equation to calculate load is

$$(C_{water-column})(volume)(10^{-6}) = load, \qquad (39)$$

where the water-column concentration is in µg/L, volume is in cubic meters (m^3), and load is in kilograms (Presser and Luoma 2006). We use this exceptionally simplified approach to consider Se loading at a site to calculate the hypothetical loads associated with the different food webs illustrated in Figure 5A. These loads (Figure 5B) are calculated based on the predicted allowable water-column Se concentrations (Figure 5A) and an assumed waste stream flow of 1.2 million m^3 (Mm^3). Under the different exposure scenarios for fish, loads vary from 0.56 to 7.0 kg depending on the choice of the invertebrate that is consumed by fish in the selected food web (Figure 5B). Of course, this is only an illustration of the ultimate linkage to source loads that modeling can provide (Figure 1). More sophisticated load models are recommended when calculating loads from concentrations and volumes, and, again, it is critical that predictions be explicit about why a specific K_d was chosen and the potential variability in that choice.

The translation approach of the ecosystem-scale model, of course, can start with any media (dissolved, particulate, diet, tissue) and translate to any other media, as long as the food web is known (or assumed; Figure 1). In all cases, it is important to connect the appropriate fish species to the appropriate food (i.e., biologically correct or observed knowledge of prey–predator pairs) to illustrate the potential for bioaccumulation within a watershed. Uncertainties can be greatly narrowed if part of the risk management strategy is for an agency or stakeholders to decide which predators are the most important to protect.

Table 5 formalizes the steps in a fish tissue water-column translation. Following these steps would facilitate risk management for Se based on a tissue guideline. As shown above, equations can be included that are appropriate for mixed invertebrate diets and longer food webs (e.g., forage fish being eaten by predatory fish). The steps in this approach (Table 5) are simple enough to be widely used in a management context but address the complexity of a specified ecosystem sufficiently to reduce uncertainty well below that of conventional approaches.

Hypothetical case studies and site-specific conceptualization. One outcome of the application of the ecosystem-scale model is explicit recognition that allowable dissolved Se concentrations and loads will vary among environments. The degree of such variability that is possible can be shown by predictions of allowable dissolved concentrations for different watershed types and food web scenarios. To illustrate a full range of possible conditions, we modeled realistic scenarios based on the previously compiled field case sites and ecosystem habitats (Figure 6). The illustrated K_d categories are broadly indicative of 1) an estuary, 2) a reservoir, 3) a mainstream river, 4) a backwater, 5) a saline lake or pond, and 6) a wetland (Table 2). Species-specific TTFs are employed based on Table 3. To illustrate the discussion here, translation is for a fish tissue guideline of 5 µg/g dw whole body and an avian egg guideline of 8.0 µg/g dw (see also under *Toxicity*). These targets are applied to starry flounder, white sturgeon, Sacramento blackfish, redear sunfish, bluegill, cutthroat trout, and largemouth bass as examples of fish species and black-necked stilt, American dipper, eared grebe, and greater scaup as examples of bird species. Some of the illustrations reflect food webs of historically contaminated sites (e.g., Kesterson Reservoir, Belews Lake, San Francisco Bay–Delta Estuary), and others reflect food webs of current areas of contamination (e.g., mountain streams in Idaho and British Columbia, Great Salt Lake).

A range of Se water-column concentrations from 0.24 to 34 µg/L is predicted as protective of the different predators that are the targets of the assumed guidelines in the illustrated exposure scenarios (Figure 6). For fish, an exposure scenario that has a very low K_d (mainstream river, 150) and low food web potential (bluegill eating amphipods, $TTF_{fish} = 1.1$, $TTF_{invertebrate} = 0.9$) predicts a water-column Se concentration of up to 34 µg/L (Figure 6A). If the river is transported through a watershed into a hydrologic area of differing K_d, for example, into a backwater where the flow is decreased ($K_d = 350$), then trout consuming insects would require a much lower Se concentration in the water column (4.6 µg/L; Figure 6B).

An exposure scenario for a reservoir with a K_d of 1800 that is reflective of more opportunities for transformation and a food web that contributes to significant accumulate of Se in prey and predators (redear sunfish eating freshwater clams, $TTF_{fish} = 1.1$, $TTF_{invertebrate} = 2.8$) predicts a water-column Se concentration of less than 1 µg/L (Figure 6C). However, if Sacramento blackfish in the reservoir are consuming only zooplankton ($TTF_{fish} = 1.1$, $TTF_{invertebrate} = 1.5$), then modeling predicts a water-column Se concentration of 1.7 µg/L. Estuaries require the lowest water-column Se concentrations

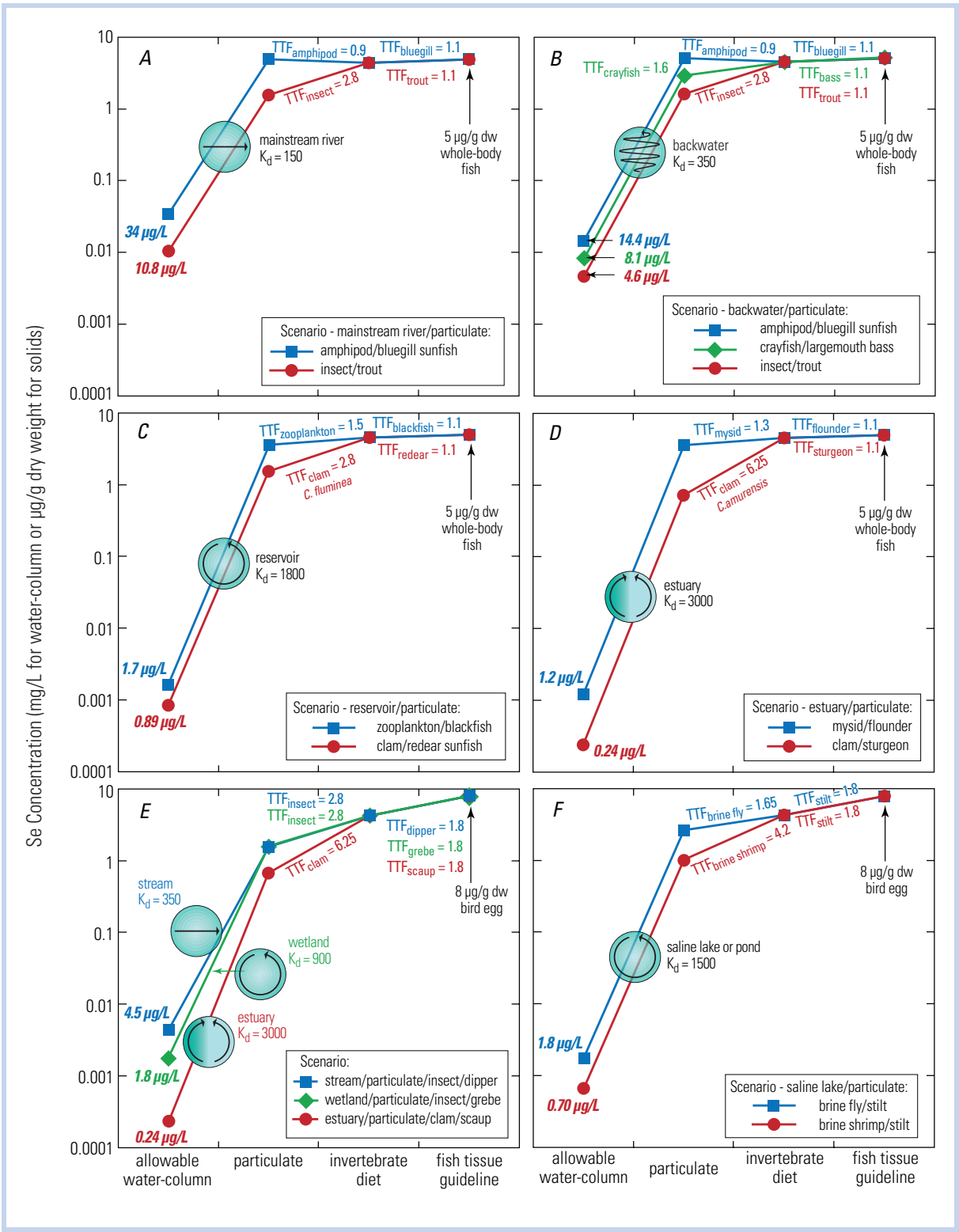

Figure 6. Range of predicted allowable water-column Se concentrations for various environmental exposure scenarios using ecosystem-scale modeling. Hydrologic environment types include an estuary, reservoir, mainstream river, backwater, saline lake, and a wetland. Food webs illustrate invertebrates as prey and fish or birds as predators. Additional food web steps can be added to illustrate more complex food webs (e.g., invertebrate through fish to bird or to include forage fish to predatory fish).

(0.24 µg/L) because of the potential for very high K_ds (Table 2) and the presence of clam-based food webs (sturgeon or scaup eating C. *amurensis*, $TTF_{fish} = 1.1$, $TTF_{invertebrate} = 6.25$; Figure 6D).

For birds, an exposure scenario similar to that at Kesterson Reservoir ($K_d = 900$) where eared grebes are feeding on aquatic insects ($TTF_{bird} = 1.8$, $TTF_{invertebrate} = 2.8$) predicts a water-column Se concentration of 1.8 µg/L (Figure 6E). A

scenario for a saline lake or pond (K_d of 1500) inhabited by black-necked stilts that are eating brine flies ($TTF_{bird} = 1.8$, $TTF_{invertebrate} = 1.65$) leads to a 1.8 μg/L water-column Se concentration, or 0.70 μg/L if stilts consume a diet of brine shrimp ($TTF_{bird} = 1.8$, $TTF_{invertebrate} = 4.2$; Figure 6F). A scenario for a mountain stream ($K_d = 350$) where American dippers are eating a diet of mayflies predicts a water-column Se concentration of 4.5 μg/L (Figure 6E).

An additional factor would be necessary to illustrate a scenario, for example, in which birds in an estuary are feeding on fish that prey on aquatic insects. If the selected fish species possesses a low food web potential ($TTF_{fish} = 1.1$) as found here, then the predicted allowable water-column Se concentration would not differ substantially from that predicted from invertebrates alone.

This exercise illustrates both the strengths and the limits of the model. Even when feeding relationships and TTFs are known, potential exists for variability in the translation from water to particulate phase. The model can provide perspective by illustrating that variability around reasonable scenarios for that watershed, but the model is not suitable for explicitly defining one number that will be protective in any habitat. Documenting all decisions, whether mathematical or policy choices, throughout modeling will record all considered pathways between dissolved Se and tissue Se and their outcomes.

Limitations and uncertainties. No model can incorporate all the complexities of nature or make exact predictions of outcomes. The approach presented in this paper is no exception. However, models can provide new insights that advance understanding of value to both science and management. The greatest values of the present model are that it shows why allowable water-column concentrations differ among aquatic environments and that it advances our ability to explain food web bioaccumulation of Se. The combined mechanistic and empirically based approach provides a unified methodology for evaluating how interactions of hydrology, biogeochemistry, biology, ecology, and toxicology affect ecological risks from Se at any given location. However, as with every model, forecasts from the model have limitations and uncertainties, most of which were detailed above.

Sensitivity analyses in earlier work compared the influence of uncertainty on different terms used in kinetic biodynamic modeling (see, e.g., Wang et al. 1996). Variability in TTFs reflects the outcome of those uncertainties when summed for an individual species. Experimentally determined TTFs appear to have low uncertainties judged by repeatable results in different studies. For example, TTFs for estuarine or marine zooplankton range from 1.3 to 1.5 in repeated experiments; TTFs for barnacles range from 15.8 to 20.3 (Table 3). We might expect the most uncertainty in TTFs derived from field observations given the complexity of field variables, but field- and laboratory-derived TTFs for individual species also appear to agree well (within 2-fold) in the few cases in which comparisons are possible. For example, TTFs calculated from Conley et al. (2009) for mayflies (combined mean 2.2) are very similar to the average TTF of 2.8 derived here for larvae of aquatic insects in general (Table 3).

Such conclusions are consistent with the strong correspondence between model-predicted Se concentrations for a specific environment and independent determinations of bioaccumulated Se concentrations in the same species from that environment (Figures 3 and 4). The approximately 2-fold or lower difference between predictions and independent observations for individual species of invertebrates or fish 1) is similar to the degree of uncertainty found with biodynamic modeling of a variety of metals and metalloids (including Se) in earlier studies (Luoma and Rainbow 2005) and 2) is given by Landrum et al. (1992) as sufficient accuracy to define a useful relationship within aquatic modeling. Much greater uncertainties are found in the conventional BAF approaches to modeling Se bioaccumulation at least because they are not typically species, food web, or habitat specific. The 38-fold variability in TTFs observed among invertebrate species illustrates one reason for the poorer performance of the conventional approaches than the present model. The mechanistic reasons for the similarities within taxa and the differences among some taxa are not fully known and deserve further investigation.

The relatively low uncertainty in TTFs and the validation comparisons at least partially result from recognition that such values are species specific, require the appropriate predator–prey match-ups, and should be made within the same or similar environments. The methodology recognizes that modeling of Se partitioning from the dissolved phase into the particulate material phase (transformation) and Se distribution among particulate phases (bioavailability) must mimic adequately the conditions typical of an environmental site to yield results that can be widely extrapolated to nature. Thus, if particulate Se concentrations are known for an environment and trophic transfer pathways are carefully chosen to match nature, then predictions of Se bioaccumulation can be expected that are within an acceptable uncertainty for toxicokinetic modeling (Landrum et al. 1992). Similarly, if tissue concentrations in a fish predator are known, reasonable predictions of the particulate-material Se concentrations in that environment should be feasible (recognizing the caveats described above in defining particulate material).

The concentration dependence of TTFs, as a source of uncertainty, remains largely unstudied. However, the large database of TTFs reported here was derived from a variety of habitats with different degrees of contamination, so this limitation may not normally be of concern for model application except at the extremes of possible system status for Se. Uncontaminated situations and their inhabitants are underreported in our compilation and, as noted above, elevation of TTFs in uncontaminated circumstances might be expected if Se is physiologically regulated at low environmental concentrations. Hence, further direct investigation of this premise is needed to be able to apply the model with certainty across the full spectrum of investigated sites and predators. Use of TTFs and K_ds developed from studies of only systems that fall into the same order-of-magnitude range of Se contamination as the one that someone wants to model may further mitigate this uncertainty.

The greatest potential for variability in predictions and forecasts is the choice of a factor to describe transformation of Se from dissolved to particulate phase (K_d). Representing a hydrologic system in terms of the dynamics of transformation is complex. Geochemical models (equilibrium-based) cannot describe transformation outcomes well because transformation processes are biogeochemically driven. Meseck and Cutter (2006) incorporated hydrodynamic processes, redis-

tribution from sediment to the water column, and the influence of primary productivity in describing the fate and speciation of Se in San Francisco Bay. However, the complexity of this type of modeling, uncertainties about boundary conditions, and lack of consideration of multifaceted but influential aspects of hydrodynamics limit the applications of such models to date and the questions even such admirable efforts can address. For the present model, we chose a more parsimonious approach, relying on empirical knowledge of the site or watershed to limit uncertainties. Collection of sets of well-matched samples for analysis of dissolved and particulate Se concentrations can document variability within an ecosystem, especially if hydrologic characteristics and speciation are taken into account in the interpretation. For example, data collection that divides modeling efforts into subareas and temporal cycles of rainfall or flow might be employed to reduce uncertainties, even without complex modeling. It is also possible to illustrate potential variability by computing predictions using alternative choices of K_d bracketed by the variability empirically observed in the environment of choice. The database of K_ds derived here from matched data sets shows less variability within broad categories of aquatic systems (lotic, lentic, estuaries) than across the entire data set. Information on speciation may also be another way to constrain the choice of K_d in the absence of empirical data (see above under *Partitioning and transformation environments*). However, the database of K_ds suggests that uncertainties in the transformation coefficient could range from 2-fold to 10-fold in the absence of local data.

The methodology here uses partitioning and food web scenarios to combine variables and illustrate uncertainty. For example, under conditions of an assumed global TTF_{fish} of 1.1 and a backwater K_d of 350 (Figure 6B), a high degree of certainty exists that fish eating an exclusive diet of amphipods will require a less stringent water-column Se concentration (14 μg/L) than if fish are exclusively eating aquatic insects (5 μg/L), given the magnitude of the difference in trophic transfer at the prey level (0.9 vs. 2.8). If a K_d of 500 were chosen for the example, the allowable water-column Se concentrations would be 10 μg/L and 3.2 μg/L, respectively. The exact number may differ in these examples, but the tenets remain unchanged.

A requirement to measure dissolved-phase Se concentrations rather than total water-column Se concentrations would rectify the geochemical inaccuracy of including a suspended-particulate-material Se fraction in a dissolved-phase modeling parameter. Further development of methods for differentiation of particulate material type and for dissolved and particulate speciation is also important to improving the accuracy of this final step in translation.

Quantitative modeling does produce quantitative outcomes, leading to the potential for overexpectations from a model. Given the uncertainties described above, the present model is more suitable for illustrating the implications of different choices of, for example, a site-specific water quality guideline for Se than it is for choosing any specific number for that guideline, but realistically the outcomes of guideline development depend on decisions in addition to mathematical ones. Policy choices based on what scenario or food web the regulator wishes to manage toward are also important decision points. Additional detailed analysis of ecological and hydrological variations for the site (i.e., site-specific conceptualization) could address uncertainty within mathematical choices or ranges but at a level of reasoning different from mathematics (Table 1). For example, 1) clearly defining food webs in conceptual models of fauna and their feeding relationships from empirical knowledge of the investigated site can identify details of species-specific exposure, 2) life cycles of habitat species can be displayed on a yearly basis to identify details of spatial and temporal exposure, 3) identifying feeding areas for wildlife can help determine what percentage of diet comes from the polluted site, 4) dissolved- and particulate-material Se speciation can be related to hydrologic conditions (e.g., high- or low-flow season or residence time), and 5) bioaccumulation dynamics can be related to particulate material characterization. As development of Se protection proceeds, a compilation of site-specific derivations of water-column Se concentrations from diverse sites and their validation through monitoring could ultimately address the sufficiency of data requirements for ecosystem-scale modeling.

Further work is needed to expand the database available for use in quantitative models. Continued work on quantitatively modeling transformation from dissolved to particulate Se under different circumstances is essential. More data are needed on physiological TTFs for invertebrates, fish, and bird species derived from kinetic experiments. Comparisons are also needed for experimental vs. field-derived TTFs (with the latter derived from matched data sets across different field sites). Few biodynamic studies are available for different fish species, so determining the range of TTFs from experimental studies would further assess the importance of the role of fish physiology in understanding food webs. Biodynamic kinetic studies are not available for avian species, and data available for derivation of TTF for different bird species in different dietary settings are limited, so further experiments to develop egg–diet relationships are needed with particular attention to mimicking the bioavailability of a diet found in nature. Inclusion of a database of factors for translation to fish ovary Se concentrations would be an important addition to allow connection of modeling of fish directly to reproductive effects. Developing TTFs specific to the dietary exposure concentration being modeled would require systematic experimental studies of common food web species to generate a set of generalized TTF equations as a function of dietary Se.

In the end, if we are to protect ecosystems with defensible assessment procedures, then the only choice is to incorporate the complexity of multiple route exposures, whatever the challenges. Thus, ecosystem-scale modeling offers a major step forward in terms of confronting and defining uncertainty by formalizing the knowledge necessary to understand the basis of protective criteria for Se. This formalization of knowledge, including choices used to initiate or limit modeling scenarios, thus clearly documents pathways that connect dissolved and tissue Se concentrations and provides a record of supporting data throughout decision-making phases.

Complementary approach: Wildlife criteria

A wildlife criterion (sometimes referred to as a wildlife value or tissue residue guideline, TRG) is the dietary concentration of an element necessary to keep the daily ingested amount of a contaminant at or below a level at which no adverse effects are expected (USEPA 1989; Sample et al. 1996; CCME 1999; USFWS 2003). The use of dietary

toxicity testing is one common link with the ecosystem-scale approach. In regulatory terminology, a wildlife criterion is analogous to a tissue residue concentration (TRC) for human health criterion. A common focus for these types of criteria is consumption of fish either by wildlife or by humans (USEPA 2001). The steps for deriving this type of wildlife criterion and applying it in modeling are shown in Table 6 and discussed further in the Supplemental Data. This approach to deriving a wildlife criterion uses body weight (BW, kg wet weight), food ingestion rate (IR, g food/d), and a reference dose (RfD, $\mu g \cdot kg^{-1} d^{-1}$) determined by dietary toxicity testing (Nagy 1987; USEPA 1993; Sample et al. 1996). In effect, the wildlife criterion converts an RfD into a species-specific allowable dietary uptake rate, if 100% assimilation efficiency is assumed, or into an allowable Se concentration in food for each species. In modeling here for birds, an Se wildlife criterion is referred to as an allowable C_{food} ($\mu g/g$) and is defined by the equation

$$\text{allowable } C_{food} = (\text{RfD})(\text{BW}) \div \text{IR}. \quad (40)$$

An allowable Se dose, or exposure rate, is defined by the equation

$$\text{allowable dose} = (\text{RfD})(\text{BW}). \quad (41)$$

An allowable Se concentration in food for predators (i.e., wildlife criterion) can be written in terms of allowable dose as

$$\text{allowable } C_{food} = \text{dose} \div \text{IR}. \quad (42)$$

If a Se RfD is assumed for modeling of effects to birds, then an allowable C_{food} for various species of birds can be calculated (see Supplemental Data). For watershed evaluation, the allowable C_{food} is used as a dietary target and compared with 1) existing Se concentrations in dietary items in biologically appropriate food webs, or 2) predicted concentrations as a result of food web modeling. Equations can be added to consider mixtures of food (Table 6).

The wildlife criteria approach and the ecosystem-scale approach could easily be combined by adding values for assimilation efficiency and considering K_d, for example, in the translation to dissolved Se. Validation would be important; uncertainties in the relationship of body weight and ingestion rate, for example, would have to considered, but the combination might be helpful in assessing a watershed in terms of threatened and endangered avian species. A list of species can be developed, wildlife criteria calculated, and species-specific dietary guidelines applied in modeling (USFWS 2003). Steps such as this in the methodology could also serve to harmonize regulation, a goal long sought in obtaining consensus and understanding (Reiley et al. 2003).

Table 6. Steps in Wildlife Value derivation (aquatic birds) and dietary application (invertebrate or fish diet for aquatic birds) for ecosystem-scale Se methodology

Wildlife Value and Dietary Modeling (acquatic bird example)
Develop a conceptual model of food webs in watershed
Choose avian RfD, endpoint, and uncertainty factor · RfD = NOEC or LOEC ÷ uncertainty factor
Choose bird species
Choose body weight and ingestion rate for selected bird species
Calculate allowable concentration in food of selected bird species (i.e., allowable Se C_{food} or species-specific RfD or Wildlife Value) · Wildlife Value = (RfD)(BW) ÷ IR
Identify species-specific diet
Choose dietary items
1. Compare to available food in ecosystem
2. Compare to predicted Se concentrations in invertebrate diet for aquatic birds
Identify food web(s)
Solve equation(s) for dietary Se concentration in invertebrates
If single invertebrate species diet and known particulate Se concentration or K_d and C_{water} · $C_{invertebrate} = (TTF_{invertebrate})(C_{particulate})$ or $C_{invertebrate} = (TTF_{invertebrate})(K_d)(C_{water})$
If sequential bioaccumulation in longer food webs contributes to diet · $C_{invertebrate\ b} = (C_{particulate})(TTF_{invertebrate\ a})(TTF_{invertebrate\ b})$
3. Compare to predicted Se concentrations in fish diet for aquatic birds
Identify food web(s)
Solve equation(s) for dietary Se concentration in fish
If a single invertebrate species and known particulate Se concentration or K_d and C_{water} · $C_{fish} = (TTF_{invertebrate})(C_{particulate})(TTF_{fish})$
If several invertebrate species contribute to diet · $C_{fish} = TTF_{fish}$ $(C_{particulate})[(TTF_{invertebrate\ a})$ (prey fraction)] + [$(TTF_{invertebrate\ b})$ (prey fraction)] + [$(TTF_{invertebrate\ c})$ (prey fraction)]
If assume sequential bioaccumulation in longer food webs contribute to diet · $C_{fish} = (C_{particulate})(TTF_{TL2\ invertebrate})(TTF_{TL3\ invertebrate})$ $(TTF_{TL3\ fish})(TTF_{TL4\ fish})$

NOEC = no observable effect level; LOEC = lowest observable effect level.

CONCLUSIONS

Consideration of each step in the sequence that links environmental Se concentrations to Se toxicity is fundamental to deriving effective Se criteria or guidelines for the protection of aquatic life and aquatic-dependent wildlife (Figures 1 and 2). Ecosystem-scale Se modeling provides a context for establishing these linkages and a set of model parameters for common food webs that can be used to predict species-specific responses. A high degree of correlation ($r^2 = 0.9$) is shown between observed bioaccumulation in invertebrates and fish from 29 field locations and bioaccumulation predicted based on particulate-material Se concentration and our compiled TTFs (Figures 3 and 4). This model validation illustrates how variability in food webs result in widely different Se concentrations in different predators in a contaminated ecosystem, but those differences can be explained and quantified using this relatively simple protocol.

The validation also establishes the adequacy of the type of knowledge compiled to represent a specific occurrence of Se.

Analysis from the model shows that 1) a crucial factor ultimately defining Se toxicity is the link between dissolved and particulate phases at the base of the food web (i.e., K_d); 2) collection of particulate material phases and analysis of their Se concentrations are key to representing the dynamics of the system; 3) bioaccumulation in invertebrates is a major source of variability in Se exposure of predators within an ecosystem, although that variability can be explained by invertebrate physiology (i.e., $TTF_{invertebrate}$; Figure 5); 4) TTF_{fish} is relatively constant across all species considered here; and 5) Se concentrations are at least conserved and usually magnified at every step in a food web (Figure 6).

Application of the model to habitat-specific and species-specific exposure scenarios illustrates how, if a desired Se concentration is chosen to protect predators, allowable dissolved Se concentrations will vary among sites depending on how phase transformation and food webs are linked (Figure 6). Much of the controversy about a proper dissolved Se guideline for regulating the chemical, therefore, stems from unavoidable biogeochemical and food web differences within and among environments. The mechanistic aspects of the model and the flexibility of model components in terms of portraying the realities of exposure in nature all increase the reliability of model predictions over traditional approaches that tie water-column concentrations directly to tissue concentrations. Details of hydrology and ecology added to modeling through conceptualization of seasonal hydrologic cycles, food webs, life cycles of predators, and feeding possibilities create several levels of confidence in model outcomes based on mathematics and realistic ecology. Thus, the model can confront complexity to account directly for critical sources of variability and uncertainty in assessing Se effects. The model can run either backward or forward to verify choices and develop scenarios based on knowledge of food webs, hydrology, or proposed management.

The methodology also shows the need for a better understanding of the aspects of ecosystems, such as water residence time and dissolved and particulate speciation, that contribute to the environmental partitioning and bioavailability of Se. In lieu of this, determining Se concentrations in the suspended particulate material phase is the preferred measure of the complex water, sediment, and particulate milieu that forms the base of the food web and is consumed as food by invertebrates. Monitoring invertebrate Se concentrations in food webs that are the most likely to be heavily contaminated may be a practical initial step in a monitoring plan, because the first and second most variable aspect of Se dynamics (i.e., K_d and $TTF_{invertebrate}$) are integrated into invertebrate bioaccumulation. Policy choices such as 1) the predator species to represent an ecosystem (e.g., toxicologically sensitive, ecologically vulnerable based on food web, resident or migratory, commercially or esthetically valuable) and 2) the food web to represent an ecosystem (e.g., potentially restored food webs in addition to current food webs) also serve as important initial inputs into the development of protective scenarios for a site or watershed.

Currently, within USEPA's Clean Water Act programs, aquatic life criteria and wildlife criteria are separate and are derived independently (see, e.g., USEPA 1995, 2004). The USEPA in 1989 identified the need for criteria to protect wildlife as an outgrowth of Se-induced deformities of aquatic birds at Kesterson Reservoir (USEPA 1989) but has not acted nationally to develop a wildlife Se criterion. The USEPA started considering development of a fish tissue aquatic-life criterion for Se in 1998 and proposed a national fish whole-body Se criterion of 7.9 μg/g dw to protect freshwater fish in 2004 (USEPA 1998, 2004). That criterion is now under revision. Our model can be a useful tool in determining scientifically integrated protection for both aquatic life (such as fish) and aquatic-dependent wildlife (such as waterfowl). For example, based on typical TTFs for Se, USEPA's proposed whole-body fish tissue criterion of 7.9 μg/g dw (USEPA 2004) would also allow Se concentrations in aquatic invertebrates that, when eaten by breeding waterbirds, would pose a substantively higher hazard (see, e.g., Ohlendorf 2003; EC50) for avian toxicity than the designed level of protection for fish (USEPA 2004; EC20).

Our ecosystem-scale model for Se is applicable to connecting fish and bird tissue to environmental concentrations in a rigorous way and to providing perspective when deriving site-specific or broader Se guidelines. We now have the knowledge necessary to understand the basis of protective water-quality criteria for Se for fish and birds. Species-specific diets and reference doses for wildlife can also be used to determine an allowable Se concentration in food (i.e., a wildlife criterion or value) using a few outlined supplemental steps. As we noted above, the set of choices to initiate ecosystem-scale modeling implicitly suggests that management of Se requires consideration of biology, ecology, biogeochemistry, and hydrology along with ecotoxicology. Intuitively, this seems an obvious requirement. In practice, it provides a means to move beyond the traditional objections (see, e.g., Cairns and Mount 1990) that we can never understand enough about ecology and hydrology to include them in chemical regulation.

SUPPLEMENTAL DATA

Methodology for ecosystem-scale modeling of selenium: Data and references.

Supplemental Data Table A. Water-column Se concentrations, particulate Se concentrations (dw), and calculated K_ds from field studies.

Supplemental Data Table B. Experimental data for invertebrate physiological parameters and calculated kinetic TTFs for invertebrates (particulate to invertebrate in dw).

Supplemental Data Table C. Calculated TTFs from field studies for invertebrates (particulate to invertebrate in dw).

Supplemental Data Table D. Calculated kinetic or field TTFs for fish (invertebrate to fish in dw except where noted as fish to fish in dw).

Supplemental Data Table E. Model validation for prediction of invertebrate and fish (whole-body or muscle) Se concentrations.

Supplemental Data Table F. Model validation for prediction of invertebrate and bird egg Se concentrations.

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Open-File Report 2020–1098

U.S. Department of the Interior
U.S. Geological Survey

Cover. Photograph showing Libby Dam on the Kootenai River near Libby, Montana. Photograph by U.S. Army Corps of Engineers, Seattle District Public Affairs Office.

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
square mile (mi^2)	259.0	hectare (ha)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
acre-foot (acre-ft)	1,233	cubic meter (m^3)
acre-foot (acre-ft)	0.001233	cubic hectometer (hm^3)

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Flow rate	
cubic meter per second (m^3/s)	70.07	acre-foot per day (acre-ft/d)
	Mass	
kilogram (kg)	2.205	pound avoirdupois (lb)

Supplemental Information

Concentrations of chemical constituents in water are given in either micrograms per gram (µg/g) or micrograms per liter (µg/L).

A water year is the period from October 1 to September 30 and is designated by the year in which it ends; for example, water year 2019 was from October 1, 2018, to September 30, 2019.

Abbreviations

>	greater than
<	less than
AE	assimilation efficiency
BAP	bioaccumulation potential
CF	tissue-to-tissue conversion factor
dw	dry weight
EC	effect concentration or level of toxicity (for example, an EC10 is the toxicant concentration that causes a 10-percent effect in the endpoint of interest such as incidence of larval fish deformity in the case of selenium
ESA	Endangered Species Act
GSI	gonadosomatic index
IFM	invertebrate to fish model
K_d	environmental partitioning factor
MTDEQ	Montana Department of Environmental Quality
MTFWP	Montana Department of Fish, Wildlife and Parks
n	number of samples
RG_DSELK	downstream Elk River
SOE	south of Elk
SPM	suspended particulate material
TFM	trophic-level (predator to forage) fish model
TL	trophic level
TTF	trophic transfer factor
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
wb	whole body

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Abstract

Modeling of ecosystems is a part of the U.S. Environmental Protection Agency's protocol for developing site-specific selenium guidelines for protection of aquatic life. Selenium as an environmental contaminant is known to bioaccumulate and cause reproductive effects in fish and wildlife. Here we apply a modeling methodology—ecosystem-scale selenium modeling—to understand and document the scientific basis for predicting and validating ecological protection for Lake Koocanusa, a transboundary reservoir between Montana and British Columbia. A comprehensive set of site-specific data compiled from public databases (Federal, State, and Provincial) and reports by Teck Coal Ltd., is available in a companion U.S. Geological Survey data release. The tissue guideline used within modeling here to assess protection is the U.S. Environmental Protection Agency's national selenium guideline for whole-body fish (dry weight); however, other numeric values for a whole-body guideline or other tissue types may be assumed if applicable tissue-to-tissue conversion factors are available.

We consider the report assembled here as a working document that presents a model that can effectively address and structure the needs of (1) scientific understanding in representing the lake's ecosystem and selenium biodynamics and (2) policy and management development during a decision-making process, but it is open to modification and updating as more ecologically detailed data become available. The approach brings together the main concerns involved in selenium toxicity: likelihood of high exposure, inherent species sensitivity, and close connectivity of ecosystem characteristics and behavioral ecology of predators. Detailed site-specific modeling equations are provided to document the linked factors that determine the responses of ecosystems to selenium. A series of scenarios quantifies the implications of choices of site-specific variables including food-web species, bioavailability of particulate material, and partitioning between the dissolved and particulate phases at the base of food webs. A gradient mapping tool applied to Lake Koocanusa provides a precedent for ecosystem-scale modeling of lakes by recognizing the importance of lake strata and hydrodynamics as components of modeling.

Data requirements for ecosystem modeling, including ecological and hydrological process information fundamental to the dietary biodynamics of selenium in site-specific food webs, were assessed as a precursor to model validation for Lake Koocanusa. Understanding these relationships is necessary to connect modeling outcomes to reproductive effects and establish boundaries, in the case of Lake Koocanusa, for the influences of dam operation, fish-community viability, and its Clean Water Act impaired 303(d)-listing status on ecosystem function.

We find that an assemblage of conditions affects the representation of Lake Koocanusa's ecosystem within modeling scenarios but that the constructed gradient maps, mechanistic model, and associated bioaccumulation potentials portray and quantify the variables that are determinative to protection of predator species. Ecological and hydrological sorting of compiled individual data points on a site- and species-specific basis helps identify and address model uncertainties. Sources of uncertainty include (1) the scarcity of data for some environmental media compartments across time and locations, (2) the complexity of hydrodynamic conditions that can lead to seasonal ecological disconnects such as in selenium partitioning from water into particulates, and (3) the functional status of Lake Koocanusa's ecosystem because of cumulative effects of various environmental stresses (for example, fish-community changes, flow regime changes, parasites, gonadal dysfunction, and increasing mining input-selenium concentrations since 1984). To this last point, it is important to determine where Lake Koocanusa is in an impairment-restoration cycle so as not to base protection on survivor bias, the maintenance of a currently degraded ecosystem, or normalized toxicity. In a broader context, one of the overall consequences of revised selenium regulations is that their derivation is now dependent on being able to define and understand the status of the ecosystem on which protection is based.

Introduction

The objective of this work is to document the scientific basis of selenium ecological protection in support of the development of selenium guidelines for Lake Koocanusa (fig. 1). Both the British Columbia Ministry of Environment and Climate Change Strategy (hereafter referred to as "BCMOE") and the U.S. Environmental Protection Agency (hereafter referred to as "USEPA") provide guidance for recently revised selenium regulations that recognizes the importance of linking the primacy of a fish tissue toxicity guideline with the practicality of a water-column guideline through a site-specific modeled ecosystem (BCMOE, 2014; USEPA, 2016a). The overall goal of this work is to provide an ecosystem-scale model that illustrates the site-specific range of potential selenium exposure and bioaccumulation that can inform the basis for regulatory decision-making by the State and the Province.

Explicit goals related to modeling as expressed at the conception of this work and embedded in a cooperative funding agreement between the U.S. Geological Survey (USGS) and the Montana Department of Environmental Quality (MTDEQ) are as follows:

- consideration of ecologically significant species and those important to stakeholders;
- protection of 100 percent of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding in an ecosystem that functions as a lentic reservoir;
- long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all selenium loading profiles, and all water years (precipitation/runoff scenarios);
- protection of ecosystems during maximum dietary selenium exposure (that is, feeding within a benthic food web); and
- protection of downstream uses including protection of the endangered Kootenai River *Acipenser transmontanus* (white sturgeon).

As a working document, the report integrates information, data, and graphs from the following:

- Teck Coal Ltd., Lake Koocanusa Monitoring Reports, 2014–16 and 2018 (Teck Coal Ltd., 2018a, 2019);
- Lotic Environmental Ltd., Lake Koocanusa Food Webs (Baranowska and Robinson 2017; Baranowska, 2018);
- USGS data release (Presser and others, 2018; https://doi.org/10.5066/P9HB5S5F) that includes data from sample collection in 2015–17 and a plan designed for ecosystem-scale selenium sample collection for Lake Koocanusa;
- a modeling methodology that is well documented in the literature (for example, Presser and Luoma, 2010a); and
- a methodology for sampling and analysis of suspended particulate material (SPM) that was implemented specifically for Lake Koocanusa (Presser and others, 2018).

Presented here in support of modeling are the following:

- USGS data release (Presser and Naftz, 2020; https://doi.org/10.5066/P9VXYSNZ) that is specific to the basis of ecosystem-scale selenium modeling;
- summary tables that characterize and justify site- and species-specific choices in model development;
- spreadsheets that show model scenarios and computations for validation of ecosystem selenium concentrations and prediction of protective dissolved selenium concentrations based on those scenarios;
- a series of lake contours or gradient maps for 2017 showing observed dissolved and SPM selenium concentrations and calculated environmental partitioning factors (K_d values);
- observed dissolved selenium concentration cross sections specific to lake strata and location that address both north-to-south and depth selenium gradients for April–October (2016–19); and
- supplemental data that may help identify additional predator, contaminant, and stressor pathways in the future (Presser and Naftz, 2020).

Hence, there are a number of ways to build upon the structure and templates presented here as data-collection efforts go forward and model runs are needed to test the implications of additional site-specific variables.

This report is in answer to a specified need by MTDEQ and other stakeholders concerning the derivation of site-specific aquatic-life protection for Lake Koocanusa. That need was to model the ecosystem in terms of selenium dietary biodynamics and provide modeling spreadsheets as an interactive way for stakeholders to participate in scenario building within a decision analysis structure (Jenni and others, 2017). The audience for this work is expected to be somewhat familiar with the environmental setting, regulatory issues, and the transboundary nature of the work. As such, this work cuts across Federal, State and Provincial boundaries and, hence, agency protocols. In this regard, terms such as criterion, standard, and guideline, which are specific to regulatory agencies cited in this report, are collectively referred to here as "guideline." The guidelines cited are those recommended on a national basis by the USEPA through the Clean Water Act section 304(a) or on a Provincial basis by BCMOE (USEPA, 2016a; BCMOE, 2014). For fish tissue, the whole-body (wb) selenium guidelines are as follows: USEPA, 8.5 micrograms

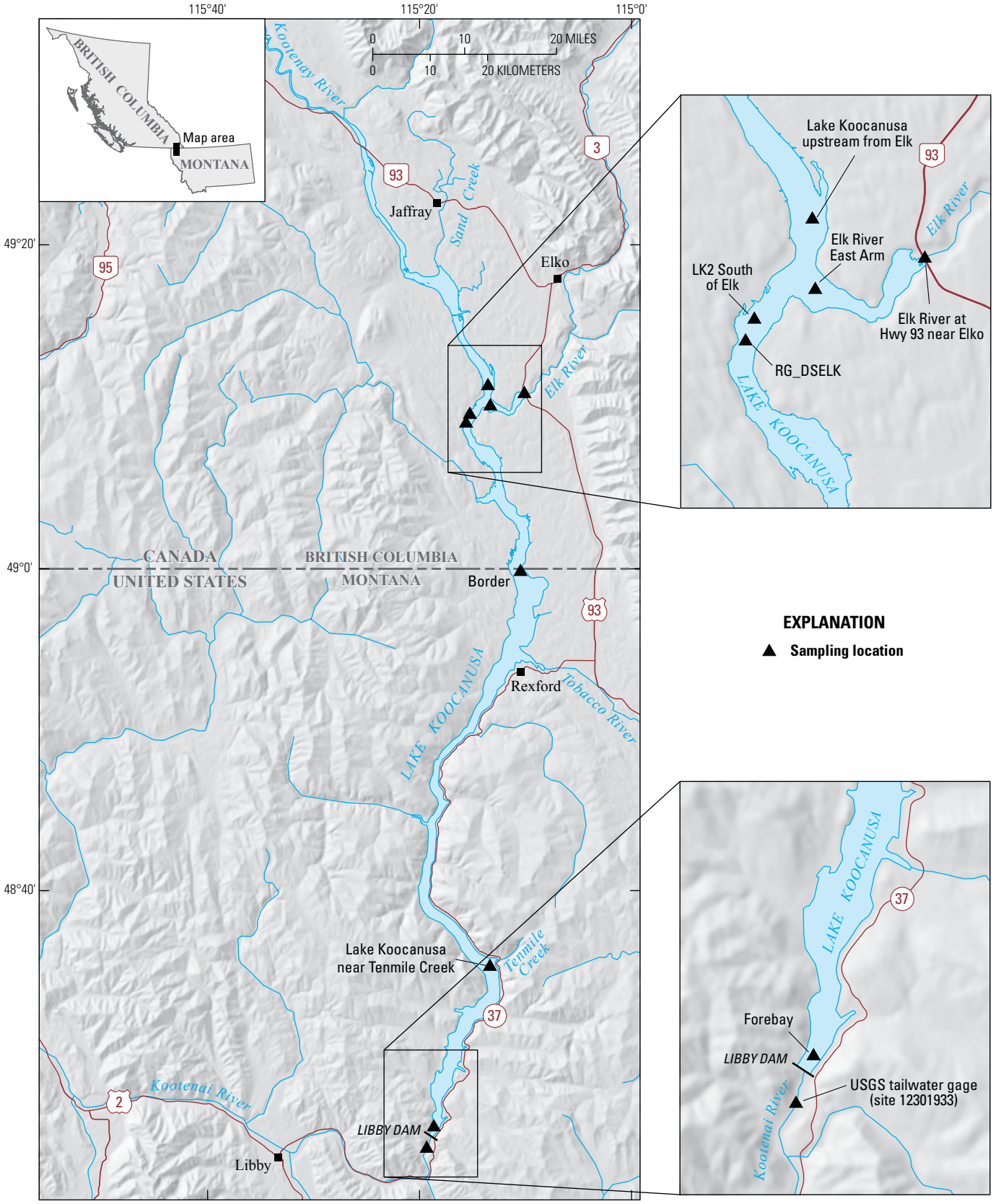


Figure 1. Study areas map of Lake Koocanusa showing locations of Libby Dam, the border between Montana and British Columbia, and the Elk River, which discharges selenium from five coal mines in British Columbia into the reservoir (see figures 11*A*–*F* for all sampling locations).

per gram dry weight (µg/g dw), and BCMOE, 4 µg/g dw. These regulatory guidelines and their derivation (for example, exposure-response curves) are not addressed here in any detail. However, it should be kept in mind when modeling that protection goals associated with these guidelines differ (for example, through application of an uncertainty factor; consideration of wildlife in addition to fish). The citations mentioned previously also document additional recommended national or Provincial water-column and diet guidelines for selenium that are used in this report.

Integration of available datasets within modeling for Lake Koocanusa was affected as we attempted to sort through fundamental landscape and ecosystem function alterations known to be caused by Libby Dam itself to establish sensitive locations and timing with which to inform regulatory and management actions. New sampling methods and tools also were implemented during the length of this project in response to ecosystem conditions assessed during our study. For example, collection of SPM, as the phase of particulate material most relevant to modeling of the base of food webs, was adapted for an oligotrophic lake setting of as much as 315 feet (ft) of depth. Additionally, a gradient mapping tool was formulated and applied to spatially integrate the influence of variables such as hydrodynamics and selenium source inputs within the lake. The generated plots illustrate the type of conceptualization and quantification that is possible to support food-web modeling of lake settings in comparison to stream-network or estuarine systems (Presser, 2013; Presser and Luoma, 2013). Overall, some tolerance is needed to accept these interim efforts as they are meant, as a way to move forward when encountering unforeseen uncertainty in ecosystem functioning and health.

As part of future monitoring to support ecosystem-scale modeling and constrain uncertainty within regulatory and management actions, the USGS deployed a monitoring platform in August 2019 at the U.S. and Canada border (fig. 1). In addition to standard, hourly limnological profiles that can be viewed in near-real time, filtered water samples are collected on a daily time step from four depths and preserved for selenium analysis (USGS site 12300110; https://nwis.waterdata.usgs.gov/usa/nwis/qwdata/?site_no=12300110). Similar equipment was deployed within the existing tailwater gaging station below Libby Dam (USGS site 12301933; https://nwis.waterdata.usgs.gov/usa/nwis/qwdata/?site_no=12301933). This station provides (1) coordinated monitoring for a second downstream location of the reservoir and (2) information on the variability of selenium inputs to the downstream lotic habitats of the Kootenai River. Hence, with the addition of these datasets in the future, the distribution of selenium concentrations with time and depth during varying hydrodynamic conditions (for example, spring freshet, fall turnover, density driven currents) can inform modeling scenarios.

Setting and Ecosystem

Lake Koocanusa is a transboundary reservoir between Montana and British Columbia created by the impoundment of the Kootenai River when Libby Dam was built in 1972 (Bonneville Power Administration and others, 1995) (fig. 1). Its construction was authorized as part of a treaty between the United States and Canada for cooperative development of flood storage, hydroelectric power generation, and recreation within the Columbia River Basin. The drainage area captured by the dam is 8,985 square miles (mi^2) and reservoir capacity is 6.0 million acre-feet (https://www.nwd-wc.usace.army.mil/dd/common/projects/www/lib.html). The reservoir is 90 miles (mi) in length, with 48 mi on the Montana side and 42 mi on the British Columbia side. Full-pool elevation is 2,459 ft, with a maximum depth of 350 ft at the forebay (Easthouse, 2013). Minimum pool is 2,287 ft, with drawdown occurring approximately from November to May (see also Presser and others, 2018, figs. 11, 13, 15). As part of the Columbia River Basin, many ecological studies were completed before and after impoundment. The status of the lake is currently monitored by (1) the U.S. Army Corps of Engineers for parameters critical to meeting management goals including flow, water quality, and productivity (https://www.nwd-wc.usace.army.mil/ftppub/water_quality/tdg/#LBQM) and (2) the USGS for outflow (river discharge) from the dam (https://waterdata.usgs.gov/nwis/uv/?site_no=12301933&agency_cd=USGS). Beginning in 2019, the USGS also monitors water quality and selenium concentrations at the international boundary (site number 12300110) and below Libby Dam (site number 12301933).

The Kootenai River drainage basin has its origin in British Columbia and is located within the Northern Rocky Mountain physiographic province (Woods and Falter, 1982). The basin lies within a latitude range of 48–51 degrees north, which is at the edge of the boreal (that is, high latitude) zone of climate classification. Lake Koocanusa's Trophic State Index fluctuates between oligotrophic and mesotrophic. This index compares lakes according to their summer (June–September) biological productivity on a scale of 0–100. At the lower end of the scale (oligotrophic, less than [<] 40), a lake would have low productivity, high water clarity, and low nutrients.

The Fording River and Elk River watersheds (240 and 1,718 mi^2, respectively) of the Kootenai River Basin are the sources of selenium to Lake Koocanusa. Drainage from five surface coal mines is transported by these rivers under permits administered by the BCMOE (Teck Coal Ltd., 2014; BCMOE, 2018). Mining of this mineral resource is through a technique similar to mountaintop removal that requires large-scale storage of selenium-enriched waste rock in valley drainages or open pits. Management of selenium risk is necessary once these waste rocks are exposed, oxidation to mobile selenate occurs, and the resulting leachate interfaces with the environment (Presser and others, 2004; Presser, 2013). Since 1984, selenium concentrations in the Elk River measured at a station 2.2 mi above its discharge into Lake Koocanusa

(that is, at Highway 93) show a continuing increase as mines have expanded (https://www.canada.ca/en/environment-climate-change/services/freshwater-quality-monitoring/online-data.html) (fig. 2*A*). Selenium concentrations in the Elk River have exceeded BCMOE's Provincial guideline of 2 micrograms per liter (µg/L) for protection of aquatic life (Nagpal and Howell, 2001; BCMOE, 2014) since 1993 and the USEPA's guideline of 3.1 µg/L for lotic waters (USEPA, 2016a) since 2002 on a seasonal basis. In terms of maxima, selenium concentrations at this site exceeded 8 µg/L in February 2014 and April 2018. This upward trend has created a nonsteady state for dissolved selenium in the lake that the ecosystem is responding to throughout this 35-year period. The USEPA (2016a) describes concerns about site-specific derivations under new input conditions that lead to nonsteady-state conditions and recommends a series of steps that involve investigating the dynamics of selenium bioaccumulation within such an ecosystem. Expansion of mining is ongoing, and management plans for selenium call for a doubling of the amount of waste-rock storage by 2023 (Teck Coal Ltd., 2014).

A snapshot in 2014 of selenium concentrations farther downstream (1) in the Elk River at its mouth (fig. 1) shows a range of concentrations of 3.2–8.5 µg/L at the surface from January to December (fig. 2*B*) and (2) in the east arm (fig. 1), where the Elk River discharge enters into the reservoir, shows a range of 0.8–3.8 µg/L at different depths in May–November of 2014 (fig. 2*C*). A study in 2018 addressed riverine mixing within the reservoir during three pool conditions (low, intermediate, high). Specific conductance and temperature elucidated the density gradient from the river input to the order station (that is, permitted water-quality site; RG_DSELK [downstream Elk River]) at approximately 4.4 mi downstream (fig. 1) (Teck Coal Ltd., 2018b). The Elk River discharge is characterized by elevated specific conductance and cooler water temperatures compared to reservoir waters, thus setting up the potential for characteristic density-layering effects. Profiles of the east arm of the reservoir indicate that the Elk River waters were generally confined to bottom contours, but downstream mixing took place as river water rose to midcolumn. Confinement of river-water mixing to the eastern side of channelized flow also occurred until below RG_DSELK.

Lake Koocanusa's highly modified hydrological and ecological systems contribute to the interpretation of how its ecosystems are functioning, especially here, in terms of the fundamental processing of selenium. In general, Lake Koocanusa has the traditional problems associated with dam management for power generation in that large elevation changes (for example, 140–170 ft below full pool) occur during drawdown and refilling operations (Bonneville Power Administration and others, 1995). A series of hydrographs illustrate the magnitude of these types of changes (that is, elevation, discharge) specific to Lake Koocanusa in 2015–17 (Presser and others, 2018, figs. 11–16). An example of this type of disturbance specific to the species of the lake is that

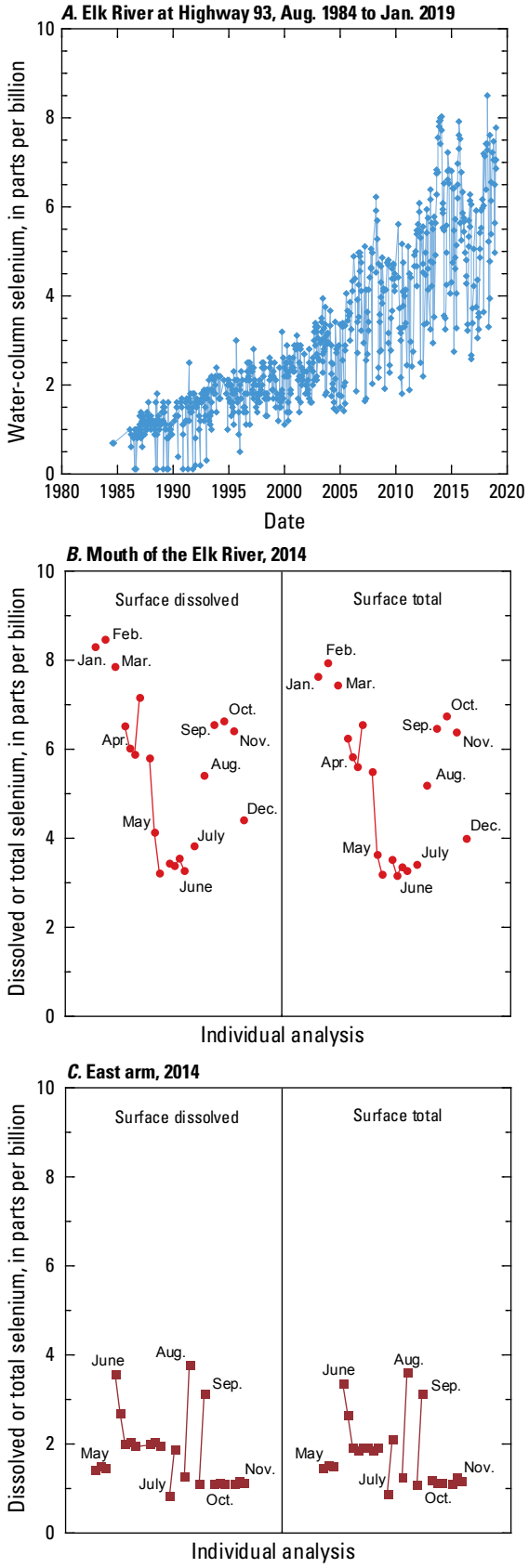


Figure 2. Water-column selenium concentrations for sites on the Elk River. *A*, for the Elk River at Highway 93 from 1984 to 2019; *B*, the mouth of the Elk River in 2014; *C*, the east arm in 2014.

the spawning time of *Lota lota* (burbot) coincides with the timing of maximum drawdown in Lake Koocanusa (Teck Coal Ltd., 2015).

Given the variable retention and flushing times, residence time of water in the lake also varies. Woods (1982) calculated a range of annual hydraulic residence times of 1.7–7.5 months during initial conditions after dam construction (1972–80). Holderman and others (2009) cite 5.5 months based on a mean annual discharge of 440 cubic meters per second. The U.S. Army Corps of Engineers (Easthouse, 2013) cites a mean water residence time of approximately 9 months. Residence time profiles, if measured on a monthly time scale, could further elucidate a connection to lake productivity and, hence, ecosystem function (see later discussion, "Transboundary Metadata and Suspended Particulate Material Sampling" section).

Hauer and Stanford (1997) referred to the large-scale effects of river regulation on the ecology of the Kootenai River Basin as a functional reset of a river continuum. Impacts include decreases in (1) production of zooplankton and benthic invertebrates and (2) availability of terrestrial insects, especially when refill is not achieved (Bonneville Power Association and others, 1995, appendix K). Decreases in prey organisms can, in turn, lead to decreased fish growth. Impacts also affect the extent and quality of shoreline and littoral habitats, which can contribute to reduced nesting for birds and spawning areas for fish.

Phytoplankton and invertebrate densities (Presser and Naftz, 2020) elucidate shifts in taxa and provide measures of seasonal productivity for Lake Koocanusa (figs. 3–5). For example, diatoms dominated in 2014 and 2015 with a substantial shift to dominance by dinoflagellates in 2016 and 2017 (fig. 3). Yearly macrozooplankton (that is, excluding rotifers) densities (150-micron net) show dominance by the copepod Cyclops from 2009 to 2015 (fig. 4; Dunnigan and others, 2017). Mean densities for that period show productivity peaks in May and August, but the most recent profile in 2015 shows a series of peaks occurring during May, July, August, and September. Zooplankton densities (63-micron net) at three reservoir locations show rotifers dominate most profiles as increases occur from the border location to sites farther downstream towards the forebay of the dam (fig. 5*A*, *B*, and *C*). Experimental data for farm-raised fish show rotifers to be a nonnutrient food, low in selenium (for example, Mæhre and others, 2012); therefore, dilution of selenium in a composite sample of zooplankton could take place if a large number of rotifers were present compared to other zooplankton taxa.

Abundance data for macroinvertebrates in Lake Koocanusa are less available. Chisholm and others (1989) report large density peaks of aquatic macroinvertebrates in April–May, and to a lesser extent in August, based on a seasonal study of limnetic and nearshore habitats in 1983–87. For terrestrial macroinvertebrates, a peak in August dominates the 1983–87 profile. Also for this period, the density of individual invertebrate taxa, in decreasing order, was Hymenoptera (ants), aquatic dipterans (midges), Homoptera (leafhoppers), Coleoptera (beetles), Hemiptera (corixids, aphids), and Arachnida (spiders, ticks, mites) (Richards, 1997).

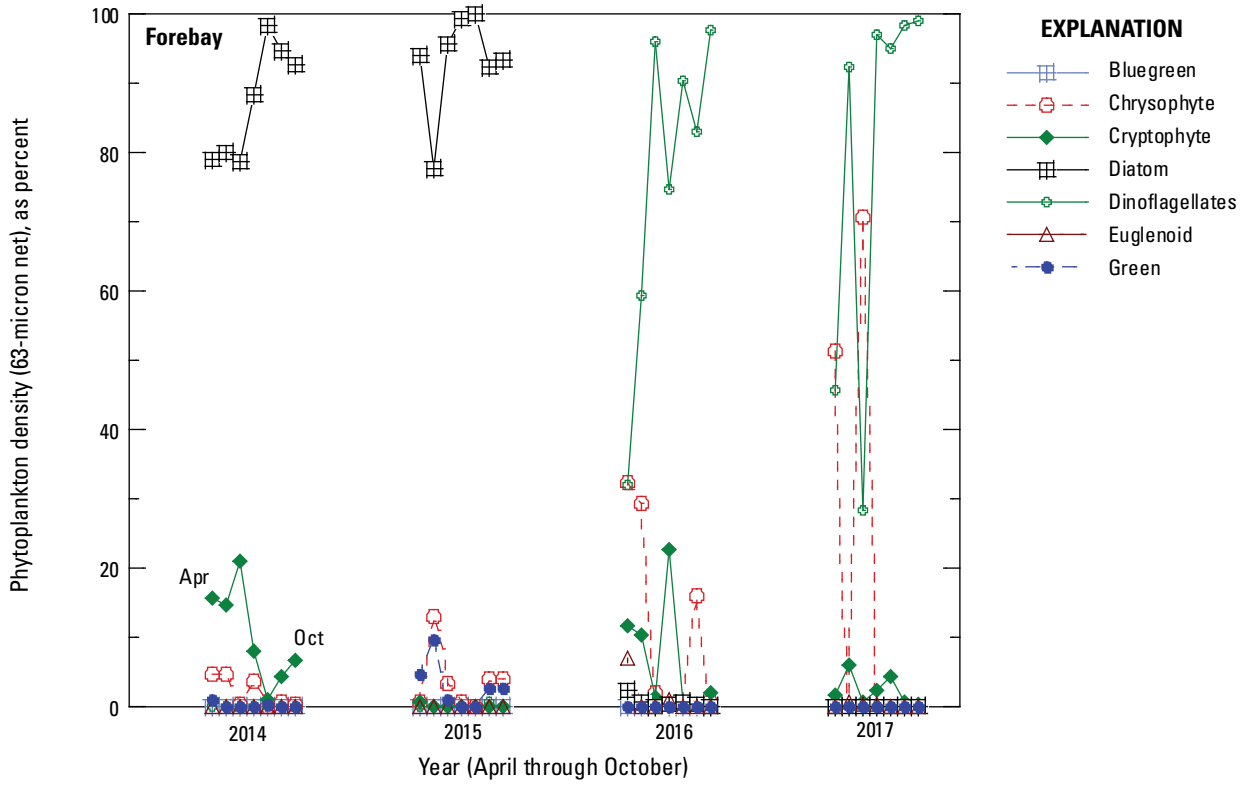


Figure 3. Phytoplankton profiles at the forebay for 2014–17.

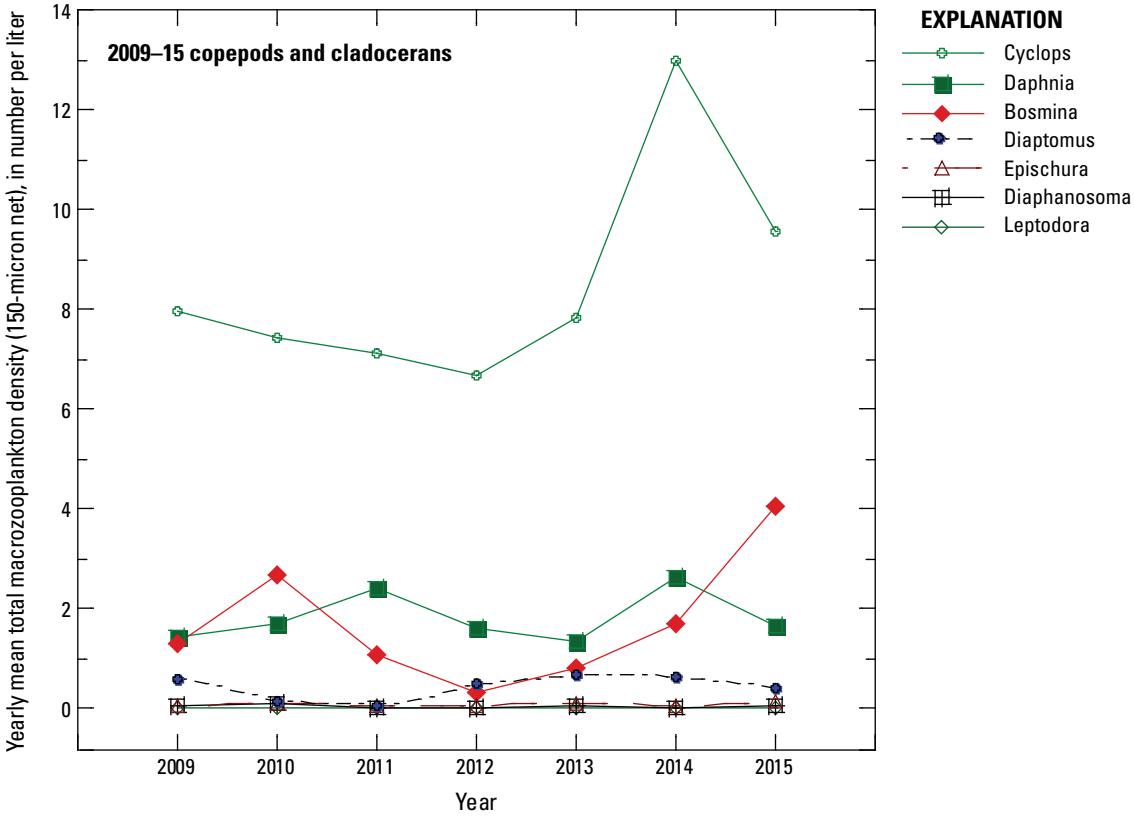


Figure 4. Macrozooplankton profiles (150-micron net) for 2009–15.

Specific to Lake Koocanusa for fish-community fluctuations, *Oncorhynchus mykiss* (rainbow trout), *Oncorhynchus clarki lewisi* (Westslope cutthroat trout), and *Prosopium williamsoni* (mountain whitefish) all demonstrated substantial decreases in abundance after construction of the dam caused a habitat shift from riverine to lacustrine, whereas *Mylocheilus caurinus* (peamouth chub) and *Ptychocheilus oregonensis* (northern pikeminnow) (that is, smaller bodied fish) have gone from rare during predam conditions to abundant during postdam conditions (Dunnigan and others, 2017). During the period 1979–85, inadvertent introduction of nonnative *Oncorhynchus nerka* (kokanee), stocking of hatchery rainbow trout, and invasion by *Perca flavescens* (yellow perch) all have added to the disruption of fish-community profiles and of species composition of zooplankton and invertebrate food sources. In 1985, kokanee accounted for 96 percent of the number of fish harvested from the reservoir (Chisholm and Hamlin, 1987). The Montana Department of Fish, Wildlife and Parks (MTFWP) introduced rainbow trout to feed on kokanee with goals of (1) reducing the number of kokanee and thereby increasing the size of trout and (2) providing a trophy fishery as trout attained an ultimate large size. Kokanee are also an important food source for *Salvelinus confluentus* (bull trout) and burbot.

Species of fish currently monitored, their histories, and important traits and characteristics are listed in table 1 (available for download at https://doi.org/10.3133/ofr20201098) and discussed in more detail later. Percentage composition of species in spring and fall from 2009 to 2016 is shown in figure 6*A* and *B* (Presser and Naftz, 2020). Peamouth chub, northern pikeminnow, and *Catostomus macrocheilus* (largescale sucker) are dominant in spring, with the remaining species making up <10 percent of totals. In fall, peamouth chub, northern pikeminnow, and kokanee dominate, with the remaining species again making up <10 percent of totals. Tracking of hatchery rainbow trout shows few fish of the approximately 30,000 to 90,000 stocked per year since 2001 are caught in nets (2.2 fish, mean) (Dunnigan and others, 2017, 2020; Presser and Naftz, 2020), which leads to the supposition that hatchery fish do not thrive or recruit.

Mitigation of the effects of Libby Dam on the lake's ecosystem is an ongoing effort since its construction in 1972. Focus in terms of fish is on (1) stream habitat enhancement or restoration; (2) removal of nonnative species; (3) protection of subwatersheds supporting native species; (4) assessment of burbot stock, restored natives, and hatchery fish (for example, hybridization between Westslope cutthroat trout and rainbow trout); and (5) increasing the productivity of fish populations

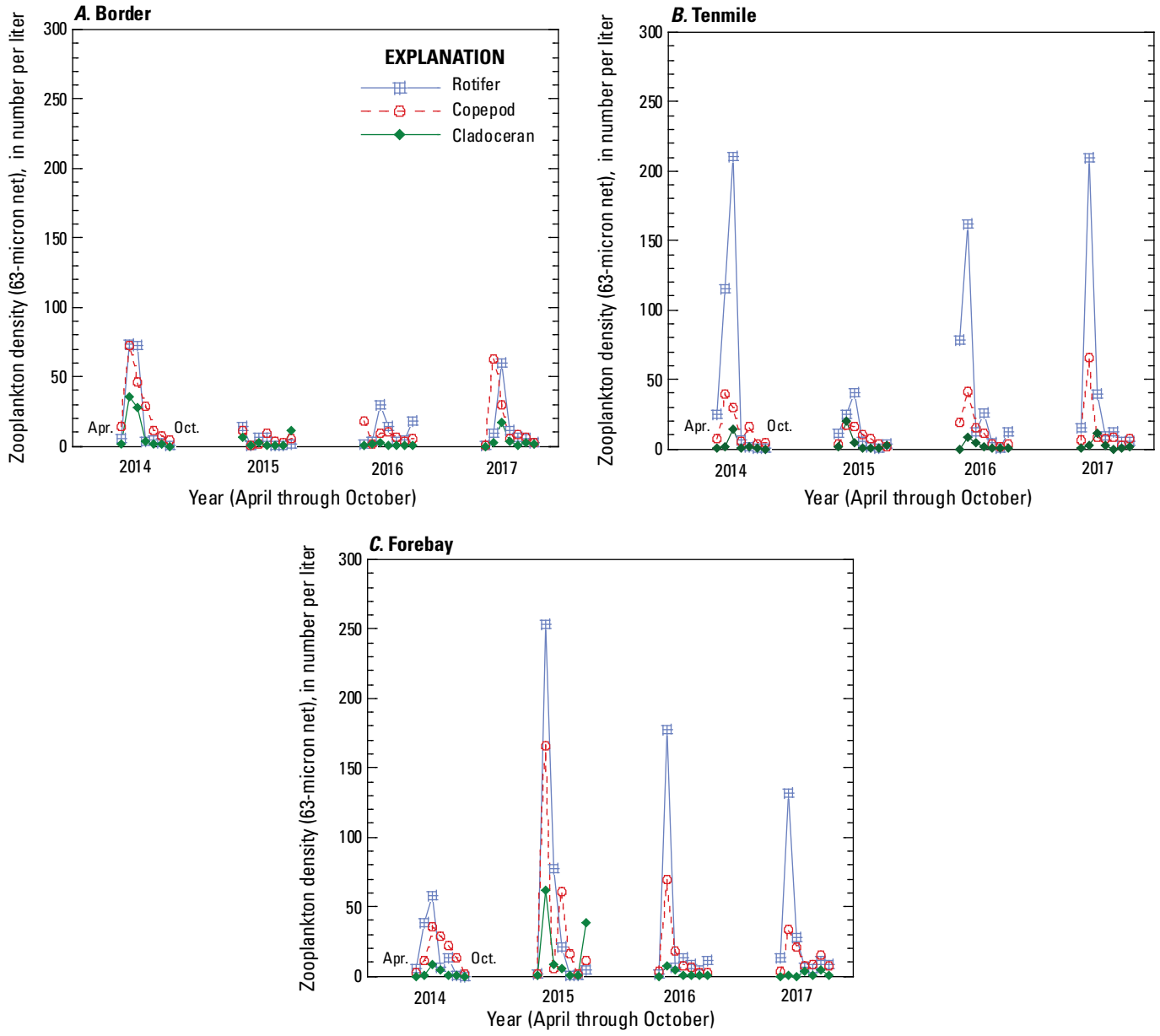


Figure 5. Zooplankton densities for 2014–17 (63-micron net). *A*, at the border; *B*, at Tenmile; *C*, at the forebay.

to help recruitment to adult stages (Dunnigan and others, 2017). The Kootenai River below the dam (that is, tailwater fishery) benefits from entrainment of kokanee through the dam because the fish become food for the river's population of rainbow trout; however, entrainment is detrimental to kokanee themselves.

Mitigation actions for minimizing the effects of Libby Dam operations that are temporal in nature include managing (1) downstream effects in the Kootenai River where the endangered white sturgeon and culturally sensitive burbot reside (for example, U.S. Fish and Wildlife Service [USFWS], 1999; Idaho Department of Fish and Game, 2008) and (2) tributary habitats for spawning (Dunnigan and others, 2017). Libby Dam has a selective withdrawal system that allows dam operators to mimic the natural annual temperature regime in the dam discharge in comparison to a traditional dam release of the hypolimnial layer (that is, bottom water). Hence, overall management of the Lake Koocanusa ecosystem is highly dependent on actions directed towards downstream mitigation.

The British Columbia side of Lake Koocanusa offers comparatively less elevation changes than the Montana side; hence, the ecological values of the northern reservoir shoreline (that is, foreshore as described here) areas for birds

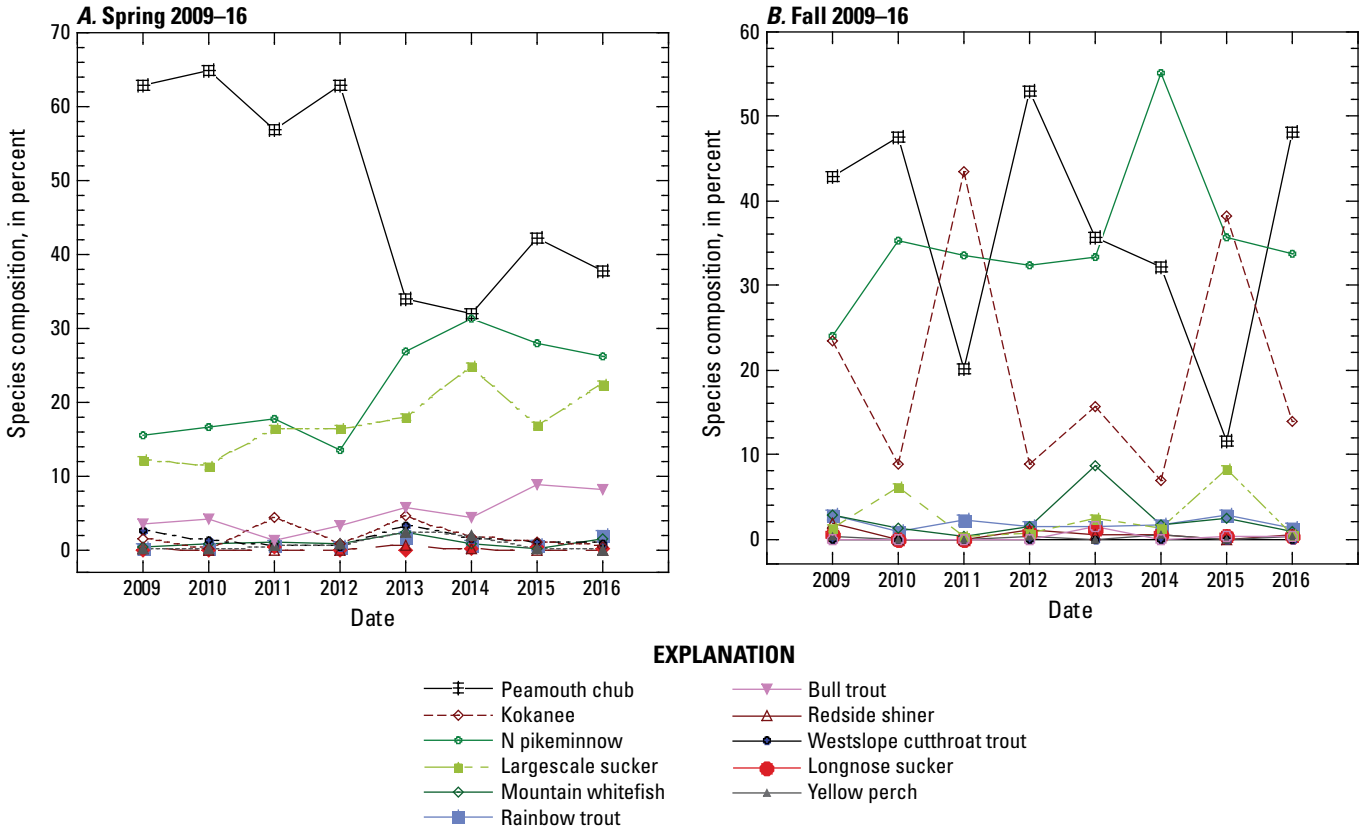


Figure 6. Percent composition of major fish species. *A*, in spring 2009–16; *B*, in fall 2009–16.

increase for such activities as foraging, migratory staging, and nesting/breeding (VAST Resource Solutions Inc., 2017). A list of recently observed birds for the British Columbia side foreshore areas includes species such as geese, grebes, loons, and mergansers and many migrating duck species (VAST Resource Solutions Inc., 2017, appendix C). A listing of bird species from eBird in 2016 for sites across the entire reservoir including Libby Dam (VAST Resource Solutions Inc., 2017, appendix D) shows an extensive array of aquatic, riparian, shore, and wetland species that are protected under the Migratory Bird Treaty Act.

The lake encompasses transboundary migratory routes and is a part of the Pacific Flyway. Northbound migration occurs from mid-April through mid-May. Southbound migration begins in mid-August for many shorebirds such as sandpipers, plovers, and dowitchers. Species that breed close to the lake's shores are *Actitis macularius* (spotted sandpiper), *Charadrius vociferus* (killdeer), and *Numenius americanus* (long-billed curlew). Upland wildlife that use the foreshore include songbirds (warblers, sparrows, flycatchers). Raptors utilizing the lake are *Falco peregrinus* (peregrine falcon), *Haliaeetus leucocephalus* (bald eagle), and *Pandion haliaetus* (osprey). Zones of sensitivity include specific protection rationales: active nesting for bank swallow and long-billed curlew; and designated Wildlife Habitat Areas for *Melanerpes lewis* (Lewis's woodpecker) and long-billed curlew (VAST Resource Solutions Inc., 2017). Selenium was analyzed in (1) spotted sandpiper eggs at the mouth of the Elk River in 2013–14 and (2) killdeer eggs in 2016 (Presser and Naftz, 2020). The maximum concentration in spotted sandpiper eggs was 8.4 µg/g dw (number of samples [*n*] =17), which exceeds the USFWS EC00 of 5.5 µg/g dw and EC10 of 7.7 µg/g dw for sensitive species (Presser and Luoma, 2010b). The maximum concentration in killdeer eggs was 3.7 µg/g dw (*n*=4).

Overarching Federal and State Policies for Ecosystem Setting and Species

The MTDEQ has identified Lake Koocanusa as threatened by selenium sources outside the State of Montana's jurisdiction or borders and listed the water body as impaired under section 303(d) of the U.S. Clean Water Act in 2012 (MTDEQ 303(d) list [MTDEQ, 2018]). The lake was identified as not fully supporting aquatic life as a beneficial use because of selenium, but also because of flow regime modifications. The current State regulation for selenium is through an aquatic-life standard of 5 µg/L (chronic) and 20 µg/L (acute) (MTDEQ, 2019). For comparison, the current

aquatic-life guidelines are 1.5 µg/L for lentic waters of the United States (USEPA, 2016a) and 2 µg/L for waters of British Columbia (Nagpal and Howell, 2001; BCMOE, 2014).

In the 303(d)-list assessment record for Lake Koocanusa, selenium loads were estimated to increase by an average of 376 kilograms per year (kg/yr) or 19.7 percent per year, with planned mine expansions and new mines adding to that trend in the future. Loads measured in 2017 in connection with British Columbia's permit monitoring show a total input of 10,398 kg/yr based on monthly selenium concentrations at the mouth of the Elk River and extrapolated daily flow measurements (Teck Coal Ltd., 2018b). The two highest monthly inputs are approximately 2,400 kilograms during May and June. Establishment of a total maximum daily load as part of State regulatory actions is required, but the status of attainment is currently unassigned, and its priority is listed as low. The assessment unit is only that part of the lake located in the United States. Additionally, in consideration of permitting, a mixing zone of approximately 4.4 mi extends between the Elk River input and the permit sampling location RG_DSELK (fig. 1). Technically, mixing zones are not applicable in U.S. regulation of contaminants that bioaccumulate such as selenium (USEPA, 2016d).

Qualifying statements about the regulatory status of Lake Koocanusa (that is, the source of pollution is not controlled by actions of a locally issued discharge permit and pollutant loads are increasing) introduce the question of whether permits for the coal mines in British Columbia will be adjusted based on selenium guidelines adopted by the State of Montana. In this regard, Lake Koocanusa is subject to transboundary regulations: namely, the Boundary Waters Treaty Act of 1909 between Canada and the United States. The treaty establishes (1) an International Joint Commission to carry out the provisions of the act when requested by national governments and (2) a framework for dealing with disputes. The U.S. State Department, U.S. Department of the Interior, and USEPA, along with their Canadian counterparts, are actively engaged in this transboundary issue, but not to the point of referring the issue to the International Joint Commission.

The USFWS lists bull trout as threatened and designated Lake Koocanusa as critical habitat for this species (USFWS, 1998). In Montana, this species is considered a "species of concern," and in British Columbia, bull trout is blue listed (that is, a species of special concern). Westslope cutthroat trout is a species of concern in Montana and is blue listed in British Columbia. Burbot is red listed (that is, potentially extirpated, endangered, or threatened) in British Columbia, with lower Kootenay River populations designated as critically imperiled (Teck Coal Ltd., 2018a; BCMOE, 2015). Burbot also is considered culturally important to native Tribes, with resources being actively dedicated to its preservation, especially below Libby Dam (Idaho Department of Fish and Game, 2008).

White sturgeon do not inhabit Lake Koocanusa, but in 1999, the USFWS published a recovery plan for the Kootenai River population of endangered white sturgeon that depends on the implementation of a long-term flow strategy for Libby Dam (USFWS, 1999). Thus, the selenium guidelines for Lake Koocanusa are required to protect the downstream use of provision of habitat for white sturgeon under the requirements of the Endangered Species Act (ESA). For ESA-listed species, a site-specific guideline can only be approved by the USEPA if the guideline either would not harm a single individual of any listed species or would harm so few individuals as to avoid a jeopardy biological opinion under section 7 of the ESA. If even one individual could reasonably be expected to be harmed (as might be the case for a guideline that is based on an EC10 level of toxicity), at a minimum, a formal biological opinion would need to be prepared by USFWS and an incidental-take statement would be required. The opinion could include perpetual-monitoring requirements for the documentation of compliance with the incidental-take statement. Additionally, a guideline could not adversely modify any critical habitat, which would include perturbations of food webs.

Methods—Modeling, Contours, and Cross Sections

The derivation of the methodology used here and the need for regulatory revision of selenium protection is extensively documented in Reiley and others (2003), Luoma and Rainbow (2005), Luoma and Presser (2009), Chapman and others (2010), and Presser and Luoma (2010a). Examples of the site-specific application of ecosystem-scale selenium modeling are available for the coal mining regions of southern West Virginia and several regions of San Francisco Bay affected by discharges from oil refineries, agriculture, and municipalities (Presser, 2013; Presser and Luoma, 2013; Luoma and Presser, 2018). The USEPA's national guidelines (2016a) and those developed for California (USEPA, 2018) used an approach based on ecosystem-scale modeling.

Jenni and others (2017) describe an ecosystem-scale modeling framework applicable to Lake Koocanusa. Their report (1) serves as a coherent and consistent structure for organizing relevant scientific information within modeling parameters, (2) provides an appropriate context for interpreting new information as datasets and site parameters are developed, and (3) identifies data and science gaps that limit understanding of the implications of alternative selenium guidelines. Also available is an initial assessment of a site-specific monitoring design that meets the data needs of modeling selenium in Lake Koocanusa (Presser and others, 2018). This sampling matrix focuses on (1) spatially and temporally paired samples of environmental media (water, particulates, prey and predator tissue), (2) a site nearest the source of selenium entering Lake Koocanusa from the Elk River in British Columbia, and (3) sites at the forebay of Libby Dam and at the international border in the United States.

Ecosystem-scale selenium modeling conceptualizes and quantifies the site-specific variables that determine the effects of selenium (Presser and Luoma, 2010a) (fig. 7). Used optimally, the model provides a tool for framing a site-specific ecological problem or occurrence of selenium exposure, quantifying exposure within that ecosystem, and narrowing uncertainties about how to protect it by understanding the specifics of the underlying system ecology, biogeochemistry, and hydrology.

This mechanistic approach uses dietary selenium biodynamics to explain bioaccumulation and predict responses in ecosystems to selenium (note: all environmental media selenium concentrations are expressed as micrograms per gram dw except dissolved selenium, which is expressed as micrograms per liter). Dietary selenium biodynamics establish that (1) invertebrates biomagnify selenium (influx is greater than efflux) from particulate material at the base of the food web by as much as 38-fold depending on species-specific differences in dietary assimilation efficiency (AE) and the rate constant of loss and (2) dietary transfer of invertebrate selenium to fish species (as measured in wb dw tissue) has a median of approximately one, which reflects a cumulative preservation of selenium. Thus, trophic transfer from particulate material, based on dietary selenium concentrations and feeding, controls bioaccumulation within an ecosystem. The mechanistic dietary transfer model was validated through use of datasets from 29 historical and recent field case studies of selenium-exposed sites (Presser and Luoma, 2010a).

Choices explicit to running the model that are critical to deriving site-specific protection are (1) the choice of fish species, which determines the food web through which selenium is modeled; (2) the choice of food web, which determines the particulate material to prey kinetics of bioaccumulation; (3) the characterization of the type (for example, SPM, bed sediment) and bioavailability (for example, selenium speciation) of particulate material, which determines exposure at the base of the food web; and (4) the metric describing partitioning between particulate material and dissolved selenium concentrations, which is specific to the attributes of the hydrologic setting. Overall, the approach illustrates that environmentally safe dissolved selenium concentrations will differ among ecosystems depending on the ecological pathways and biogeochemical conditions in that system.

The constructed model here is first run in validation mode. For Lake Koocanusa, this model uses an observed SPM selenium concentration and categorized food-web types, with their associated trophic transfer factors (*TTFs*), to predict selenium concentrations in prey and predators. The predictions are then compared to observed selenium concentrations. The equation as applied to Lake Koocanusa is

$$C_{Se\ fish\ wb} = (C_{Se\ SPM})\ (TTF_{invertebrate})\ (TTF_{fish\ wb}) \quad (1)$$

where

$C_{Se\ x}$ is the selenium concentration in SPM, invertebrate, or fish (wb);

$TTF_{invertebrate} = C_{Se\ invertebrate} \div C_{Se\ SPM}$; and

$TTF_{fish\ wb} = C_{Se\ fish\ wb} \div C_{Se\ invertebrate}$.

As stated previously, the main mathematically determinative variables are the type of particulate material used to represent the ecosystem, the selenium concentration in the particulate material, and the taxa or species of the invertebrate consuming that food. The effect of multistep food webs that include forage fish as additional prey (that is, trophic level 3 to trophic level 4 [TL3 to TL4] food webs) and the bioavailability of selenium to the invertebrate (that is, selenium speciation of the particulate material) also may be quantified through additional equation components (Presser and Luoma, 2010a).

The model is then run in translation mode, which addresses the regulatory aspects of modeling. Here, an assumed fish tissue selenium guideline ($C_{fish\ Se\ guideline\ wb}$) and an observed environmental partitioning factor [$K_{d\ SPM}$ = ($C_{Se\ SPM}/C_{Se\ dissolved}$) (1,000)] indicative of hydrological setting characteristics are used to predict a dissolved selenium concentration ($C_{Se\ dissolved}$) that is protective of the site-specific ecosystem. The equation as applied to Lake Koocanusa is

$$C_{Se\ dissolved} = C_{fish\ Se\ guideline\ wb} \div [(TTF_{fish\ wb})\ (TTF_{invertebrate})\ (K_{d\ SPM})] \quad (2)$$

where

$(K_{d\ SPM})\ (C_{dissolved})$ is substituted for $C_{Se\ SPM}$ and the equation is solved for $C_{Se\ dissolved}$.

$K_{d\ SPM}$ reflects the efficiency of environmental partitioning of selenium from the dissolved phase to the chosen particulate phase (Presser and Luoma, 2010a). In the modeling here, wb fish tissue is used as the endpoint ($C_{fish\ Se\ guideline\ wb}$), but egg-ovary tissue can be substituted if tissue-to-tissue conversion factors (CFs) are available (Janz and others, 2010; USEPA, 2016a, b). Thus, the predicted dissolved selenium concentration would quantify the ecosystem condition where fish would adhere to the assumed tissue guideline.

A series of modeling scenarios are used throughout the modeling process based on a range of choices that are informed by selenium science and stakeholders' decisions. Through both data utilization, as described below, and scenario development, sensitive locations, timing, and food webs can be identified to understand the maximum selenium bioaccumulation potential (BAP) and, hence, the focus of effective regulation and management.

The contouring package in OriginPro (v. 9.1, 2014) software was used to construct gradient plots of monitoring data from Lake Koocanusa. The triangulation method was used to contour the data. Selected polygons (for example, dissolved selenium concentrations greater than [>] 1.0 µg/L) from the gradient plots were extracted with the digitizer tool for x- (distance from Libby Dam) and y- (depth below water surface) coordinates from each monthly concentration gradient map. The absolute area of each polygon was calculated using the polygon area calculator.

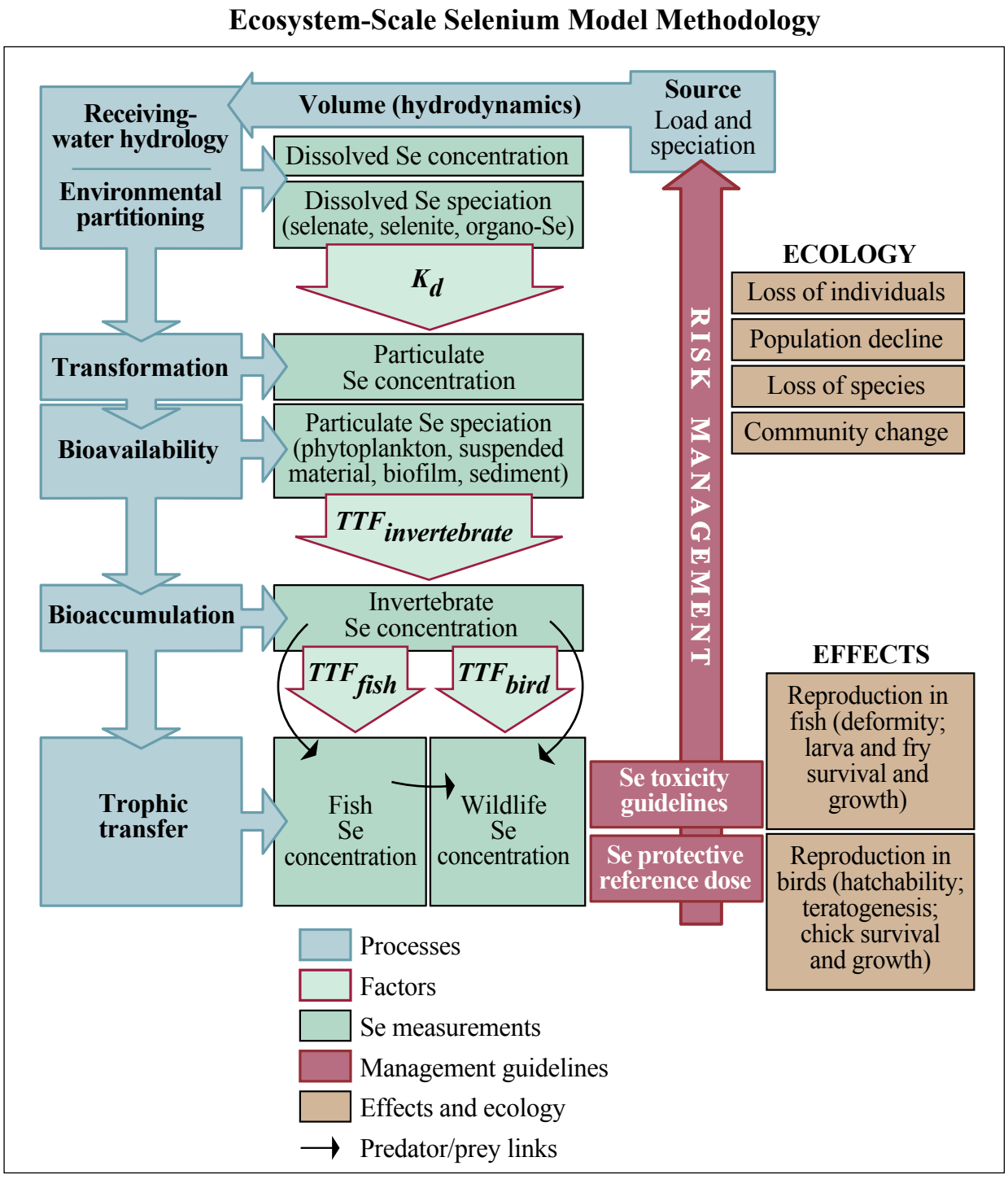


Figure 7. Ecosystem-scale selenium model methodology. The model conceptualizes processes and parameters important for quantifying and understanding the effects of selenium in the environment. The model can be applied to forecast exposure and to evaluate the implications of management or regulatory choices. [K_d, empirically determined environmental partitioning factor between water and particulate material; *TTF*, biodynamic food-web transfer factor between an animal and its food; Se, selenium (adapted from Presser and Luoma, 2010a)]

Supporting Data—Scope of Studies and Study Area

Sets of site-specific data support and give context to ecosystem-scale selenium modeling in this interpretative report (Presser and Naftz, 2020). These data were compiled from publicly available databases of Federal, State, and Provincial agencies and reports by Teck Coal Ltd. Some sets of raw data that helped inform the data presented in Presser and Naftz (2020) are available from MTDEQ (lakekoocanusaconservation.pbworks.com). The spreadsheets within our compilation are designed in an ecosystem-component format to assist modeling. Water-quality data are generically termed as "water column" for descriptive purposes below, but the data themselves are specific to dissolved (filtered) or total (unfiltered) samples. Reservoir sampling sites extend from the Elk River input in the north to the forebay in the south (see fig. 1 and figs. 8–11 for detailed maps of water-quality, invertebrate, and fish sampling locations).

Categories of data compiled in the companion USGS data release (Presser and Naftz, 2020) are as follows:

1. water-column selenium concentrations (2008–18),
2. recent USGS border platform water-column selenium concentrations (2019),
3. water-column selenium concentrations for the Elk River at Highway 93 (August 1984–January 2019),
4. spatially and temporally paired water-column and SPM selenium concentrations (2015–18),
5. recent detailed south of Elk (SOE) water-column selenium concentrations (2017–18),
6. dissolved selenium speciation (2015–16),
7. productivity (phytoplankton density) (2014–17),
8. zooplankton selenium concentrations (three net sizes) (2008, 2004–19),
9. zooplankton taxa metrics (2014–18),
10. invertebrate selenium concentrations (2013–16; 2018),
11. invertebrate taxa metric (2014–16; 2018),
12. dietary metrics for fish (percent of diet; relative importance index) (historical),
13. invertebrate taxa in fish stomachs (2017–18),
14. fish selenium concentrations (egg-ovary) (2008; 2013; 2014–18),
15. fish species abundance (2009–16) and fish catches (2014–16; 2018),
16. number of stocked fish (1988–2016),
17. annex—bird egg selenium concentrations (2013, 2014, 2016),

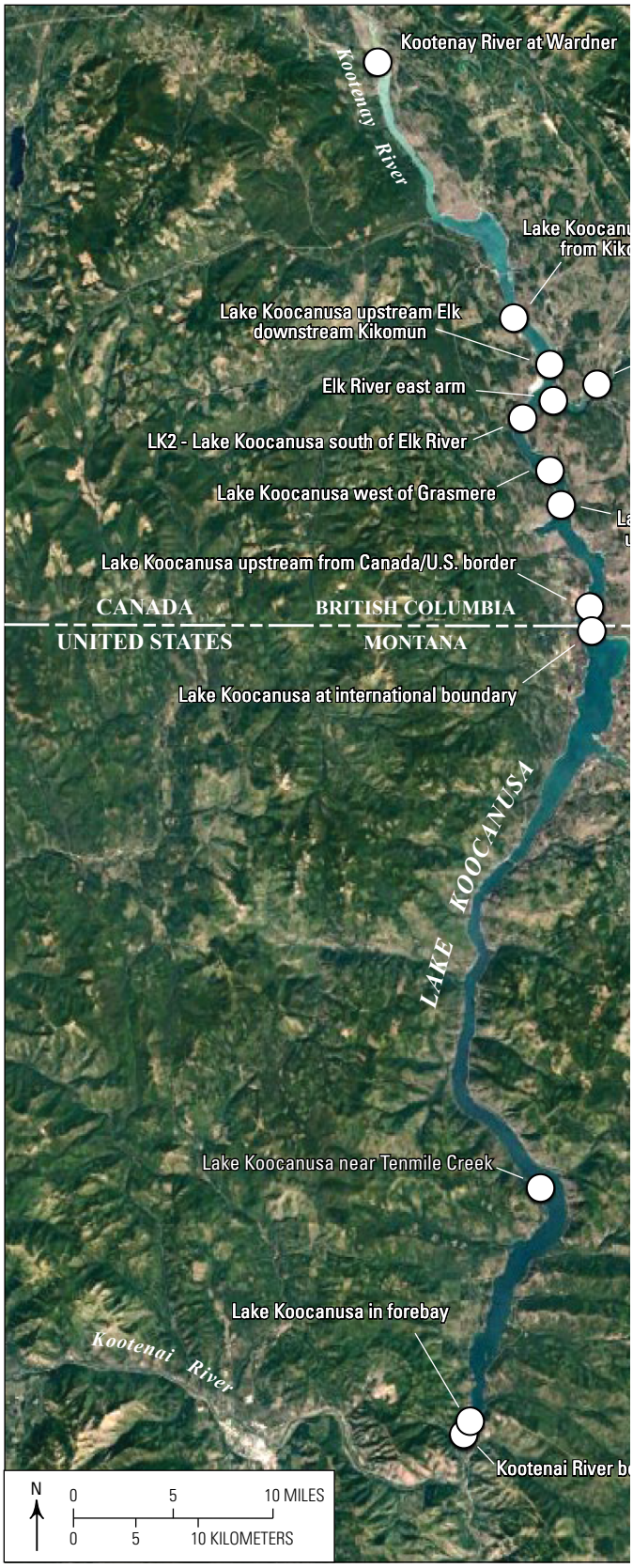


Figure 8. Location of sites where water quality and (or) suspended particulate material samples were collected from Lake Koocanusa, Elk River, and the Kootenai River, Montana, and Kootenay River, British Columbia.

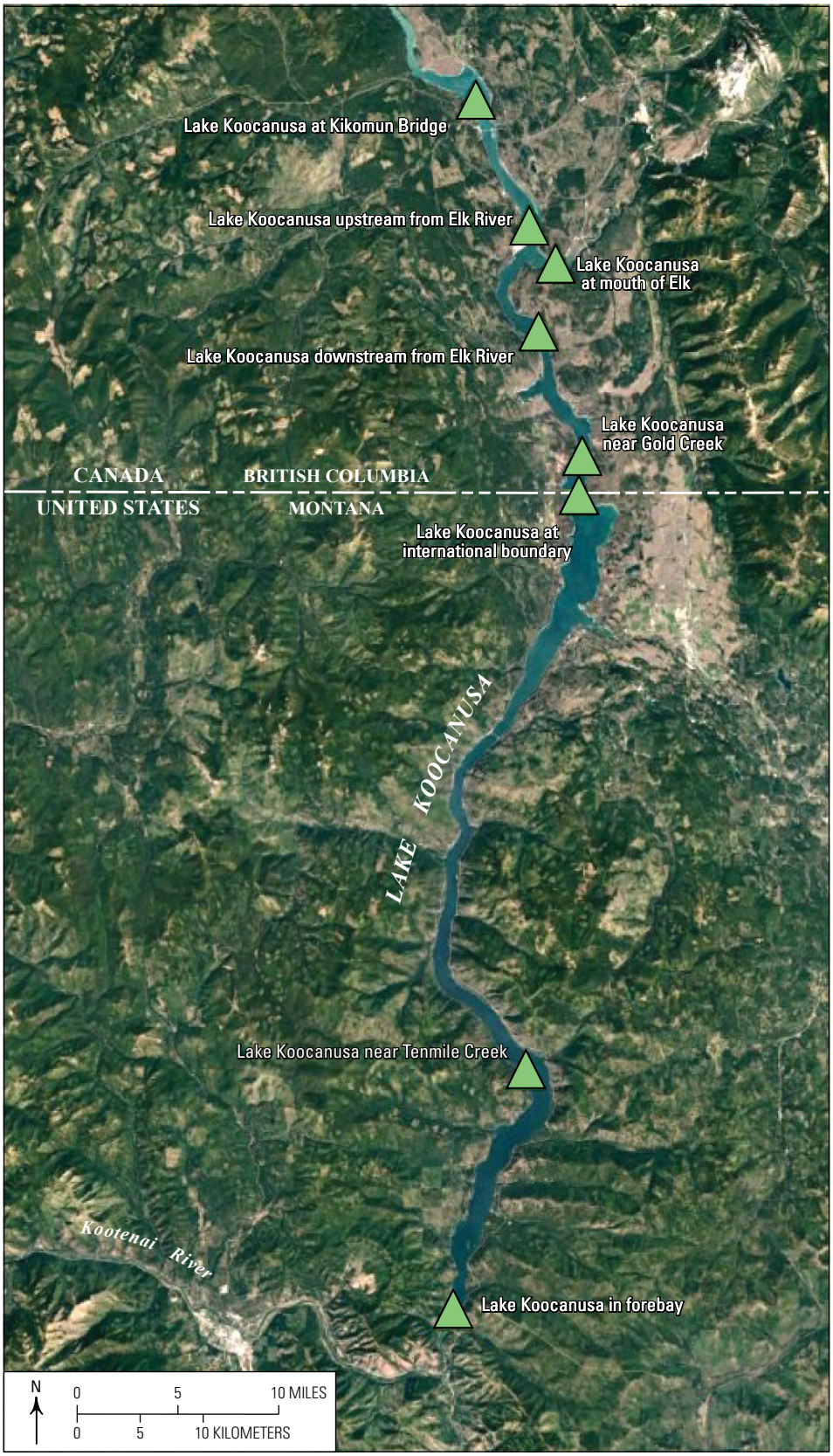


Figure 9. Location of sites where zooplankton samples were collected from Lake Koocanusa, Montana and British Columbia. Sample locations north of the international boundary may vary slightly depending on reservoir levels and other variables when the sample(s) were collected.

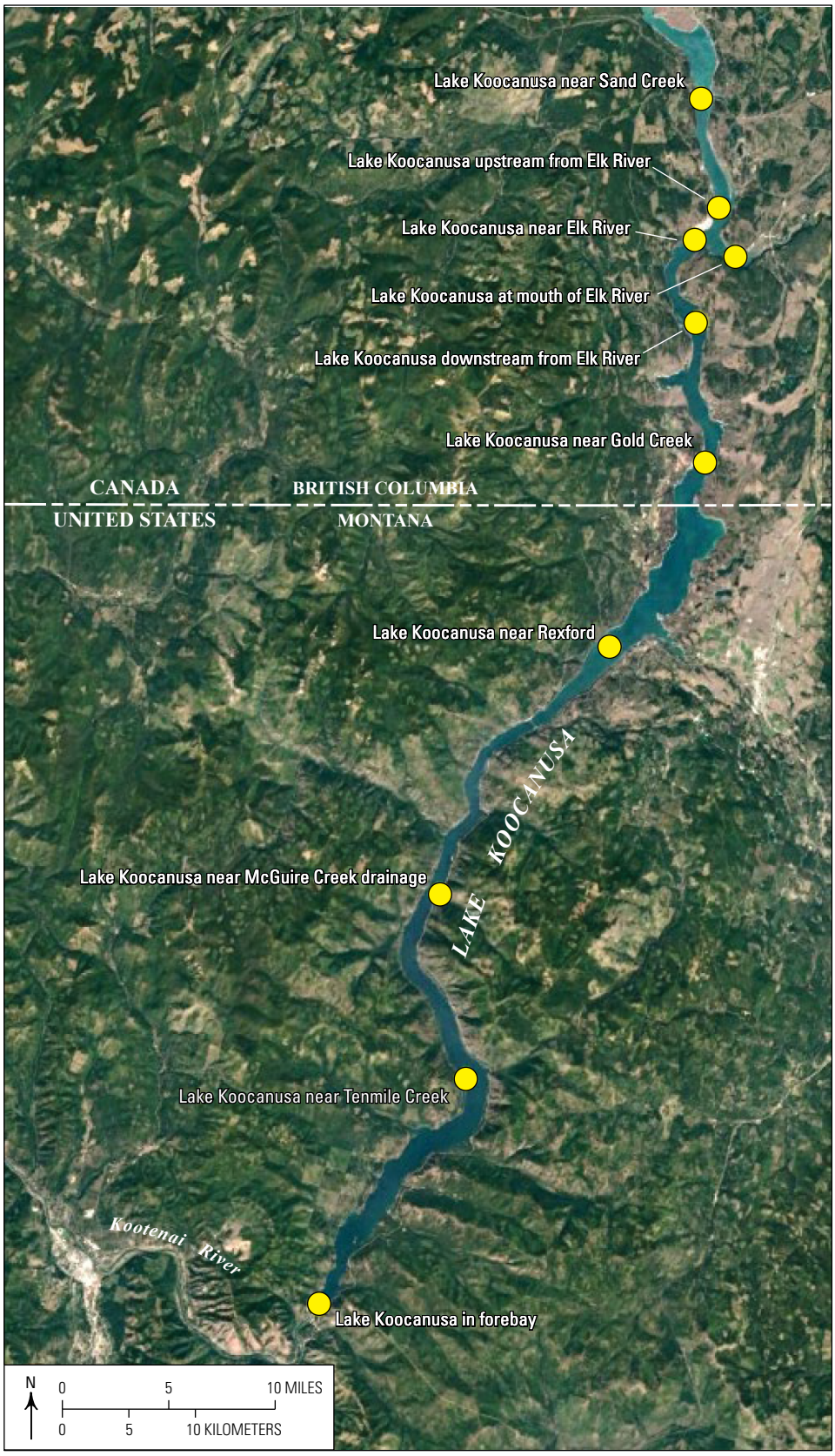


Figure 10. Location of sites where invertebrate samples were collected from Lake Koocanusa, Montana and British Columbia. Sample location(s) may vary slightly depending on reservoir levels and other variables when the sample(s) were collected.

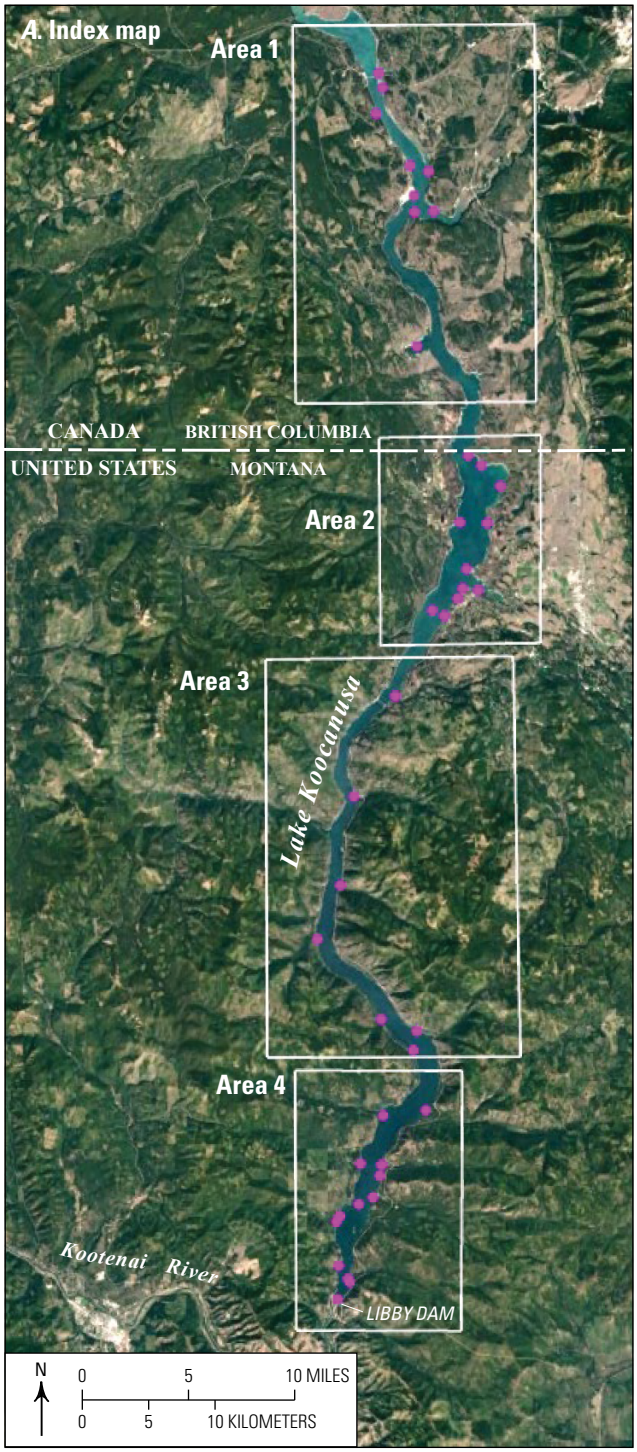
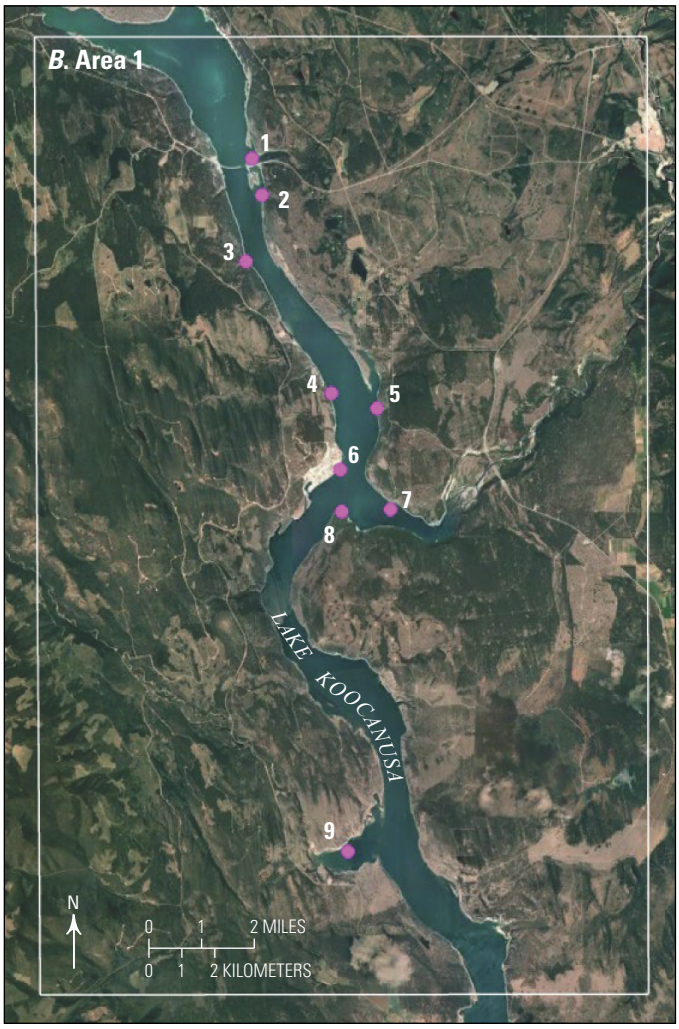
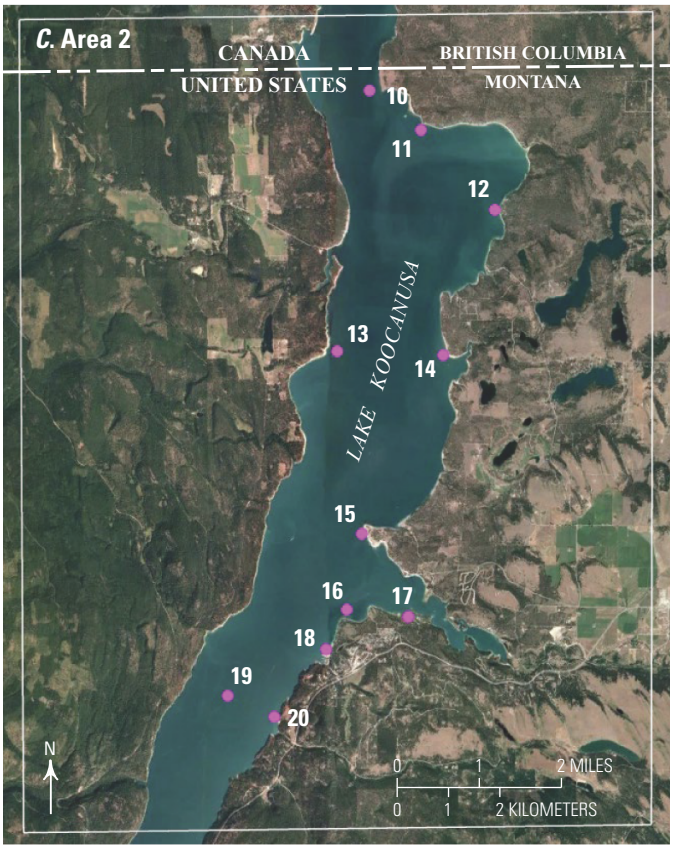


Figure 11. Location of sites (circles) where fish samples were collected from Lake Koocanusa. *A*, index map; *B*, Lake Koocanusa, British Columbia, area 1; *C*, Lake Koocanusa, Montana, area 2; *D*, Lake Koocanusa, Mont., area 3; *E*, Lake Koocanusa, Mont., area 4; *F*, Lake Koocanusa, British Columbia and Montana, areas 1–4 showing gill-net locations (triangles) used by Montana Fish, Wildlife and Parks.



EXPLANATION

1. Lake Koocanusa near Sand Creek
2. Lake Koocanusa south of Kikomun Road bridge
3. Lake Koocanusa near Kikomun Creek Provincial Park
4. Lake Koocanusa north of cabin
5. Lake Koocanusa across from campground
6. Lake Koocanusa at cabin
7. Lake Koocanusa south of Elk River
8. Lake Koocanusa at south point of Elk River drainage
9. Lake Koocanusa near Gold Creek



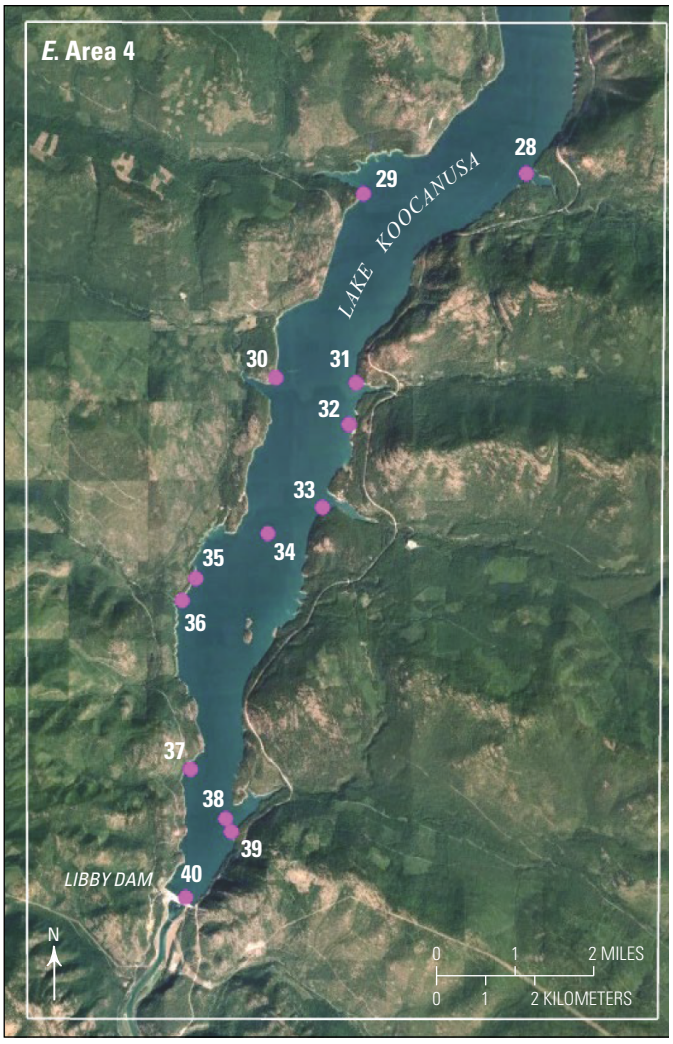
EXPLANATION

10. Lake Koocanusa at international boundary
11. Lake Koocanusa north of Sophie Creek drainage
12. Lake Koocanusa south of Sophie Creek drainage
13. Lake Koocanusa near Young Creek drainage
14. Lake Koocanusa North Point Murray
15. Lake Koocanusa at north point of Tobacco River drainage
16. Lake Koocanusa at south point of Tobacco River drainage
17. Lake Koocanusa near Tobacco River drainage
18. Lake Koocanusa farther south of Tobacco River drainage
19. Lake Koocanusa near Rexford
20. Lake Koocanusa North Black Lake

Figure 11. —Continued

EXPLANATION

21. Lake Koocanusa near Pinkham Creek drainage
22. Lake Koocanusa near Sutton Creek drainage
23. Lake Koocanusa near McGuire Creek drainage
24. Lake Koocanusa near Parsnip Creek drainage
25. Lake Koocanusa near Ural Creek drainage
26. Lake Koocanusa near Tenmile Creek drainage
27. Lake Koocanusa near Tenmile Creek



EXPLANATION

28. Lake Koocanusa near Fivemile Creek drainage
29. Lake Koocanusa near Bristow Creek drainage
30. Lake Koocanusa north of Barron Creek drainage
31. Lake Koocanusa north of Warland Creek drainage
32. Lake Koocanusa near Warland Creek drainage
33. Lake Koocanusa near Cripple Horse Creek drainage
34. Lake Koocanusa near McGillivray campground
35. Lake Koocanusa north of Jackson Creek drainage
36. Lake Koocanusa near Jackson Creek drainage
37. Lake Koocanusa near Peace Creek drainage
38. Lake Koocanusa near Canyon Creek drainage
39. Lake Koocanusa south of Canyon Creek drainage
40. Lake Koocanusa in forebay

Figure 11. —Continued

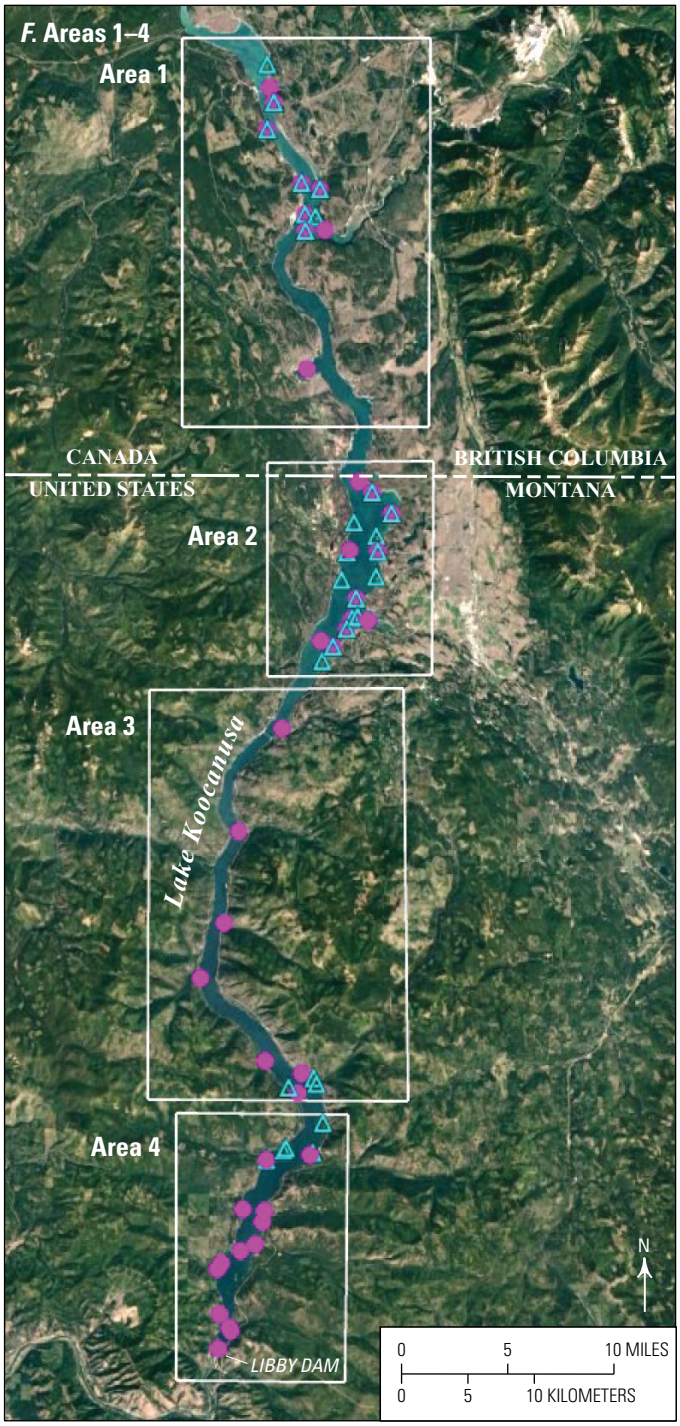


Figure 11. —Continued

18. annex—fish mercury concentrations (muscle) (2013–18), and

19. annex—the State of Montana's human health consumption advisories for mercury and selenium (2014).

Transboundary Metadata and Suspended Particulate Material Sampling

Metadata within Presser and Naftz (2020) give details of sampling and analysis methodologies for each category of data. Methodologies are specific to collection agency because of the transboundary nature of monitoring. Discussions are currently proceeding among Federal, Provincial, and State agencies about a potential transition to applying consistent monitoring protocols to ensure consistency in sample and data collection on both sides of the border.

It is important to note here that key to the data-collection effort to support modeling of Lake Koocanusa was the introduction of sampling of SPM on the Montana side in 2015 and on the British Columbia side in 2017. A selenium concentration in SPM is used to initiate modeling as the diet of invertebrates at the base of the food web, and its characterization is important in representing the dynamics of the system (Presser and Luoma, 2010a). The oligotrophic/mesotrophic nature of Lake Koocanusa requires collecting large-volume water samples and using an efficient concentrating technique (that is, ultracentrifugation) to obtain a sufficient mass of SPM for chemical analysis (Horowitz,1986; Horowitz and others, 2001, 2008). This approach provides an integrated range of particle sizes representational of (1) seasonal sources, (2) hydrological transport conditions, and (3) the dynamics of ecological selenium processing within Lake Koocanusa.

SPM samples were spatially and temporally matched to water samples to enable calculation of K_d (Presser and others, 2018; Presser and Naftz, 2020). K_d operationally defines the instantaneous partitioning of selenium between particulate and dissolved phases. This metric is driven by the residence time of water and, as such, offers a quantitative factor representing competing hydrologic and biogeochemical processes at work in complex aquatic systems (Presser and Luoma, 2010a). Monthly dissolved selenium concentrations, even if not paired with an SPM concentration, also are compiled as part of Presser and Naftz (2020) to further elucidate the impacts of hydrodynamics.

From 2015 to 2017 on the Montana side, paired samples were collected at two locations (forebay and international boundary) and at two depths (epilimnion, 3 meters (m) below lake surface; hypolimnion, 3 m above lake bottom). The number of SPM sampling times per year varied (2015, n=3; 2016, n=4; 2017, n=7), thus limiting direct comparative profiles. In 2018, paired sampling on the Montana side focused on concentrations for the epilimnion at the border, Tenmile, and forebay sites in May, June, and September (n=9). In 2019, sampling occurred at the border and forebay for May, July, and September for two depths except for the forebay in September (n=11). For the British Columbia side, sampling events at SOE occurred in June, July, and September in 2017 at three depths (n=9) and in June–October in 2018 at the epilimnion (n=5). SPM data for 2019 were delayed and are not included here.

A Lake-Gradient Approach to Support Modeling and Resulting Decisions on Data Reduction

A dynamic, working system view of Lake Koocanusa during 2017 was developed from (1) detailed bar graphs for observed dissolved and SPM selenium concentrations specific to epilimnion and hypolimnion strata for SOE, border, and forebay sites (Presser and others, 2018; figs. 5–9) and (2) depth-dimensional gradient maps of observed dissolved and SPM selenium concentrations and calculated K_d values for sites at the SOE, border, and forebay (fig. 12*A*, *B*, *C*). The distribution of selenium in the lake, as illustrated in these figures, is influenced by hydrodynamics and source loading conditions of the lake. Specifically these conditions (1) drive environmental partitioning and may under some instances disconnect the particulate material and the water column as rapid flow transitions occur (Presser and others, 2018, figs. 11–16) or riverine conditions interface with the reservoir (for example, extensive range of K_d values for SOE) and (2) underlie the fundamental functioning of ecosystems that are portrayed and quantified in selenium modeling. These data and figures illustrate that (1) a distinct spring contaminant plume in dissolved selenium occurs at 13 m, with a similar plume occurring in fall at 21 m; (2) for both the epilimnion and hypolimnion, selenium concentrations in SPM at the forebay are generally greater than those at the border, with the trend reversing for dissolved selenium (that is, border is greater than forebay); (3) for SPM selenium concentrations in the forebay, the epilimnion generally exceeds the hypolimnion, but for dissolved selenium concentrations, the hypolimnion generally exceeds the epilimnion; and (4) patterns for K_d are similar to that for selenium concentrations in SPM.

The dynamic nature of Lake Koocanusa is an important consideration because (1) modeling is initiated through choice of SPM selenium concentrations and (2) site-specific water-column selenium concentrations are derived for regulatory purposes that are a function of both the choice of K_d and the food web. Hence, concentration gradient maps specific to lake strata and location can narrow uncertainty both in terms of selenium source and hydrodynamic effects. These gradient maps also help explain temporal variability of (1) dissolved selenium concentrations during April and May when snowmelt and drawdown are occurring and (2) SPM selenium concentrations during June, July, and August when biological productivity is elevated.

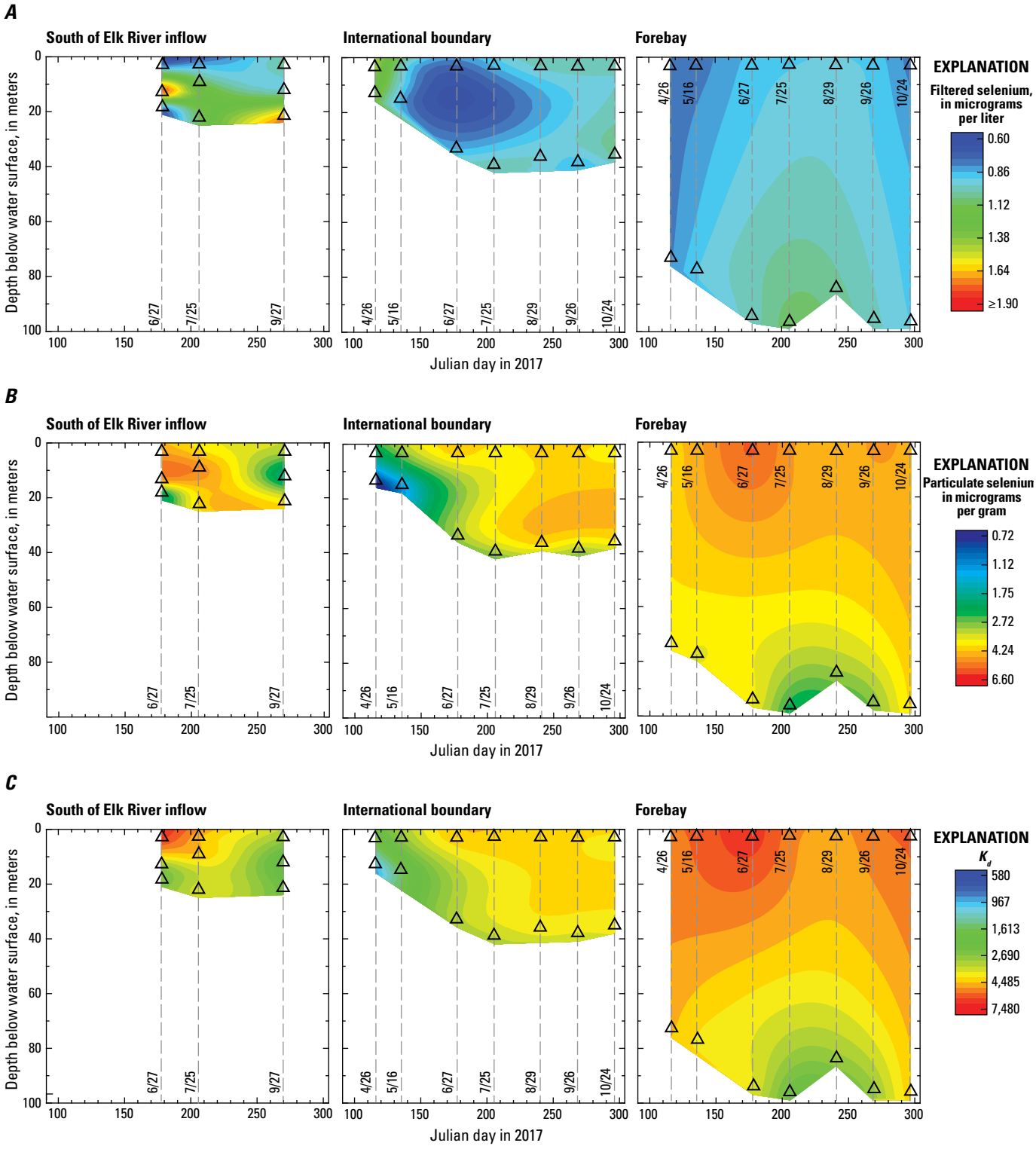


Figure 12. Depth-specific gradients across three reservoir monitoring sites in 2017. *A*, for dissolved selenium concentrations; *B*, for suspended particulate material selenium concentrations; *C*, for environmental partitioning factor (K_d) values (that is, partitioning factor between the dissolved phase and particulate phase). [triangles denote sample depth; dashed lines denote when samples were collected: date (mm/dd) and Julian day (2017)]

Given the importance of the dimensional nature of dissolved and SPM selenium concentrations in the lake as delineated above, the decision here, at this stage of knowledge for the lake, was not to statistically reduce datasets but rather to consider each paired sampling event and consequent K_d calculation as an independent scenario. Overall, a gradient-specific approach helps conceptualize potential seasonal and spatial interactions with food webs and inform modeling in terms of sensitive times and locations when considering regulatory and management options.

Data Utility for Modeling—Field Collection and Selenium Analysis of Invertebrates and Fish

Divergent goals for monitoring of Lake Koocanusa impinged on data usefulness for the dietary biodynamic modeling of selenium. In general, existing monitoring programs were not adjusted to protocols based on the ecosystem aspects of selenium modeling and, thus, do not provide essential spatially and temporally coordinated samples of ecosystem compartments that are important to narrowing modeling uncertainty. Further, species or taxa specificity, sampling of reproductive tissue in game fish species, and a gradient approach for a defined source of selenium contamination were not important design elements of monitoring. Rather, sampling programs were maintained at current objectives that pertain to (1) age, health, and recruitment of fish species; (2) fish-community aspects; (3) set times and sites for reservoir-reach scale sampling or broadly defined upstream-to-downstream sampling at the Elk River input; and (4) separate schedules and sites for sampling each media type. For example, fish sampling by MTFWP took place at a site designated as "Canada," which is mainly above the Elk River discharge, the source of selenium (Presser and Naftz, 2020) (fig. 11*F*). On the British Columbia side, monitoring of zooplankton and invertebrate as food sources identified taxa, but selenium concentrations were analyzed on composited samples. Fish monitoring, which occurs on a 3-year cycle in Montana, also was not adjusted to accommodate matching fish samples with the scheduled intensive sampling of SPM in 2017. Additionally, selenium speciation for particulate material was not analyzed as part of monitoring. This parameter is important for determining the bioavailability of SPM selenium to prey species within a biodynamic model (Presser and Luoma, 2010a).

Listed in table 1 are the species of fish important to the Lake Koocanusa ecosystem and past decisions concerning field sampling of fish (for example, seasonal timing, type of tissue, number of samples per species) that took place during 2008–18 at sites on both sides of the border. The components of table 1 also address the broader context of connecting to the reproductive nature of selenium's toxicity as a requirement of regulatory policy. Specifically, as described by Janz (2011), selenium is an environmental reproductive toxicant for fish with a characteristic suite of early life-stage deformities resulting from maternal transfer of selenium-methionine to yolk proteins during vitellogenesis and subsequent selenium exposure during yolk resorption by developing larvae.

There is an important distinction in terms of derivation of tissue selenium guidelines themselves (that is, construction of toxic-response curves) and their subsequent connection to bioaccumulation equations in order to derive or predict site-specific dietary and dissolved selenium concentrations. The primacy of egg tissue as a guideline is related to its reliability for prediction of effects (Lemly and Skorupa, 2007; Chapman and others, 2010; USFWS, 2012; USEPA, 2016a). The interconnectedness or partitioning of selenium concentrations among diet, egg, and wb selenium concentrations in fish is complex, although there is causality between diet and both egg and wb tissue selenium concentrations. The derived connection used by the USEPA in modeling and translating tissue guidelines is mainly between diet and wb concentrations (for example, 89 percent in field exposure scenarios) with a CF that is species specific in order to convert wb to egg-ovary concentrations (USEPA 2016a, b, c, d, 2018). Hence, the practical consequence of the USEPA's approach is that the diet to egg relationship is not defined as a primary relationship in fish when modeling the dietary transfer of selenium to connect to a water-column selenium concentration. However, studies have addressed the necessity of an internally consistent equilibrium relationship between wb and egg selenium concentrations based on dietary exposures, as would be expected in nature (Chapman and others, 2010). Additionally, few laboratory data are available to directly connect trophic transfer of selenium from diet to egg tissue.

To further inform our discussion here, excerpts relating the ecotoxicology of selenium to (1) fish tissue monitoring guidance for the implementation of site-specific selenium guidelines (USEPA, 2016b) and (2) tissue-to-tissue conversion relationships (Janz and others, 2010) are given in table 2. These rationales shed light on questions concerning (1) muscle tissue as an unacceptable surrogate for egg tissue and consequent connection to reproductive effects; (2) the importance of gravid females and sampling of expressed eggs; (3) problematic interpretations of data for combined egg-ovary tissue, in general, and specifically for nonsynchronous spawners like cyprinids; and (4) timing for preference of wb sample collection versus egg sample collection. The implications of the compiled information (1) as part of food-web and hydrological model inputs and (2) as to their ecological relevance in representing and quantifying fundamental processing of selenium are discussed below in the larger context of the status of the Lake Koocanusa ecosystem.

Table 2. Excerpts concerning ecotoxicology and fish tissue monitoring or tissue-to-tissue relationships.

Excerpts (U.S. Environmental Protection Agency, 2016b)
Egg-ovary sampling including temporal factors associated with spawning
When using egg-ovary tissue for the implementation of the selenium criterion, States and authorized Tribes must be careful to consider the difficulty in timing egg-ovary sampling with spawning periods.
The only appropriate time to collect egg-ovary tissue from suitable species is when the female is gravid in the pre-spawn stage, just before mating and spawning. This is typically a very small window (see appendix B) of time for most synchronous species.
An egg-ovary tissue sample from a female that is not gravid will not be representative for monitoring and assessment when compared with gravid egg-ovary results because the egg-ovary tissues represent the potential selenium load available to eggs and larvae through maternal transfer.
Egg-ovary tissue is the preferable tissue to collect because the egg-ovary tissue of pre-spawn, reproductively mature (also called "gravid" or "vitellogenic") females will give the most accurate view of potential selenium hazard to reproduction.
Egg-ovary tissue (which refers to eggs, ovaries, or both) data provide point measurements that reflect integrative dietary accumulation, transfer, and deposition of selenium over time and space in female fish at a given site.
Research has shown that selenium concentrations in egg-ovary tissue is strongly correlated with selenium in the maternal diet, which is transferred from the adult female during vitellogenesis.
It is the selenium concentration in eggs that drives early life-stage toxicity, so adult female fish must be collected during the late vitellogenic or preovulatory periods of oogenesis for this criterion to be scientifically and toxicologically meaningful.
Timing errors related to fish reproduction may result in data that falsely indicate the selenium criterion is being met.
Most fish species that are synchronous spawners do so in the spring, whereas fish tissue collection for advisories typically occurs in the late summer or early fall, when contaminant loads in the edible portion of the fish are highest. If an agency is limited to sampling outside of the pre-spawning period due to resource constraints, that will need to be considered when incorporating selenium fish tissue monitoring into the existing programs, or when developing a new program (for example, sampling whole-body or muscle tissue instead of egg-ovary tissue).
For egg-ovary tissue sampling, agencies with fish tissue monitoring responsibilities should consult with a State fisheries biologist to determine the appropriate time for sampling specific species in their region in order to capture the specimens in their pre-spawning phase. These regional experts will be familiar with the local species and able to use their best professional judgment to determine which are appropriate for selenium sampling and the appropriate sampling time frame based on spawning season.
Monitoring programs should sample for reproductively mature females from iteroparous fish species (that is, fish that have multiple reproductive life cycles over the course of its lifetime) that are single batch (synchronous) or multiple batch (asynchronous) spawners.
Fish species that spawn multiple times per season (asynchronous; for example, species in the family Cyprinidae) have variable cycles of oogenesis and, thus, special care should be taken when using these for egg-ovary monitoring as the pre-spawn window can be hard to predict.
Egg maturation may occur well before, immediately prior to, or during the spawning season.
For example, *Lepomis cyanellus* (Green Sunfish) can spawn multiple times per season (Osmundson and Skorupa 2011, Chapman and others, 2010).
For many fish species, vitellogenesis can occur over several months prior to spawning, with a relatively large amount of yolk deposited into eggs (Osmundson and Skorupa 2011).
It is also possible that species with relatively large eggs and yolks deposit more selenium in their eggs than species with smaller eggs and yolks (Osmundson and Skorupa 2011).
Selenium concentrations in the eggs and ovarian tissues are expected to be at their maximum level when eggs have maximum levels of vitellogenin prior to spawning; therefore, egg-ovary tissue samples collected outside of the pre-spawn window are not suitable for assessment in comparison to the national egg-ovary fish tissue criterion element.
Reproductively mature females of most fish species, except indeterminate spawning species and viviparous species (that is, live bearing), will produce eggs that can be sampled for selenium. Appendix A of this document (i.e., USEPA, 2016b) presents egg and ovary collection and sample preparation methods.
The egg-ovary tissue element has primacy over all other elements; thus, when available, it is the ultimate arbiter for compliance with the selenium water-quality criterion. Most States and authorized Tribes do not currently collect egg-ovary tissue as part of their regular monitoring programs. The USEPA recognizes that many States and authorized Tribes may not have the resources to augment their existing monitoring programs to include egg-ovary tissue collection. Although egg-ovary tissue remains the preferable tissue type, whole-body or muscle samples can be used as an alternative.

Table 2. Excerpts concerning ecotoxicology and fish tissue monitoring or tissue-to-tissue relationships.—Continued

Excerpts (U.S. Environmental Protection Agency, 2016b)
Fish species selection—Fish body size
It is difficult to collect egg-ovary (or muscle) tissue samples from small fish species (for example, certain species in the family Cyprinidae or Cyprinodontidae) because the amount of tissue available for analysis is small, and many of these species are asynchronous spawners that do not have a large number or biomass of eggs at any one time.
Whole-body sampling
Measuring selenium concentration in ovarian tissue during other periods of oogenesis will be much less informative. Summer and fall may be prime periods for whole-body and muscle tissue collection due to the engorgement of populations to replenish fat and energy reserves post-spawn.
If agency resources limit fish tissue collection to times outside of these species-specific windows connected to spawning periods, then the only appropriate samples to collect are whole-body and muscle tissue. Target fish species collected in the fall may be common to selenium monitoring and human health risk assessment. In this case, muscle tissue can be composited and evaluated for selenium in addition to contaminants of interest for fish consumption advisories.
The USEPA is aware that some States and authorized Tribes make use of muscle plugs in their monitoring programs. However, it is important to remember that contaminant concentrations can vary considerably depending on where the plug is collected. Plugs provide very small tissue quantities (about 1 gram of tissue per fish) and, therefore, not enough biomass for possible reanalysis or quality assurance/quality control considerations. In addition, relatively small individuals may not recover from a muscle plug biopsy punch. Care should be taken to ensure that the sampling protocols involving plugs have a sound scientific basis and that there is enough tissue for the analytical method.
Seasonal considerations are less stringent for whole-body and muscle tissue sampling. Seasonal collection of whole-body or muscle fish tissue samples should be timed to avoid the pre-spawning influence on selenium tissue concentrations, particularly for females, since enhanced depuration of selenium from tissue stores may occur during vitellogenesis prior to spawning (USEPA, 2016a).
Excerpts (Janz and others, 2010)
Section 6.4.5.1 Selenium Concentration Relationships between Fish Tissue (pages 167–168).
Therefore, tissue-tissue relationships should not be used generically to derive tissue-based selenium toxicity thresholds.
However, even within a species tissue-tissue, extrapolations should ideally be site-specific because individuals show considerable intraspecific variation in the ratio between egg/ovary and whole-body selenium.
If no species-specific tissue-tissue relationship is available, it is not possible to use adult tissue selenium [as opposed to eggs] to reliably estimate potential early life-stage exposure [*text in brackets is for clarification purposes*].

Influence of Ecosystem Characteristics on Selenium—Status of Ecosystems and Data Limitations for Modeling

The 12 species of fish considered for modeling are bull trout, burbot, kokanee, *Catostomus catostomus* (longnose sucker), largescale sucker, mountain whitefish, northern pikeminnow, peamouth chub, rainbow trout (wild strain), *Richardsonius balteatus* (redside shiner), Westslope cutthroat trout, and yellow perch (table 1). Fish characteristics used to ecologically sort these fish species to support modeling include (1) family; (2) origin and function (native, nonnative; game, nongame; introduced, invasive); (3) association of dietary habitat and life cycle; (4) species status, history, and abundance; (5) sampled tissue variations; (6) number of egg-ovary samples per species; (7) observations concerning female ripeness; (8) availability of a gonadosomatic index (GSI) metric; and (9) spawning type (for example, nonsynchronous spawning for cyprinids), location, and timing (table 1). Additionally, a diagram explicit to the life cycle of Lake Koocanusa fish species and their potential exposure opportunities was constructed by Baranowska and Robinson in 2017. This array of information (that is, a "fish grid") helps identify the who, when, where, and why of exposure during the life cycle of species that can inform goals for monitoring and help interface with modeling (Presser and Luoma, 2013).

Restricting available observed data based on these listed fish factors yielded few species and few selenium concentrations per species (that is, a reduced fish-selenium-concentration database in Presser and Naftz, 2020) on which to validate model predictions. Species-specific factors that were found to limit the utility of available data as the basis of a dietary biodynamic selenium model are included in table 1. Overarching concepts that limit data utility relate to (1) dysfunctional selenium dietary bioaccumulation and tissue partitioning that affect tissue-to-tissue relationships, (2) effects to gonadal development, (3) uncertainty in connections to reproductive effects, and (4) sampling protocols not being designed for a known source gradient (that is, geographic focusing). Species-specific limiting factors are (1) timing of egg sampling when sampling is through net hauls of many species; (2) muscle plug sampling of game fish species, rather than egg or wb sampling; (3) egg-ovary selenium concentrations reported for

nonripe or undeveloped ovaries as indicated by field observation or GSI values <1, especially in nonsynchronous spawners; (4) a high incidence of intestinal tapeworms in cyprinids; (5) ingestion of nematodes in trout; (6) a low number of samples for species important to representation of the lake's ecosystem; (7) no fish samples at ecologically important sites (for example, forebay, SOE) for species important to Lake Koocanusa; and (8) elevated mercury concentrations (Teck Coal Ltd., 2018a, 2019) that are known to cause antagonistic/synergistic effects within fish affected by selenium (for example, redistribution of selenium within tissues) (table 1). Individual discussions of these topics are beyond the scope of this report; however, selected literature citations concerning GSI, parasitism, and mercury/selenium interactions are included in a separate "Supplementary References" section in the appendix.

In sum, these identified ecosystem influences define and justify the constraining choices that were made for use of selenium concentration data for various fish species. For the future, modifying sampling protocols to accommodate these types of identified vulnerabilities and focusing modeling on exposures important to species of known concern would lower uncertainty and identify potential ecological bottlenecks (most sensitive species at time and place of greatest exposure) for Lake Koocanusa. To this point, selenium concentrations in ovaries of the few samples available of Westslope cutthroat trout (n=2) and rainbow trout (n=11) ranged from 5.6 to 19.8 µg/g dw, but none were downstream from the Elk River. Even fewer samples are available to assess bull trout (n=2) and burbot (n=3).

Diet Component Analysis and Categorization of Fish Species

Stomach content data for fish collected by the MTFWP from 1982 to 1992 are summarized in Baranowska and Robinson (2017) and Baranowska (2018). Results are either based on calculation of a relative importance index or percentage of biomass for each prey item. Detailed field methods and calculations are given in Dalbey and others (1998) and Chisholm and others (1989) and are summarized in Baranowska and Robinson (2017) and Baranowska (2018).

Species-specific dietary data considered for each fish species modeled here are summarized as percentage of taxa-specific invertebrate biomass and relative importance index (Presser and Naftz, 2020). Percentage of biomass data are the most applicable for biodynamic modeling (Presser and Luoma, 2010a) and are used here as the basis for categorizing Lake Koocanusa fish species for modeling (table 3, available for download at https://doi.org/10.3133/ofr20201098). Recent selenium concentrations for invertebrate taxa in 2018 and a study of the contents of the stomachs of fish species caught in 2017 were also used to inform the categorization for modeling (Presser and Naftz, 2020). Chironomids in particular were key components of benthic Diptera larvae sampling. Teck Coal Ltd. (2018a, 2019) also found chironomids and mayflies as the dominant taxa for sites on the British Columbia side of the reservoir including the Elk River sites (Presser and Naftz, 2020). Finally, qualitative information on the density of different invertebrate taxa at certain sites were included as an additional basis for diet component analysis (Presser and Naftz, 2020).

The number of fish species of interest necessitated each fish species being assigned to a generalized food-web category for modeling to reduce the number of scenarios (table 4, available for download at https://doi.org/10.3133/ofr20201098). The resultant initial diet categorizations for invertivores (aquatic insects and zooplankton to fish) are as follows: 100-percent aquatic insect (rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker), 50-percent aquatic insect and 50-percent zooplankton (peamouth chub, largescale sucker, mountain whitefish), 75-percent zooplankton and 25-percent aquatic insect (rainbow trout December–March), and 100-percent zooplankton (kokanee). Categorization for TL4 (predator fish) to TL3 (forage fish) is specific to bull trout, burbot (winter and summer), and northern pikeminnow. In these scenarios, forage fish ingest a range of higher risk insectivores and lower risk planktivores (that is, 100-percent insectivores, 50-percent aquatic insect and 50-percent zooplankton, and 100-percent planktivores). Rainbow trout could be included in the last category if a scenario was desired to include adult rainbow trout switching to a diet of 100-percent kokanee. A food web consisting of 50-percent aquatic insects and 50-percent fish also can be considered for yellow perch as a combination of assimilation from two separate food webs (that is, the average of the two outcomes) but is not included here because of noninterest in using yellow perch as a species to represent Lake Koocanusa. These initial modeling categories can then be modified based on additional data collection or specific choices pertaining to decision-makers' goals for protection and representation of the ecosystem in their efforts to narrow uncertainty.

Modeling and Fish Scenario Development

The equation for prediction of a selenium concentration in fish ($C_{Se\ fish\ wb}$) is initialized from a selenium concentration in SPM ($C_{Se\ SPM}$) as diet for an assumed invertebrate prey taxon (eq. 2). The equation modified by assigning a percentage of bioavailability to the SPM is

$$C_{Se\ fish\ wb} = [(C_{Se\ SPM})\ (\text{percent bioavailability})]\ (TTF_{invertebrate})\ (TTF_{fish}). \quad (3)$$

For an aquatic insect food web that assumes 100-percent bioavailability of SPM, the numeric version of the equation is

$$C_{Se\ fish\ wb} = [(C_{Se\ SPM})\ (1.0)]\ (2.8)\ (1.1) \quad (4)$$

where

$TTF_{invertebrate}$ is the mean aquatic insect TTF of 2.8, which here also represents the $TTF_{chironomid}$ of 2.7; and

TTF_{fish} is the mean fish (wb) *TTF* of 1.1, which is based on 25 fish species (Presser and Luoma, 2010a).

Similarly, for a zooplankton food web, the numeric version of the equation is

$$C_{Se\ fish\ wb} = [(C_{Se\ SPM})\ (1.0)]\ (1.5)\ (1.1) \quad (5)$$

where

$TTF_{invertebrate}$ is the mean freshwater zooplankton *TTF* of 1.5.

However, a more conservative choice would be a *TTF* of 1.9 that is specific to Daphnia (Presser and Luoma, 2010a).

Assimilation efficiencies (AEs) of different types of particulate matter can be considered in this type of equation to account for the site- or species-specific bioavailability of foods likely to be consumed by invertebrates. The selenium speciation of the particulate phase (that is, elemental selenium, adsorbed selenium, and organo-selenium), the type of particulate material (sediment, detritus, or algae), and the taxa of the invertebrate ingesting the particles all affect the efficiency of selenium assimilation as quantified in biodynamic modeling (Presser and Luoma, 2010a). For example, Schlekat and others (2004) determined an AE of 52 percent for the copepods *Tortanus* sp. and *Acartia* sp. at a specific efflux rate. Both elemental and adsorbed selenium are probably minor components of the food of most organisms. Assimilation of selenium is more efficient when animals ingest living food or detritus, both of which are dominated by organo-selenium. From these materials, AEs vary from 55 to 86 percent among species, with smaller differences among living food types such as different species of algae. Estimates of AE for specific types of particulate material are 15 percent for sediment, 35 percent for detritus, and 60 percent for algae.

A similar equation where an assumed, generalized SPM bioavailability of 60 percent is applied to an aquatic insect food web is

$$C_{Se\ fish\ wb} = [(C_{Se\ SPM})\ (0.6)]\ (2.8)\ (1.1). \quad (6)$$

When applied to a zooplankton food web, the equation becomes

$$C_{Se\ fish\ wb} = [(C_{Se\ SPM})\ (0.6)]\ (1.5)\ (1.1). \quad (7)$$

Modeling of selenium bioaccumulation also can represent a diet that includes a mixed proportion of prey in the diet through use of the equation

$$C_{Se\ fish\ wb} = (TTF_{invertebrate})\ [(C_{Se\ invertebrate\ a})\ (\text{prey fraction a}) + (C_{Se\ invertebrate\ b})\ (\text{prey fraction b}) + (C_{Se\ invertebrate\ c})\ (\text{prey fraction c})]. \quad (8)$$

Accommodating longer food webs that contain more than one higher TL consumer (for example, forage fish being consumed by predatory fish) can be incorporated through additional *TTF* values.

For modeling of a food web with both a TL3 forage fish and a TL4 predatory fish, the equation is

$$C_{Se\ predator\ fish\ wb} = (TTF_{invertebrate})\ (C_{Se\ SPM})\ (TTF_{forage\ fish\ wb})\ (TTF_{predator\ fish\ wb}). \quad (9)$$

Species specificity in terms of TTF_{fish} for predator and forage fish can be addressed using factors compiled by the USEPA (2016a). The range of TTF_{fish} is narrow though for the species of Lake Koocanusa (0.88–1.46), so the quantitative effect of deviating from the mean factor of 1.1 is relatively small (see later discussion).

Model Validation

For the ecosystem-scale selenium modeling methodology, validation or estimation of uncertainty is through comparison of predicted prey and predator selenium concentrations with observed selenium concentrations. For Lake Koocanusa, the availability of SPM samples establishes the modeled sites as the forebay, border, and SOE. Availability of field data from the lake (1) establishes the validation of invertebrates and zooplankton and (2) excludes comparison to observed fish because of the factors specified in table 1 and discussed above.

For sites at the forebay, border, and SOE, observed selenium concentrations for invertebrates and zooplankton (Presser and Naftz, 2020) are compared to selenium concentrations predicted using the invertebrate model equation described earlier calculated for each observed SPM selenium concentration and for two SPM bioavailabilities (100 percent and 60 percent) (table 5, available for download at https://doi.org/10.3133/ofr20201098). The SPM selenium concentrations applied in validation are specific to the time period of sample collection and encompass the entire available dataset (Presser and Naftz, 2020). Selenium concentrations are specific to taxa of invertebrates in field samples from the Montana side but are composited samples from the British Columbia side (Presser and Naftz, 2020). Zooplankton sampling was executed using three net sizes (64-, 80-, and 150-micron nets) during a variety of months (Presser and Naftz, 2020).

Given the limited amount of field data and varied sampling methods and collection sites for invertebrates and zooplankton, comparison of ranges is more appropriate than statistical comparisons. Only one set of observations (May and September 2018, n=12) for invertebrates is available from a collective Tenmile/forebay and Rexford site on the Montana side due to the small mass of material available for analysis.

For validation, comparisons are to predicted selenium concentrations for 2015–18 (table 5). The range of observed selenium concentrations is 0.4–9.1 µg/g dw in 2018, whereas the range of predictions throughout all years at 100-percent bioavailability is 1.2–18.8 µg/g dw and at 60-percent bioavailability is 0.7–11.3 µg/g dw.

For the British Columbia side, observed ranges for invertebrates at the SOE site are 5.3–9.7 µg/g dw in 2013–16 (n=8) and 4.7–12 in 2018 (n=7). Predicted selenium concentrations at 100-percent bioavailability range from 3.8 to 15.5 µg/g dw in 2017 and 9.3–21 µg/g dw in 2018. At 60-percent bioavailability, the ranges are 2.3–9.3 µg/g dw in 2017 and 5.6–12.6 µg/g dw in 2018.

Validation for zooplankton is affected by the transitory nature of the zooplankton community and the variable field dataset available for comparison (figs. 4 and 5). Specific factors involved are use of different net sizes (64-, 80-, and 150-micron nets) for sampling and the variable uptake rate of selenium for different taxa that are composited for analysis. For example, rotifers are a major component of zooplankton sampled on the Montana side with density varying by site (fig. 5*A*, *B*, and *C*). In studies where rotifers were reared as food for fish larvae, rotifers were found to be devoid of selenium both prior and after enrichment and, thus, provided little nutrition to prey (for example, Mæhre and others, 2012).

For zooplankton on the Montana side (64- and 150-micron nets), comparisons for predictions at 60-percent bioavailability based on years when the most data were collected (2017–18) showed a range of 0.7–5.9 µg/g dw compared to an observed range of 0.3–4.4 µg/g dw. For zooplankton on the British Columbia side (80-micron net), the predicted range at 60-percent bioavailability was 1.2–6.7 µg/g dw compared to an observed range of 2.2–4.9 µg/g dw.

Dietary guidelines give context for the observed selenium concentrations in the aggregate for the site-specific food sources of Lake Koocanusa. The BCMOE currently has a dietary guideline of 4 µg/g (BCMOE, 2014), which is exceeded by the ranges of observations and predictions for Lake Koocanusa. The USFWS has derived a dietary guideline of 3.6 µg/g dw for both fish and birds at the EC00 level, of <4.9 at the EC10 level, and of 5.7 µg/g dw at the EC20 level (Presser and Luoma, 2010b). Again, the ranges of observations and predictions for Lake Koocanusa exceed those values.

Prediction of Protective Dissolved Selenium Concentrations—Invertebrate to Fish Model and Trophic-Level (Predatory to Forage) Fish Model

Two models for prediction of protective dissolved selenium concentrations were developed. The equation for the invertebrate to fish model (IFM) is

predicted protective $C_{Se\ dissolved}$ = fish guideline wb /TTF_{fish} /[($TTF_{invert1}$*invert fraction1) + ($TTF_{invert2}$*invert fraction2)] / SPM % bioavailability/(K_d/1,000). (10)

The equation for the trophic-level (predatory to forage) fish model (TFM) is

predicted protective $C_{Se\ dissolved}$ = fish tissue guideline wb /$TTF_{fishTL4}$/$TTF_{fishTL3}$[($TTF_{invert1}$*invert fraction1) + ($TTF_{invert2}$*invert fraction2)]/SPM % bioavailability /(K_d/1,000). (11)

Modeled Bioaccumulation Potentials for Lake Koocanusa

Equations to calculate selenium BAPs as an intermediate numeric endpoint independent of setting (that is, K_d) for each of these models are

$$BAP_{IFM} = (TTF_{fish})\ [(TTF_{invert1}\text{*invert fraction1}) + (TTF_{invert2}\text{*invert fraction2})], \quad (12)$$

$$BAP_{TFM} = (TTF_{fishTL4})\ (TTF_{fishTL3})\ [(TTF_{invert1}\text{*invert fraction1}) + (TTF_{invert2}\text{*invert fraction2})]. \quad (13)$$

If SPM bioavailability (that is, with [w] bioavailability) is added as a factor in a composited BAP, then the equations are

BAP_{IFM} w SPM bioavailability = (TTF_{fish}) [($TTF_{invert1}$*invert fraction1) + ($TTF_{invert2}$*invert fraction2)] (SPM % bioavailability), (14)

BAP_{TFM} w SPM bioavailability = ($TTF_{fishTL4}$) ($TTF_{fishTL3}$) [($TTF_{invert1}$*invert fraction1) + ($TTF_{invert2}$*invert fraction2)] (SPM % bioavailability). (15)

Applying the BAP equations to the species and diet categorizations shown in table 4 allows development of a set of scenarios within each model (tables 6 and 7, available for download at https://doi.org/10.3133/ofr20201098). As noted previously, specific species and scenarios for the IFM model are as follows: 100-percent aquatic insect (rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker), 50-percent aquatic insect and 50-percent zooplankton (peamouth chub, largescale sucker, mountain whitefish), 75-percent zooplankton and 25-percent aquatic insect (rainbow trout December–March), and 100-percent zooplankton (kokanee). The TFM scenarios for bull trout, burbot (winter and summer), and northern pikeminnow are as follows: 100-percent insectivores, 50-percent aquatic insect and 50-percent zooplankton, and 100-percent planktivores. Rainbow trout could be included in the latter category if a scenario was desired to include adult rainbow trout switching to a diet of 100-percent kokanee compared to an insectivorous diet.

The BAPs for IFM food-web scenarios range from 3.08 to 1.65 at 100-percent bioavailability and from 1.85 to 0.99 for 60-percent bioavailability (table 6). The scenario of kokanee ingesting a diet of 100-percent zooplankton represents a scenario where no bioaccumulation (BAP <1) is taking place. For TFM food-web scenarios, BAPs range from 3.39 to 1.82 at 100-percent bioavailability and from 2.03 to 1.09 for 60-percent bioavailability (table 7). Again, this last scenario (bull trout and burbot ingesting a diet of 100-percent planktivorous forage fish) is at the cusp of no bioaccumulation occurring; hence, choice of a fish species and its food web to represent the lake is a critical decision.

Illustrated Scenarios—Prediction of Protection for Westslope Cutthroat Trout, Rainbow Trout, Redside Shiner, Longnose Sucker, Bull Trout, and Burbot

Translation model equations for the IFM and TFM food-web scenarios are then applied to sites at the forebay, border, and SOE where data for paired selenium concentrations of dissolved and SPM are available to calculate observed, instantaneous K_d values (that is, within a common set of K_d values) (tables 8 and 9, available for download at https://doi.org/10.3133/ofr20201098). Given the importance of the dimensional nature of dissolved particulate material and SPM selenium concentrations in the lake (fig. 12*A*, *B*, and *C*), the modeling here does not statistically reduce the K_d datasets; rather, each paired sampling event and consequent K_d calculation is considered as an independent scenario (n=87). Using this all-encompassing set of available K_d values specific to timing, depth, and location, the range of values shows that 69 percent of values lie between 3,017 and 5,977, with the interval between 4,071 and 4,975 containing the greatest percentage (29 percent) (tables 8 and 9). The smallest K_d value in the observed data is 424, but only three observations are less than a K_d value of 1,000. The greatest value is 7,475, and only three observations are greater than 7,000.

The predictions for the illustrated food-web scenarios focus on protection of Westslope cutthroat trout, rainbow trout, redside shiner, and longnose sucker using the IFM and protection of bull trout and burbot using the TFM in accordance with one of the objectives of the proposed modeling (that is, a maximum dietary exposure through feeding within a benthic food web). Two SPM bioavailability factors (100 percent and 60 percent) are used within each food-web scenario to quantify the efficiency of assimilation of SPM by invertebrates.

Predicted protective dissolved selenium concentrations are based on a fish tissue wb guideline of 8.5 µg/g dw as the endpoint (tables 8 and 9). Egg-ovary tissue can be substituted if applicable tissue-to-tissue CFs are available (that is, species-specific extrapolations at a minimum, but ideally site specific if ecosystems are not degraded). As stated previously, the derived dissolved selenium concentration would quantify the ecosystem condition where fish would adhere to an 8.5-µg/g wb tissue guideline. Modeling shows that 85–88 percent of predicted dissolved selenium concentrations are <1 µg/L for both the IFM and TFM with an assumption of 100-percent bioavailability. Using an assumption of 60-percent bioavailability, 46–60 percent of values are <1 µg/L. From the perspective of a dissolved selenium concentration of <1.5 µg/L, the corresponding outcome ranges would be 91–93 percent for 100-percent bioavailability and 78–85 percent for 60-percent bioavailability.

Species-Specific TTF_{fish} for Predator and Forage Fish

These models can be further refined by substituting a species-specific TTF_{fish} as provided by the USEPA (2016a). Using the IFM equation and an assumed K_d of 5,000, example scenarios for applicable fish species show the variation in predicted dissolved selenium concentrations when species-specific TTF_{fish} are applied and other modeling factors are held constant (table 10, available for download at https://doi.org/10.3133/ofr20201098).

Gradient Map Perspectives

Understanding the scientific basis of selenium ecological protection here includes connecting modeling outcomes and the representation of ecosystem conditions for Lake Koocanusa when deriving a water-column guideline or set of guidelines. The frequencies of occurrence of an assumed water-column condition, as discussed above, are only relevant within an understanding of the influences of hydrodynamics and source gradients on a chosen species and food web. Shown in figures 13–16 are a series of monthly (April–October) cross sections of observed dissolved selenium concentrations for 2016–19 that are examples of experimental plots of the type of conceptualization possible, but minimal data were available for building such plots. These constructs give time-, location-, and depth-specific contexts to conditions in the lake that overlap with modeling of food webs of the lake. The illustrated selenium cross sections are influenced by the hydrodynamic and biogeochemical cycles of the lake and the gradual dilution of selenium coming from the Elk River moving south in the lake; hence, comparison of dissolved selenium concentrations across years becomes meaningful when contoured to represent the dynamics of lake conditions. These contours also relate to interactions at the base of food webs and, hence, can elucidate modeling outcomes. These contours argue against using reductions of nonanchored (that is, nonlocation specific) dissolved selenium datasets because of the influences of the hydrologic setting of the lake, especially in terms of selection of K_d values for modeling predictions.

The constructs can be quantified by the cross-sectional area specific to a designated dissolved selenium concentration to illustrate existing conditions or adherence to a proposed guideline. In the example here, areas >1 µg/L are quantified for 2016–19 (fig. 17). Even though constructs are based on the relatively few available data points, seasonal trends are clear and the cross-sectional areas are seen to increase over the time period 2016–19. Hence, if choices need to be made as the development of guidelines proceeds, this would be a methodology to meaningfully constrain data with the goal of identifying the time and place of greatest selenium sensitivity (that is, the ecological bottleneck). Going forward, this type of contouring can be used to project the effect of increases or decreases based on the current dissolved selenium conditions developed here.

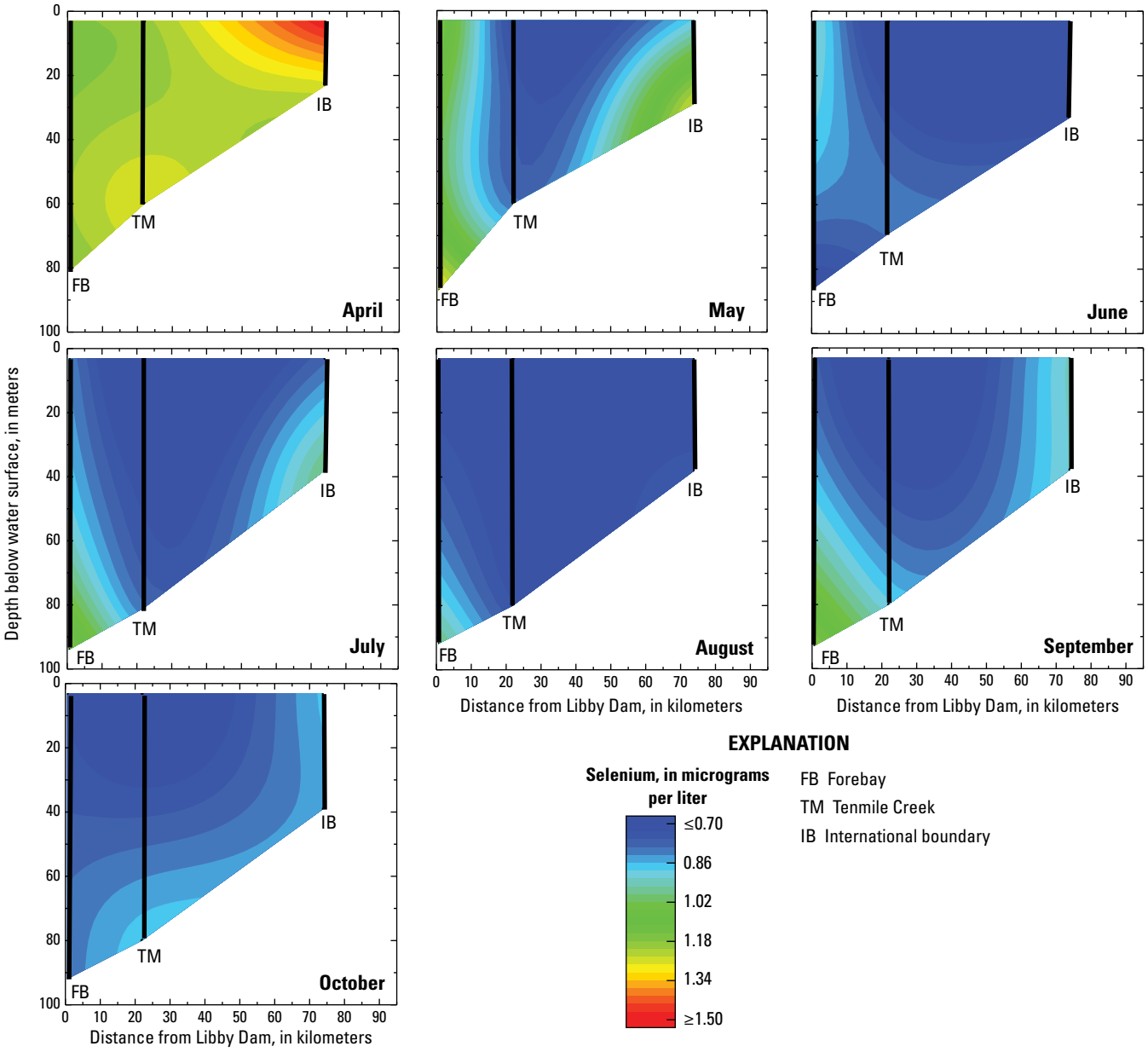


Figure 13. Cross sections of dissolved selenium concentrations from monitoring sites on Lake Koocanusa during calendar year 2016. Contours based on two samples from each site. April selenium concentrations from the international boundary site are based on unfiltered water samples.

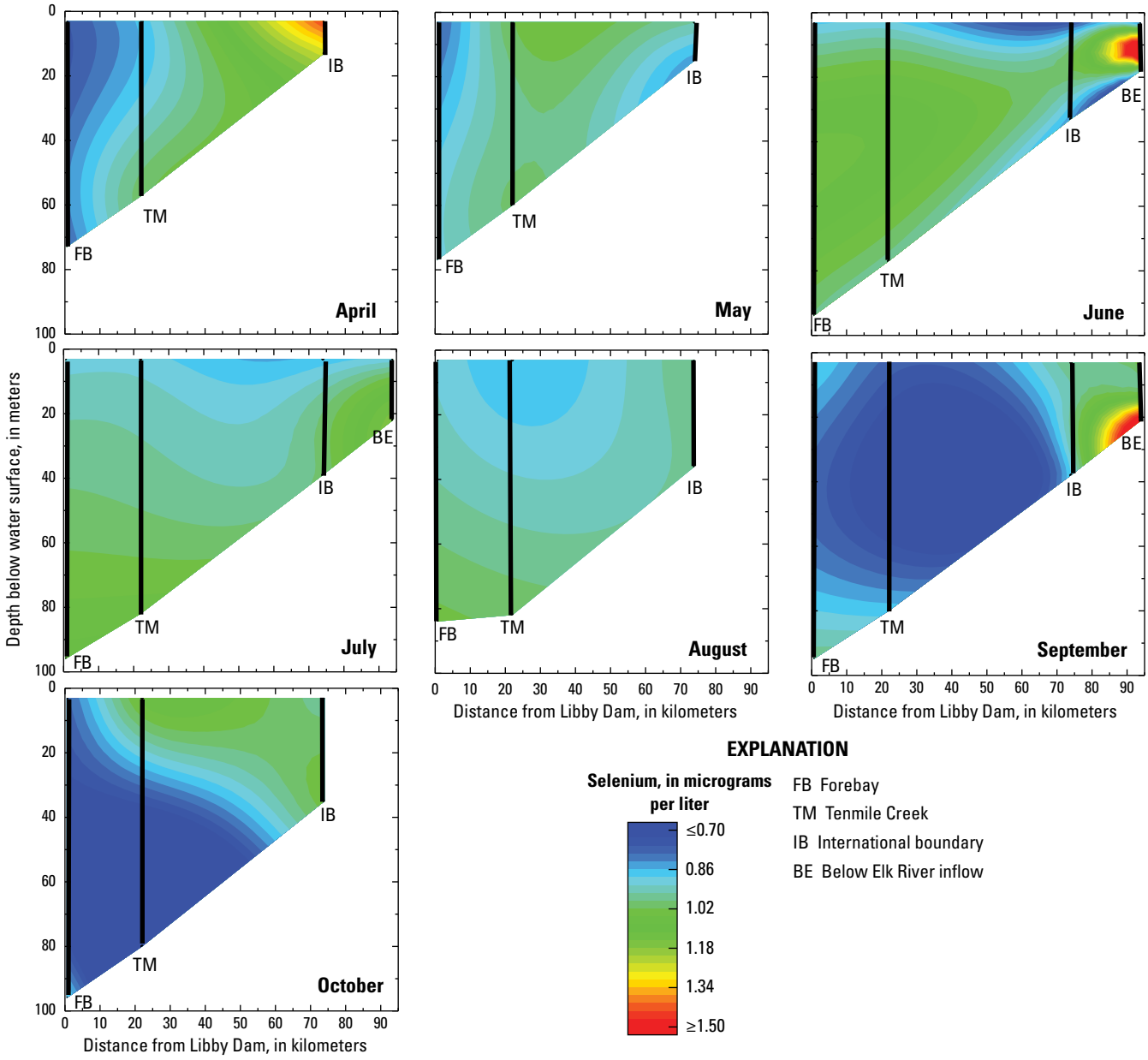


Figure 14. Cross sections of dissolved selenium concentrations from monitoring sites on Lake Koocanusa during calendar year 2017. Contours based on one to three samples from each site.

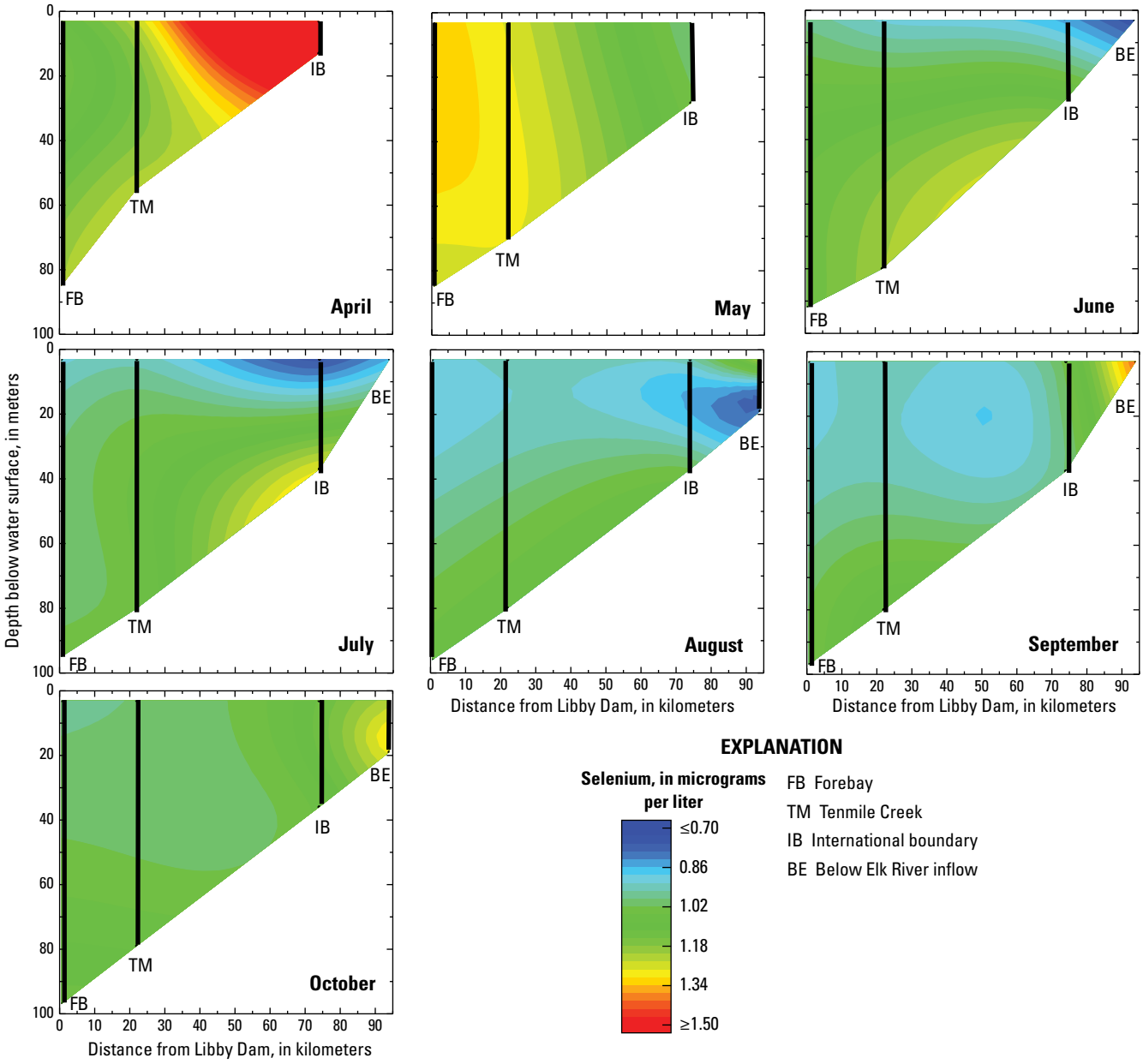


Figure 15. Cross sections of dissolved selenium concentrations from monitoring sites on Lake Koocanusa during calendar year 2018. Contours based on one to three samples from each site.

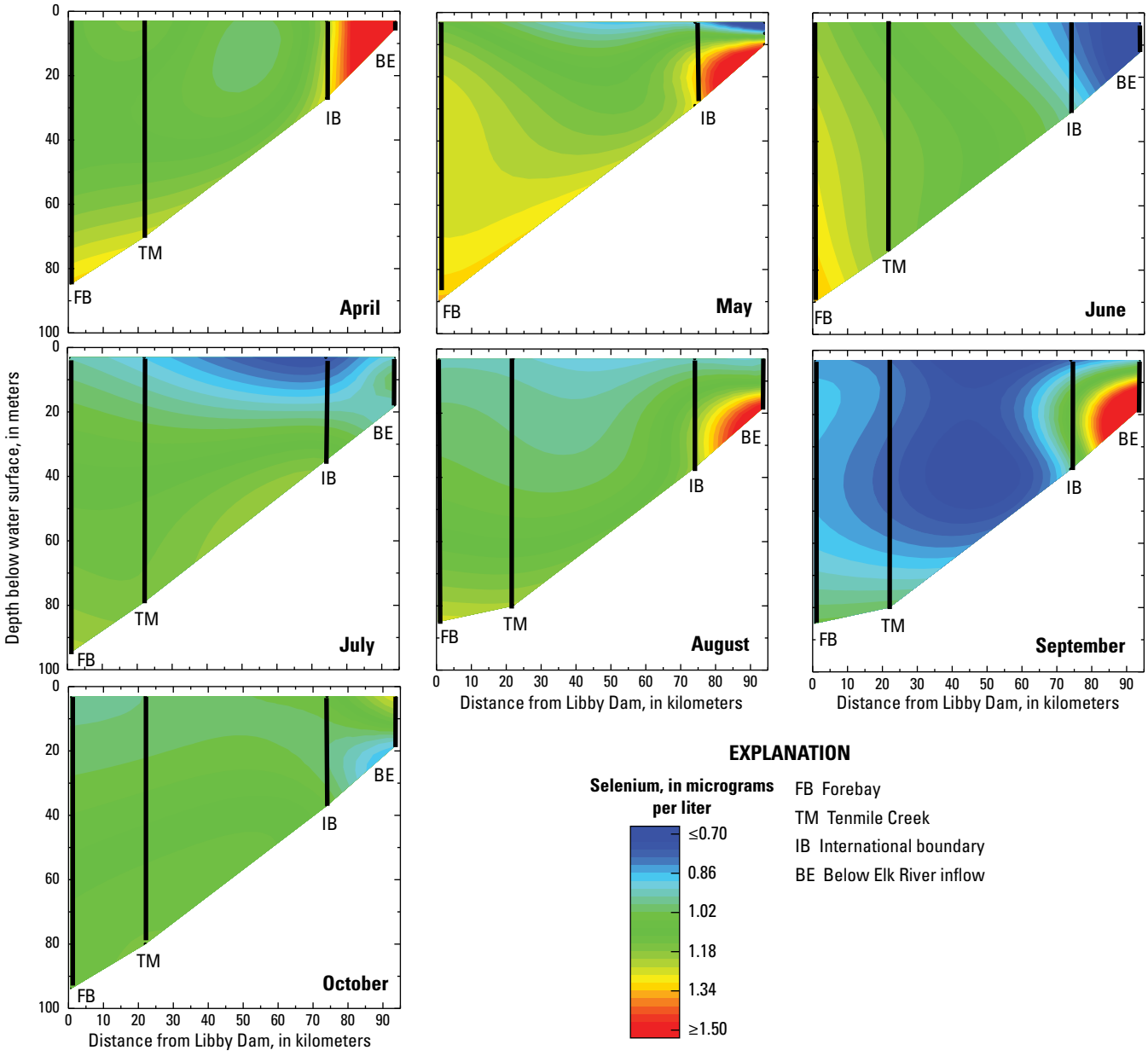


Figure 16. Cross sections of dissolved selenium concentrations from monitoring sites on Lake Koocanusa during calendar year 2019. Contours based on two to three samples from each site. No samples were collected from the Tenmile Creek site during May due to equipment problems.

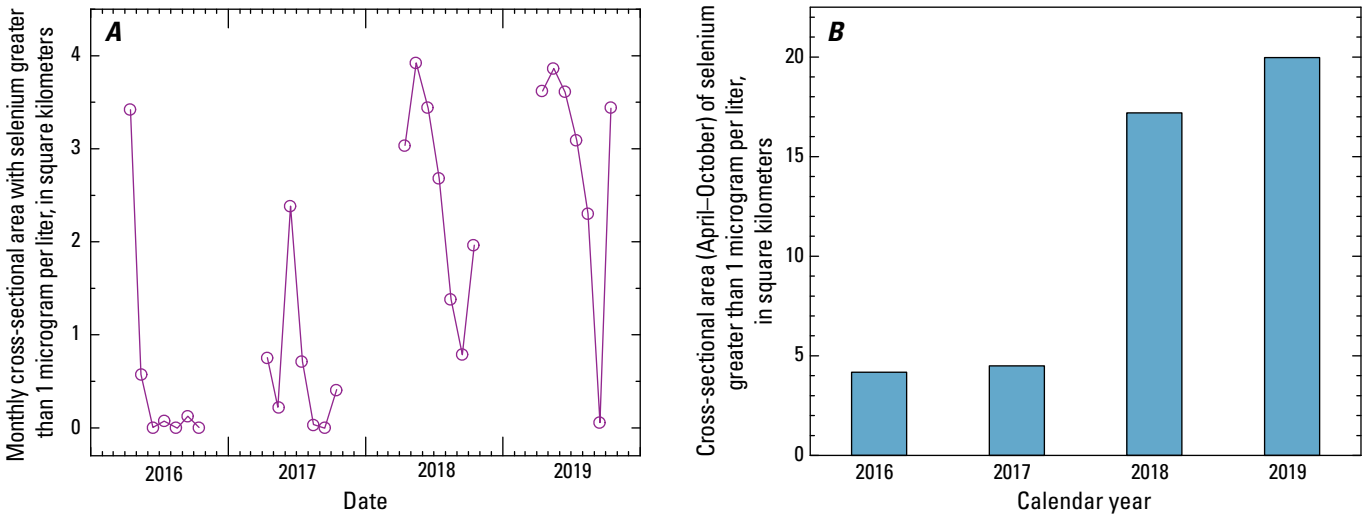


Figure 17. Graphs showing (*A*) monthly cross-sectional areas (2016–19, April–October) in Lake Koocanusa specific to a dissolved selenium concentration of greater than 1 microgram per liter for 2016–19 from the international boundary to Libby Dam and showing (*B*) annual summation of monthly cross-sectional areas specific to a dissolved selenium concentration of greater than 1 microgram per liter. Graph *B* indicates an annual increase from 2016 to 2019.

Conclusions

This working report and accompanying USGS data release (Presser and Naftz, 2020) provide the data, rationale, food-web modeling structure, and interactive spreadsheets for the quantitative derivation of site-specific selenium guidelines for Lake Koocanusa. Model scenarios and gradient maps presented here represent the lake's ecosystem that is the link between the designated protective fish-tissue selenium concentration guideline and the modeled predictions of protective dissolved selenium concentrations. Model predictions for protective dissolved selenium concentrations are specific to the USEPA's national guideline of 8.5 µg/g wb fish tissue, but the model outcomes can be modified to numerically support BCMOE's guideline of 4 µg/g wb by dividing by a factor of 2.125 (that is, 8.5/4). However, as stated previously, the tissue guidelines were derived with different protection goals.

Modeling of selenium overlays cumulative effects in the reservoir of (1) dam operation itself, (2) mitigation actions, and (3) its impaired selenium status for aquatic life designated in 2012 as coal mining continues to expand in the Elk Valley region of British Columbia over the last 35 years. Concerns encompass fish-community changes, flow regime changes, and such ecological stressors as parasitic infection, gonadal dysfunction, and mercury-selenium interactions. The cumulative effects related to fish species were found to have affected the fundamental processing of selenium through food webs, which did have ramifications for data utility, choices for model validation, and connection to a reproductive selenium endpoint.

Our previously listed goals (see "Introduction" section) guided our choices and assumptions used in illustrating Lake Koocanusa's ecosystem and modeling its dietary dynamics. Two models address protection of benthic feeders within the overall goal of identifying food webs with maximum BAPs (that is, maximum dietary exposure). As categorized and constructed, (1) the IFM protects a community of rainbow trout, Westslope cutthroat trout, redside shiner, and longnose sucker and (2) the TFM protects a community of bull trout and burbot. Constraining the variables for identification of sensitive locations and seasons to represent Lake Koocanusa led to a lake-gradient approach. In this approach, each paired sampling event of water-column and SPM and the consequent K_d calculation was considered as an independent scenario. Where data were most abundant for pivotal sites (forebay and SOE), temporal gradient maps of dissolved and SPM selenium concentrations and ecological partitioning factors (K_d values) specific to lake strata, hydrodynamics, and source gradient were constructed. These experimental plots show the kind of conceptualization possible when considering lake dynamics in general and connecting to selenium dynamics at the base of food webs in particular.

The results of our analysis and illustrated modeling scenarios show that at least 78 percent of predictions are <1.5 µg/L and at least 46 percent of predictions are <1 µg/L for protection of this community of core benthic feeders. The percentages are based on exposure through a 100-percent chironomid diet and two choices of bioavailability (100 percent and 60 percent for SPM); hence, these scenarios represent conservative, but realistic, choices within the set of 12 categorized fish species. Switching from an assumed guideline of 8.5 µg/g to 4 µg/g would decrease each individual model prediction, as stated previously, by a factor of approximately two.

To give context to this range of tested designated dissolved selenium concentrations, 1.5 µg/L is the USEPA's national guideline derived for lentic systems. The range in comparison to recent conditions in Lake Koocanusa (fig. 17)

shows that during 2016 and 2017, cumulative cross-sectional areas with selenium concentrations exceeding 1 µg/L in the reservoir south of the international border were <5 square kilometers (km^2). During 2018, a more than threefold increase in the cross-sectional area (>15 km^2) exceeding 1 µg/L of dissolved selenium was observed, followed by a more than fourfold increase (about 20 km^2) in 2019. These recent increases in the proportion of the reservoir containing selenium concentrations exceeding 1 µg/L indicate a system that is not at a steady state and raises concerns over the likelihood of continuing future increases.

The outcomes of formal protocols (for example, the USEPA's recalculation procedure [USEPA, 2016d]) for addressing the ecotoxicological sensitivity of an assemblage of site-specific fish species are not publicly available for Lake Koocanusa. However, a practical, qualitative ranking of fish species can be achieved through the metric of vulnerability, which combines inherent selenium sensitivity and exposure through diet. This assessment is informed by (1) the site-specific analysis of the fish traits and connectivity to behavioral ecology of predators shown in table 1, (2) the ranking of fish species listed in USEPA (2016a, see table 3.1) and in other reviews (for example, Janz and others, 2010, see figures 6.4), and (3) recent studies of *Danio rerio* (zebrafish) documenting the elevated sensitivity of this species of Cyprinid (Thomas and Janz, 2014, 2015; Penglase and others, 2014a,b). Our example of ranking based on species vulnerability is included as a working part of this report, which can be updated in the future when a formal analysis by regulatory agencies is prepared as guideline development proceeds.

Near the top of a vulnerability ranking would be concern for the Cyprinids of Lake Koocanusa (that is, redside shiner, peamouth chub, and northern pikeminnow) based on sensitivity and for burbot given its demersal feeding and winter spawning period. Redside shiner and peamouth chub as representative of Cyprinids would be interesting choices of species to protect as examples of small-bodied fish with 100-percent and 50-percent benthic diets, respectively (table 4). Concern for burbot is also anecdotally corroborated by the lack of fish caught in recent monitoring efforts and the fact that extensive drawdown occurs during the spawning season for this species. White sturgeon, although not residing in the lake, does inhabit waters downstream from the dam and is the most toxicologically sensitive fish as ranked by the USEPA in its national guidance (USEPA, 2016a); hence, extending protection to waters and habitat immediately downstream from the dam would seem advisable. Less concern would be for bull trout and mountain whitefish, which appear at the bottom of the sensitivity ranking (USEPA, 2016a). This leaves considering protection for remaining species such as rainbow trout and Westslope cutthroat trout, which rank near the middle of the sensitivity scale and are benthic feeders. Here too for these species, the percentages of composition among Lake Koocanusa species are low during spring and fall (fig. 6*A*, *B*).

In sum, this subset of modeling variables, species, and attributes appears to meet the specific goals set out at the beginning, which also impinge on operational interests. These considerations connect to specific scenarios and supporting rationales to represent the system. Going forward, qualifying the status of the Lake Koocanusa ecosystem within an impairment-restoration cycle would acknowledge concerns about using current conditions as a baseline for regulatory actions because of possible survivor bias and normalization of ecosystem degradation and dysfunction. Considerations for future endpoint and modeling-related actions include development of (1) a series of health endpoints related not only to long-term exposure to selenium, but also to food sources and fish communities; (2) a coordinated selenium portal where data are arranged within a set format specific to Lake Koocanusa's food webs; (3) a comprehensive plan for taxa-specific selenium analysis of invertebrates; and (4) a strategy for filling the data gaps for selenium in samples of expressed eggs for fish species and studying the occurrence of deformities in developing larvae. In reference to these latter considerations, focus for monitoring would be on the main drivers of the outcomes of ecosystem-scale selenium modeling: (1) type of particulate material (SPM in the case here), (2) selenium concentration and speciation of the particulate material that is food for invertebrates, and (3) taxa of invertebrates consuming that food. Monitoring of dissolved selenium concentrations on a high-frequency basis (for example, USGS platform data) at several key sites across the reservoir would help to further develop seasonal spatial selenium gradients and to understand process-level relationships in order to pinpoint and assess sites consequential to defining future regulatory compliance.

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Rotifers

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Table 1. Fish traits, sampling details, and species-specific limiting factors.

[USEPA, U.S. Environmental Protection Agency; MT, Montana; BCMOE, British Columbia Ministry of Environment; MTFWP, Montana Department of Fish, Wildlife and Parks; GSI, gonadosomatic index; #, number; Hg, mercury; BC, British Columbia; d/s, downstream; RBT, rainbow trout; n, number; MTDEQ, Montana Department of Environmental Quality; CCME, Canadian Council of Ministers of the Environment]

species (common name)	species (scientific name)	family	origin and utility	associations to dietary habitat or other characteristics	USEPA/MT/BCMOE species status and history	sampled tissue by MTFWP	GSI	[1] MTFWP observations: gravid when caught	MTFWP 2008-2018: # ovary samples	sampled tissue by Teck Coal Ltd (2014-2016; 2018)	GSI	2014-2016, 2018, Teck Coal Ltd: # ovary samples	spawning	[2]spawning location	species-specific limiting factors for use of data in modeling	Hg status of species (MT consumption advisory for human health/MT exceedance; BC exceedance; [3]BC listing for dietary wildlife protection)
Lake Koocanusa																
bull trout	*Salvelinus confluentus*	salmonid	native, game	[4]emigrate from tributaries (ages 3-5): sensitive to stress	[5]threatened (regulated incidental capture)/species of concern/blue listed	muscle filet	no	no samples	no samples	muscle plug	no	2	fall	tributaries	low number of samples, especially at source and forebay	listed/yes/yes/yes
burbot	*Lota lota*	lotid	native, game	self-sustaining population in Lake Koocanusa	tribally important/tribally important/red listed: critically imperiled populations	muscle plug; eggs??	no	difficult	[6]3(2016 n=2: 2017 n=1)	muscle plug	no	1 (unripe 2014.)	winter	near-shore, shallow shoals	low number of samples, especially at source and forebay	listed +Se/yes/yes/yes
kokanee	*Oncorhynchus nerka*	salmonid	nonnative, game	eeds locally; entrained through dam; food source for d/s RBT	inadvertantly introduced in 1979; important fishery	muscle filet and ovary	no	maybe	60	muscle plug: ovary 2014	no	16 (2014 only)	fall	lake littoral zones/tributaries	MT: ovary ripeness uncertainty: BC: last sampling in 2014	no/no/no/yes
longnose sucker	*Catostomus catostomus*	catostomid	native, nongame	both mountain streams and lakes	not sampled in BC	muscle filet and ovary	no	yes	12	not in BC		not in BC	spring	tributaries	no samples from BC	no/no/not sampled/?
largescale sucker	*Catostomus macrocheilus*	catostomid	native, nongame	both mountain streams and lakes	remains abundant	muscle filet and ovary	no	yes	11 (2018)	muscle filet and ovary	yes	17	spring	at lake inlets/outlets	GSI<1: spent or undeveloped females noted	not sampled/?/not sampled/?
mountain whitefish	*Prosopium williamsoni*	salmonid	native, game	both mountain streams and lakes	significant decline since impoundment	muscle filet and ovary	no	yes	4 (2018)	muscle plug	no	10 (2016)	fall	tributaries	poor correlation of Se between tissues	not sampled/?/no/yes (n=4)
northern pikeminnow	*Ptychocheilus oregonensis*	cyprinid	native, nongame	feed locally	small-bodied; feed locally; remains abundant	muscle filet and ovary	no	maybe	58	muscle filet and ovary	yes	70	spring: [7]non-synchronous	at lake inlets/outlets	GSI<1: non-synchronous cyprinid	listed/yes/yes/yes
peamouth chub	*Mylocheilus caurinus*	cyprinid	native, nongame	feed locally	small-bodied; feed locally; remains abundant	muscle filet and ovary	no	yes	70	muscle filet and ovary	yes	122	spring: [7]non-synchronous	at lake inlets/outlets	tapeworms: non-synchronous cyprinid	listed/yes/yes/yes
rainbow trout (wild strain); not claimed in BC	*Oncorhynchus mykiss*	salmonid	native, game	[4]emigrant from tributaries (ages 2-3)	introduced in 1985: compete w hybrid: MT catch/net <2 (2000-2016)	muscle filet	no	yes	1 (2013)	muscle plug	no	9 (2014 n=6: 2016 n=3)	spring	mainly tributaries	nematodes in stomachs: competing hatchery sterile strain	no/no/?/? (n=3)
rainbow trout X cutthroat (stocked hybrid)	*Gerrard strain*	salmonid	hatchery strains		MT since 1988; sterile; age 0-1; >68,000 fish/year/2015-2019; MT catch/net <1	none				none			stocked: mainly 1-year old juveniles		as juveniles, potential low Se food source: few recruit to return-to-creel	
redside shiner	*Richardsonius balteatus*	cyprinid	native, nongame	feed locally	small-bodied; feed locally	muscle filet and ovary	no	maybe	14	muscle filet and ovary	yes	83	spring: [7]non-synchronous	both	tapeworms: non-synchronous cyprinid	not sampled/?/no/yes
Westslope cutthroat trout	*Oncorhynchus clarki lewisi*	salmonid	native, game	[4]emigrate from tributaries (ages 2-3)	n/species of concern/blue listed: headwaters species: MTcatch/net <1 (2000-2016)	muscle filet	no	yes	2 (2013: 2017)	muscle plug	no	none	spring	tributaries	nematodes in stomachs: usual headwaters habitat	no/no/no/yes (high detection limit)
yellow perch	*Perca flavescens*	percid	invasive; nonnative, nongame	pioneering; tolerant to stress; community threat; fast breeding rate	[8]invasive since 1984: MT catch/net <2 (2000-2016)*	muscle filet [ovary n=1]	no	n=1	1 (2017)	muscle filet and ovary	yes	58	spring	lake	MT (n=1): sampling in BC helped eliminate invasive species	not listed/marginal/marginal/yes

[1]Working notes MTDEQ public meeting on March 10, 2020: qualitative because of lack of GSI data for fish sampled in Montana.

[2]Lotic 2017, exposure diagram (Teck Coal, Ltd., 2018a).

[3]Wildlife Hg: CCME, 2000.

[4]From tributaries: ages derived from migration classes (Chisholm and others, 1989).

[5]Lake Koocanusa is listed as critical habitat for BT.

[6]Initial dataset from 2012 to 2013 (n=98) discarded because of inaccurate percentage of moisture.

[7]Multiple spawning episodes.

[8]YP not of regulatory interest for Se monitoring.

Table 3. Dietary percentage of biomass for diet of fish species.
[%, percent; EPT, Ephemeroptera, Plecoptera, and Trichoptera; aq, aquatic; terr, terrestrial]

	fish species	zooplankton % biomass	aquatic insects [diptera, EPT] % biomass	terrestrial insects % biomass	beetles + aq + terr insect parts + other % biomass	fish % biomass	fish species	total	species % biomass of total fish	KOK	PMC	RSS	YP	NPM	suckers	other	parts
fish diet	bull trout	0	0	0	0	98.5	KOK, PMC, YP	98.5		37.2	27.6		22.8				
fish diet (percentage biomass, weighted average)	burbot	0	0.2	0	0	96.8	KOK, PMC, RSS, YP, NPM	97		8.7	27.3	10.2	22.7	6.8		10.4	8.9
	kokanee (KOK)	91.3	3.2	0.5	4.9 (parts)	0		99.9									
	longnose sucker	0	59.3	0	37.6 parts	0		96.9									
	largescale sucker	40.7	23.3	0	37.6 (parts)	0		101.6									
	mountain whitefish	34.4	29.2	0.2	34.0 (parts)	0		97.8									
	northern pikeminnow (NPM)	2.5	2.6	0.3	7.2 (parts)	86.2	KOK, PMC, suckers, others	98.8		20	20				12.5	1.5	
	peamouth chub (PMC)	32.7	24.2	4.4	24.4 (21.4 parts)	0		85.7									
	redside shiner (RSS)	0	16	0	84 (parts)	0		100									
	rainbow trout	10.3	5.6 (25% caddisfly)	16.3	59.0 (45.9 parts; 10 beetle)	6.3	PMC, RSS	97.5			3.1	2.4					
	Westslope cutthroat trout	2.2	3.6	30.3	59.8	2.3	PMC, YP	98.2			1.0		1.3				
	stocked trout hybrid (WCT X RT)	0	3.2	27.2	64.2(52 parts, 7.2 beetle)	1.5		96.1									
	yellow perch (YP)	0	47.2 (12.5 mites)	0	13.8	35		96									

Table 4. Categorization of fish species for modeling.
[%, percent; zoop, zooplankton; RII, relative importance index]

foodwebs and categories	fish species	insects % biomass	zoop % biomass	fish % biomass	seasonal	consuming insectivorous forage fish: higher risk	consuming planktivorous forage fish (e.g., kokanee): lower risk
predator fish	bull trout			98.5		PMC 28%; YP, 23%	37%
predator fish	burbot			97	winter--benthic insects	PMC 27%; YP, 23%; RSS, 10%	9%
forage fish	kokanee		91				
100% insect	longnose sucker	97					
50:50 insect:zoop	largescale sucker	61	41				
50:50 insect:zoop	mountain whitefish	63	34				
predator fish	northern pikeminnow	10	2	86		PMC, 20%; suckers, 12%	20%
50:50 insect:zoop	peamouth chub (PMC)	[1)]53	33				
100% insect	redside shiner (RSS)	100			trout fish eggs		
100% insect	rainbow trout	81	10	6	Dec-Mar 13% insect; 67% zoop; 0% fish		
100% insect	Westslope cutthroat trout	94	2	2			
100% insect	stocked trout hybrid (WCT X RT)	94					
50:50 insect:fish	yellow perch (YP)	61		35			

[1)]61% based on RII.

Table 5. Validation ranges of observed and predicted selenium concentrations in invertebrates.

[SOE, south of Elk; ppm dw, parts per million dry weight; %, percent; n, number of samples; USGS, U.S. Geological Survey; USACE, U.S. Army Corps of Engineers]

validation (aquatic insects)										
Tenmile/Forebay and Rexford		**Tenmile/Forebay and Rexford**		**Tenmile/Forebay and Rexford**		**Tenmile/Forebay and Rexford**		**SOE (South-of-Elk)**		**SOE (South-of-Elk)**	
ranges 2015 invertebrates	µg/g dw	ranges 2016 invertebrates	µg/g dw	ranges 2017 invetebrate	µg/g dw	ranges 2018 invetebrate	µg/g dw	ranges 2017 invertebrate	µg/g dw	ranges 2018 invertebrate	µg/g dw
100% predict	1.2-18.5	100% predict	8.2-18.8	100% predict	2.0-18.5	100% predict	13.7-16.4	100% predict	3.8-15.5	100% predict	9.3-21
60% predict	0.7-11.1	60% predict	4.9-11.3	60% predict	1.2-11.1	60% predict	8.2-9.8	60% predict	2.3-9.3	60% predict	5.6-12.6
observed 2015 n=0		observed 2016 n=0		observed 2017 n=0		observed 2018 n=0	0.4-9.1	observed 2017	none	observed 2018 n=7	4.7-12
observed 2018 n=12	0.4-9.1	observed 2018 n=12	0.4-9.1	observed 2018 n=12	0.4-9.1			observed 2013-2016 n=8	5.3-9.7	observed 2013-2016 n=8	5.3-9.7
validation (zooplankton)											
Tenmile/Forebay and Rexford		**Tenmile/Forebay and Rexford**		**Tenmile/Forebay and Rexford**		**Tenmile/Forebay and Rexford**		**SOE (South-of-Elk)**		**SOE (South-of-Elk)**	
ranges 2015 zooplankton	µg/g dw	ranges 2016 zooplankton	µg/g dw	ranges 2017 zooplankton	µg/g dw	ranges 2018 zooplankton	µg/g dw	ranges 2017 zooplankton	µg/g dw	ranges 2018 zooplankton	µg/g dw
100% predict	0.6-9.9	100% predict	4.4-10.1	100% predict	1.1-9.9	100% predict	7.3-8.8	100% predict	2.0-8.3	100% predict	5.0-11.2
60% predict	0.4-5.9	60% predict	2.6-6.0	60% predict	0.7-5.9	60% predict	4.4-5.3	60% predict	1.2-5.0	60% predict	3.0-6.7
observed 2015 n=0		observed 2016 n=6	0.8-2.2	observed 2017 n=21	1.9-4.2	observed 2018 n=22	0.3-4.4	observed 2017 n=0		observed 2018 n=15	2.2-4.9
USGS: 150 micron net		USGS: 150 micron net		USGS: 150 micron net		USACE: 64 micron net		observed 2016 n=5	2.4-3.7	observed 2016 n=5	2.4-3.7
						(phyto/zooplankton assemblage)		observed 2008-2015	2.4-13.2	observed 2008-2015	2.4-13.2
								Teck Coal Ltd: 80 micron net		Teck Coal Ltd: 80 micron net	

Table 6. Calculation of bioaccumulation potentials for the insect to fish model (IFM).
[TTF, trophic transfer factor; SPM, suspended particulate material; %, percent; w, with; RBT, rainbow trout; WCT, Westslope cutthroat trout; RSS, redside shiner; LNS, longnose sucker; PMC, peamouth chub; LSS, largescale sucker; MWF, mountain whitefish]

fish species	food web	TTF fish	TTF invert1	invert 1 fraction	TTF invert2	invert 2 fraction	bioaccumulation potential	SPM bioavailability fraction 100%	bioaccumulation potential w 100% SPM bioavailability	alternative scenario	SPM bioavailability fraction	bioaccumulation potential w 60% SPM bioavailablity
RBT, WCT, RSS, LNS	100% chironomid	1.1	2.8	1			3.08	1.00	3.08		0.6	1.85
PMC, LSS, MWF	50% chironomid 50% zooplankton	1.1	2.8	0.5	1.5	0.5	2.37	1.00	2.37		0.6	1.42
rainbow trout: Dec-Mar	25% chironomid 75% zooplankton	1.1	2.8	0.25	1.5	0.75	2.01	1.00	2.01		0.6	1.20
kokanee	100% zooplankton	1.1			1.5	1	1.65	1.00	1.65		0.6	0.99

Table 7. Calculation of bioaccumulation potentials for the trophic fish model (TFM).
[TTF, trophic transfer factor; TL, trophic level; SPM, suspended particulate material; w, with; %, percent; µg/L, micrograms per liter; BT, bull trout; NPM, northern pikeminnow; RBT, rainbow trout; IV, insectivore; PV, planktivore]

fish species	food web →	food web →	TTF fish1 TL4	TTF fish2 TL3	TTF invert1	invert 1 fraction	TTF invert2	invert 2 fraction	bioaccumulation potential	SPM bioavailability fraction	bioaccumulation potential w 100% SPM bioavailability	alternative scenario →	SPM bioavailability fraction	bioaccumulation potential w 60% SPM bioavailablity
BT, burbot (winter, benthic), NPM	100% TL3 fish species (100% insectivores)	100% chironomid or aquatic insect	1.1	1.1	2.8	1			3.39	1.00	3.39		0.6	2.03
BT, burbot, NPM	100% TL3 species (50% IV; 50% PV)	50% chironomid 50% zooplankton	1.1	1.1	2.8	0.5	1.5	0.5	2.60	1.00	2.60		0.6	1.56
[1)]BT, burbot (summer fish), NPM	100% TL3 species (100% planktivores)	100% zooplankton	1.1	1.1			1.5	1	1.82	1.00	1.82		0.6	1.09

[1)] Category would include RBT consuming kokanee.

Table 8. Spreadsheet scenarios and calculations for the insect to fish model (IFM) using the complete dataset for K_d (that is, predictions of protective dissolved selenium concentrations based on a guideline of 8.5 micrograms per gram whole-body fish, dry weight) (USEPA, 2016a).

[K_d, environmental partitioning factor; m, meter; TTF, trophic transfer factor; SPM, suspended particulate material; %, percent; w, with; µg/L microgram per liter; epi, epilimnion; hypo, hypolimnion; RBT, rainbow trout; WCT, Westslope cutthroat trout; RSS, redside shiner; LNS, longnose sucker; aq, aquatic; USGS, U.S. Geological Survey; SOE, south of Elk; USACE, U.S. Army Corps of Engineers; BCMOE, British Columbia Ministry of Environment]

K_d time-period and agency	site	depth (m)	date	fish species: RBT, WCT, RSS, LNS →	food web: 100% aquatic insects →	guideline µg/g wb dw	TTF fish	TTF invert1	invert 1 fraction	TTF invert2	invert 2 fraction	bioaccumulation potential	SPM bioavailability 100%	bioaccumulation potential w SPM bioavailability 100%	observed K_d	predict µg/L w SPM bioavailablity 100%	alternative scenario →	bioaccumulation potential w SPM bioavailability 60%	predict µg/L w SPM bioavailability 60%
2015	border	3-epi	5/18/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,000**	**0.55**		1.85	0.92
USGS	border	30-hypo	5/18/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,071**	**0.68**		1.85	**1.13**
	forebay	3-epi	5/18/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**545**	**5.06**		1.85	**8.43**
	forebay	88-hypo	5/18/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,579**	**0.60**		1.85	**1.00**
	border	3-epi	7/28/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**2,941**	**0.94**		1.85	**1.56**
	border	37-hypo	7/28/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,017**	**0.91**		1.85	**1.52**
	forebay	3-epi	7/28/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,367**	**0.82**		1.85	**1.37**
	forebay	84-hypo	7/28/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,796**	**0.73**		1.85	**1.21**
	border	3-epi	10/20/2015			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	34-hypo	10/20/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**1,780**	**1.55**		1.85	**2.58**
	forebay	3-epi	10/20/2015			8.5	1.1	2.8	1			3.08	1.00	3.08	**424**	**6.51**		1.85	**10.84**
	forebay	94-hypo	10/20/2015			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
2016	border	3-epi	4/12/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,076**	**0.90**		1.85	**1.50**
USGS	border	23-hypo	4/12/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,987**	**0.69**		1.85	**1.15**
	forebay	3-epi	4/12/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,642**	**0.59**		1.85	**0.99**
	forebay	81-hypo	4/12/2016			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	3-epi	5/17/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,420**	**0.62**		1.85	**1.04**
	border	29-hypo	5/17/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,730**	**0.58**		1.85	**0.97**
	forebay	3-epi	5/17/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,034**	**0.55**		1.85	**0.91**
	forebay	87-hypo	5/17/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,504**	**0.61**		1.85	**1.02**
	border	3-epi	7/26/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,715**	**0.74**		1.85	**1.24**
	border	38-hypo	7/26/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,697**	**0.59**		1.85	**0.98**
	forebay	3-epi	7/26/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,820**	**0.47**		1.85	**0.79**
	forebay	94-hypo	7/26/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,436**	**0.62**		1.85	**1.04**
	border	3-epi	9/20/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,814**	**0.47**		1.85	**0.79**
	border	38-hypo	9/20/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,086**	**0.54**		1.85	**0.90**
	forebay	3-epi	9/20/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,103**	**0.54**		1.85	**0.90**
	forebay	93-hypo	9/20/2016			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,695**	**0.48**		1.85	**0.81**
2017	border	3	4/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**1,366**	**2.02**		1.85	**3.37**
USGS	border	13	4/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**586**	**4.71**		1.85	**7.85**
	forebay	3-epi	4/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,249**	**0.53**		1.85	**0.88**
	forebay	73-hypo	4/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,962**	**0.56**		1.85	**0.93**
	border	3-epi	5/16/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,049**	**0.91**		1.85	**1.51**
	border	15-hypo	5/16/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**1,093**	**2.52**		1.85	**4.21**
	forebay	3-epi	5/16/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,340**	**0.52**		1.85	**0.86**
	forebay	77-hypo	5/16/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,377**	**0.82**		1.85	**1.36**
	border	3-epi	6/27/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**6,327**	**0.44**		1.85	**0.73**
	border	33-hypo	6/27/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,778**	**0.73**		1.85	**1.22**
	forebay	3-epi	6/27/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**7,475**	**0.37**		1.85	**0.62**
	forebay	94-hypo	6/27/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,547**	**0.61**		1.85	**1.01**
	border	3-epi	7/25/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,452**	**0.80**		1.85	**1.33**
	border	39-hypo	7/25/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**2,892**	**0.95**		1.85	**1.59**
	forebay	3-epi	7/25/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,570**	**0.60**		1.85	**1.01**
	forebay	96-hypo	7/25/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**1,418**	**1.95**		1.85	**3.24**
	border	3-epi	8/29/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,360**	**0.63**		1.85	**1.06**
	border	36-hypo	8/29/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,809**	**0.57**		1.85	**0.96**
	forebay	3-epi	8/29/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,287**	**0.64**		1.85	**1.07**
	forebay	84-hypo	8/29/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,107**	**0.89**		1.85	**1.48**
	border	3-epi	9/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,883**	**0.71**		1.85	**1.18**
	border	38-hypo	9/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**2,862**	**0.96**		1.85	**1.61**
	forebay	3-epi	9/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,782**	**0.48**		1.85	**0.80**
	forebay	95-hypo	9/26/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**2,329**	**1.18**		1.85	**1.97**
	border	3-epi	10/24/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,802**	**0.73**		1.85	**1.21**
	border	35-hypo	10/24/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,971**	**0.69**		1.85	**1.16**
	forebay	3-epi	10/24/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,503**	**0.50**		1.85	**0.84**
	forebay	96-hypo	10/24/2017			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,829**	**0.57**		1.85	**0.95**
2018	border	3	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
USGS	border	13	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	3	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	55	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	3	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	85	4/24/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	3	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,959**	**0.56**		1.85	**0.93**
	border	28	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	3	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**3,764**	**0.73**		1.85	**1.22**
	Tenmile	70	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	3	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**4,414**	**0.63**		1.85	**1.04**
	forebay	85	5/22/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	2.5	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**6,675**	**0.41**		1.85	**0.69**
	border	35	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	2.5	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**6,331**	**0.44**		1.85	**0.73**
	Tenmile	92	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	2.5	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,561**	**0.50**		1.85	**0.83**
	forebay	77	6/12/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	3	7/10/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	3	7/10/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	3	7/10/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	3	8/28/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	3	8/28/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	3	8/28/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	3	9/18/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,602**	**0.49**		1.85	**0.82**
	Tenmile	3	9/18/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**5,571**	**0.50**		1.85	**0.83**
	forebay	3	9/18/2018			8.5	1.1	2.8	1			3.08	1.00	3.08	**6,464**	**0.43**		1.85	**0.71**
	border	3	10/23/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	Tenmile	3	10/23/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	3	10/23/2018			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
2019	border	epi	4/16/2019			8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
USACE	border	hypo				8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	epi				8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	forebay	hypo				8.5	1.1	2.8	1			3.08	1.00	3.08				1.85	
	border	epi	5/21/2019			8.5	1.1	2.8	1			3.08	1.00	3.08	**6,454**	**0.43**		1.85	**0.71**

	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08	**4,543**	**0.61**	1.85	**1.01**
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08	**5,286**	**0.52**	1.85	**0.87**
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08	**4,548**	**0.61**	1.85	**1.01**
	border	epi	6/11/2019	8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	epi	7/23/2019	8.5	1.1	2.8	1	3.08	1.00	3.08	**6,964**	**0.40**	1.85	**0.66**
	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08	**4,517**	**0.61**	1.85	**1.02**
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08	**4,130**	**0.67**	1.85	**1.11**
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08	**3,179**	**0.87**	1.85	**1.45**
	border	epi	8/20/2019	8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	epi	9/25/2019	8.5	1.1	2.8	1	3.08	1.00	3.08	**6,301**	**0.44**	1.85	**0.73**
	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08	**5,977**	**0.46**	1.85	**0.77**
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08	**7,139**	**0.39**	1.85	**0.64**
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	epi	10/30/2019	8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	border	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	epi		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	forebay	hypo		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
2017	SOE	3 mean	6/27/2017	8.5	1.1	2.8	1	3.08	1.00	3.08	**6,281**	**0.44**	1.85	**0.73**
BCMOE	SOE	13		8.5	1.1	2.8	1	3.08	1.00	3.08	**2,646**	**1.04**	1.85	**1.74**
TECK Coal Ltd	SOE	18		8.5	1.1	2.8	1	3.08	1.00	3.08	**2,259**	**1.22**	1.85	**2.04**
	SOE	3 mean	7/25/2017	8.5	1.1	2.8	1	3.08	1.00	3.08	**4,758**	**0.58**	1.85	**0.97**
	SOE	9		8.5	1.1	2.8	1	3.08	1.00	3.08	**4,810**	**0.57**	1.85	**0.96**
	SOE	22		8.5	1.1	2.8	1	3.08	1.00	3.08	**3,667**	**0.75**	1.85	**1.25**
	SOE	3 mean	9/26/2017	8.5	1.1	2.8	1	3.08	1.00	3.08	**2,537**	**1.09**	1.85	**1.81**
	SOE	12		8.5	1.1	2.8	1	3.08	1.00	3.08	**1,709**	**1.61**	1.85	**2.69**
	SOE	21		8.5	1.1	2.8	1	3.08	1.00	3.08	**2,241**	**1.23**	1.85	**2.05**
													0.00	
2018	SOE	3 mean	6/13/2018	8.5	1.1	2.8	1	3.08	1.00	3.08	**4,647**	**0.59**	1.85	**0.99**
BCMOE	SOE	3 mean	7/31/2018	8.5	1.1	2.8	1	3.08	1.00	3.08	**7,413**	**0.37**	1.85	**0.62**
TECK Coal Ltd	SOE	3	8/29/2018	8.5	1.1	2.8	1	3.08	1.00	3.08	**5,205**	**0.53**	1.85	**0.88**
	SOE	13		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	SOE	19		8.5	1.1	2.8	1	3.08	1.00	3.08			1.85	
	SOE	3 mean	9/4/2018	8.5	1.1	2.8	1	3.08	1.00	3.08	**5,170**	**0.53**	1.85	**0.89**
	SOE	3 mean	10/16/2018	8.5	1.1	2.8	1	3.08	1.00	3.08	**4,975**	**0.55**	1.85	**0.92**
	SOE	16		8.5	1.1	2.8	1	3.08	1.00	3.08				
	SOE	19		8.5	1.1	2.8	1	3.08	1.00	3.08				

Table 9. Spreadsheet scenarios and calculations for the trophic fish model (TFM) using complete dataset for K_d (that is, predictions of protective dissolved selenium concentrations based on a guideline of 8.5 micrograms per gram whole-body fish, dry weight) (USEPA, 2016a).

[K_d, environmental partitioning factor; m, meter; TTF, trophic transfer factor; TL, trophic level; SPM, suspended particulate material; %, percent; w, with; µg/L, micrograms per liter; epi, epilimnion; hypo, hypolimnion; BT, bull trout; BB, burbot; NPM, northern pikeminnow; USGS, U.S. Geological Survey; USACE, U.S. Army Corps of Engineers; BCMOE, British Columbia Ministry of Environment]

K_d time-period and agency	site	depth (m)	date	fish species: BT, BB (winter, benthic), NPM	food web: 100% TL3 fish species (100% insectivores)	food web: 100% chironomid or aquatic insect	guideline µg/g wb dw	TTF fish1 TL4	TTF fish2 TL3	TTF invert1	invert 1 fraction	TTF invert2	invert 2 fraction	bioaccumulation potential	SPM bioavailability 100%	bioaccumulation potential w SPM bioavailability 100%	observed K_d	predict µg/L w SPM bioavailablity 100%	alternative scenario	bioaccumulation potential w SPM bioavailability 60%	predict µg/L w SPM bioavailability 60%
2015	border	3-epi	5/18/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,000**	0.50		2.03	0.84
USGS	border	30-hypo	5/18/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,071**	0.62		2.03	1.03
	forebay	3-epi	5/18/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**545**	4.60		2.03	7.67
	forebay	88-hypo	5/18/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,579**	0.55		2.03	0.91
	border	3-epi	7/28/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,941**	0.85		2.03	1.42
	border	37-hypo	7/28/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,017**	0.83		2.03	1.39
	forebay	3-epi	7/28/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,367**	0.75		2.03	1.24
	forebay	84-hypo	7/28/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,796**	0.66		2.03	1.10
	border	3-epi	10/20/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	34-hypo	10/20/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**1,780**	1.41		2.03	2.35
	forebay	3-epi	10/20/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**424**	5.91		2.03	9.86
	forebay	94-hypo	10/20/2015				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
2016	border	3-epi	4/12/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,076**	0.82		2.03	1.36
USGS	border	23-hypo	4/12/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,987**	0.63		2.03	1.05
	forebay	3-epi	4/12/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,642**	0.54		2.03	0.90
	forebay	81-hypo	4/12/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	3-epi	5/17/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,420**	0.57		2.03	0.95
	border	29-hypo	5/17/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,730**	0.53		2.03	0.88
	forebay	3-epi	5/17/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,034**	0.50		2.03	0.83
	forebay	87-hypo	5/17/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,504**	0.56		2.03	0.93
	border	3-epi	7/26/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,715**	0.68		2.03	1.13
	border	38-hypo	7/26/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,697**	0.53		2.03	0.89
	forebay	3-epi	7/26/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,820**	0.43		2.03	0.72
	forebay	94-hypo	7/26/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,436**	0.57		2.03	0.94
	border	3-epi	9/20/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,814**	0.43		2.03	0.72
	border	38-hypo	9/20/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,086**	0.49		2.03	0.82
	forebay	3-epi	9/20/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,103**	0.49		2.03	0.82
	forebay	93-hypo	9/20/2016				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,695**	0.44		2.03	0.73
2017	border	3	4/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**1,366**	1.84		2.03	3.06
USGS	border	13	4/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**586**	4.28		2.03	7.14
	forebay	3-epi	4/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,249**	0.48		2.03	0.80
	forebay	73-hypo	4/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,962**	0.51		2.03	0.84
	border	3-epi	5/16/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,049**	0.82		2.03	1.37
	border	15-hypo	5/16/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**1,093**	2.30		2.03	3.83
	forebay	3-epi	5/16/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,340**	0.47		2.03	0.78
	forebay	77-hypo	5/16/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,377**	0.74		2.03	1.24
	border	3-epi	6/27/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,327**	0.40		2.03	0.66
	border	33-hypo	6/27/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,778**	0.66		2.03	1.11
	forebay	3-epi	6/27/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**7,475**	0.34		2.03	0.56
	forebay	94-hypo	6/27/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,547**	0.55		2.03	0.92
	border	3-epi	7/25/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,452**	0.73		2.03	1.21
	border	39-hypo	7/25/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,892**	0.87		2.03	1.45
	forebay	3-epi	7/25/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,570**	0.55		2.03	0.91
	forebay	96-hypo	7/25/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**1,418**	1.77		2.03	2.95
	border	3-epi	8/29/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,360**	0.58		2.03	0.96
	border	36-hypo	8/29/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,809**	0.52		2.03	0.87
	forebay	3-epi	8/29/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,287**	0.59		2.03	0.98
	forebay	84-hypo	8/29/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,107**	0.81		2.03	1.35
	border	3-epi	9/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,883**	0.65		2.03	1.08
	border	38-hypo	9/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,862**	0.88		2.03	1.46
	forebay	3-epi	9/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,782**	0.43		2.03	0.72
	forebay	95-hypo	9/26/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,329**	1.08		2.03	1.80
	border	3-epi	10/24/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,802**	0.66		2.03	1.10
	border	35-hypo	10/24/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,971**	0.63		2.03	1.05
	forebay	3-epi	10/24/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,503**	0.46		2.03	0.76
	forebay	96-hypo	10/24/2017				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,829**	0.52		2.03	0.87
2018	border	3	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
USACE	border	13	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	3	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	55	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	forebay	3	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	forebay	85	4/24/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	3	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,959**	0.51		2.03	0.84
	border	28	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	3	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,764**	0.67		2.03	1.11
	Tenmile	70	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	forebay	3	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,414**	0.57		2.03	0.95
	forebay	85	5/22/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	2.5	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,675**	0.38		2.03	0.63
	border	35	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	2.5	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,331**	0.40		2.03	0.66
	Tenmile	92	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	forebay	2.5	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,561**	0.45		2.03	0.75
	forebay	77	6/12/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	3	7/10/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	3	7/10/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	forebay	3	7/10/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	border	3	8/28/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	
	Tenmile	3	8/28/2018				8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39				2.03	

	forebay	3	8/28/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	3	9/18/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,602**	0.45	2.03	0.75
	Tenmile	3	9/18/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,571**	0.45	2.03	0.75
	forebay	3	9/18/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,464**	0.39	2.03	0.65
	border	3	10/23/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	Tenmile	3	10/23/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	3	10/23/2018	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
2019	border	epi	4/16/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
USACE	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	epi	5/21/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,454**	0.39	2.03	0.65
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,543**	0.55	2.03	0.92
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,286**	0.47	2.03	0.79
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,548**	0.55	2.03	0.92
	border	epi	6/11/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	epi	7/23/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,964**	0.36	2.03	0.60
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,517**	0.56	2.03	0.93
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,130**	0.61	2.03	1.01
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,179**	0.79	2.03	1.32
	border	epi	8/20/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	epi	9/25/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,301**	0.40	2.03	0.66
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,977**	0.42	2.03	0.70
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**7,139**	0.35	2.03	0.59
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	epi	10/30/2019	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	border	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	epi		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	forebay	hypo		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
																0.00	
2017	SOE	**3 mean**	**6/27/2017**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**6,281**	0.40	2.03	0.67
BCMOE	SOE	**13**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,646**	0.95	2.03	1.58
Teck Coal Ltd	SOE	**18**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,259**	1.11	2.03	1.85
	SOE	**3 mean**	**7/25/2017**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,758**	0.53	2.03	0.88
	SOE	**9**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,810**	0.52	2.03	0.87
	SOE	**22**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**3,667**	0.68	2.03	1.14
	SOE	**3 mean**	**9/26/2017**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,537**	0.99	2.03	1.65
	SOE	**12**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**1,709**	1.47	2.03	2.45
	SOE	**21**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**2,241**	1.12	2.03	1.87
				8.5	1.1	1.1	2.8	1	0				0.00			0.00	
2018	SOE	**3 mean**	**6/13/2018**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,647**	0.54	2.03	0.90
BCMOE	SOE	**3 mean**	**7/31/2018**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**7,413**	0.34	2.03	0.56
Teck Coal Ltd	SOE	**3**	**8/29/2018**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,205**	0.48	2.03	0.80
	SOE	**13**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	SOE	**19**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39			2.03	
	SOE	**3 mean**	**9/4/2018**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**5,170**	0.49	2.03	0.81
	SOE	**3 mean**	**10/16/2018**	8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**	3.39	**4,975**	0.50	2.03	0.84
	SOE	**16**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**					
	SOE	**19**		8.5	1.1	1.1	2.8	1	0	0	3.39	**1.00**					

Table 10. Spreadsheet example of comparative scenarios using species-specific trophic transfer factors for fish species.
[K_d, environmental partitioning factor; TTF, trophic transfer factor; SPM, suspended particulate material; %, percent; µg/L, micrograms per liter]

fish species	TTFfish (Presser and Luoma, 2010)	match or surrogate	TTFfish [1](USEPA, 2016a)	match or surrogate	food web: 100% chironomid or aquatic insects	guideline µg/g wb dw	assumed TTF fish	match or surrogate	TTF invert1	invert 1 fraction	TTF invert2	invert 2 fraction	SPM bioavailability fraction	[2]K_d	predict µg/L
generic TTF_{fish}	1.1	all fish	1.21	all fish		8.5	1.1	all fish	2.8	1	0	0	1.00	5,000	0.55
longnose sucker	0.97	Utah and mountain	0.90	match		8.5	0.9	match	2.8	1	0	0	1.00	5,000	0.67
redside shiner	1.5	match	1.08	match		8.5	1.46	match	2.8	1	0	0	1.00	5,000	0.42
Westslope cutthroat trout	1.0	cutthroat	1.2	cutthroat trout		8.5	1.2	cutthroat	2.8	1	0	0	1.00	5,000	0.51
rainbow trout	0.98	match	1.19	match		8.5	1.19	match	2.8	1	0	0	1.00	5,000	0.51
largescale sucker	0.97	Utah and mountain	1.11	white sucker		8.5	1.05	genus match (*Catostomus*)	2.8	1	0	0	1.00	5,000	0.58
mountain whitefish	1.3	match	[3]1.38	match		8.5	1.38	match	2.8	1	0	0	1.00	5,000	0.44
kokanee						8.5	1.1	genus match (*Oncorhynchus*)	2.8	1	0	0	1.00	5,000	0.55
peamouh chub	1.2	Utah	1.12	creek chub		8.5	1.12	creek chub	2.8	1	0	0	1.00	5,000	0.54
bull trout						8.5	0.88	genus match (*Salvelinus*)	2.8	1	0	0	1.00	5,000	0.69
northern pikeminnow						8.5	1.46	family match Cyprinid	2.8	1	0	0	1.00	5,000	0.42
yellow perch			1.42	match		8.5	1.42	match	2.8	1	0	0	1.00	5,000	0.43
burbot						8.5	1.1	all fish	2.8	1	0	0	1.00	5,000	0.55

[1] Tables 3.11 and median factor of 1.21.

0.42-0.67 range

[2]Assumed Kd=5,000, see rows 5P or 5R in modeling spreadsheets (tables 8 and 9, respectively).

[3]Higher values are hypothesized (for example, Janz and others, 2010a).