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United States
Environmental Protection
Agency

Office of Water
4304T

EPA 822-P-14-001
May 2014

External Peer Review Draft
Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater
2014

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

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Notices

This document has been reviewed by the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, and is approved for publication.

When published in final form, this document will provide guidance to States and Tribes authorized to establish 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 establish 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 draft 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 draft 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

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Foreword

Section 304(a)(l) of the Clean Water Act of 1977 (P.L. 95-217) 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 is a new proposal of an 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 and comments received from U.S. EPA staff.

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 stream uses are adopted by a state as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state. Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state water quality standards that criteria become regulatory. Guidelines to assist the states and Indian tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994). This handbook and 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 draft document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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Acronyms

AE	Assimilation Efficiency
AWQC	Ambient Water Quality Criteria
BAF	Bioaccumulation Factor
CCC	Criterion Continuous Concentration
CF	Conversion Factor
CV	Chronic Value (expressed in this document as an EC20 or MATC)
CWA	Clean Water Act
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
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
TMDL	Total Maximum Daily Load
TRAP	EPA's Statistical Program: Toxicity Relationship Analysis Program (Version 1.21)
TTF	Trophic Transfer Factor
WB	Whole body
WQBLS	Water Quality-based Effluent Limitations
WQC	Water Quality Criteria
WQS	Water Quality Standards

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1 Executive Summary

This document sets forth the basis for and derivation of the Clean Water Act, Section 304(a) water quality criterion for protecting 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 quantifying the toxicity of selenium to aquatic organisms, and provides a basis for a criterion that will assure protection of population assemblages of fish, amphibians, aquatic invertebrates, and plants.

Although selenium may cause acute toxicity, the most deleterious effect on aquatic organisms is due to its bioaccumulative properties. Organisms in aquatic environments exposed to selenium accumulate it primarily through their diet, and not directly through water (Chapman et al. 2010). It is also recognized 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 the U.S. EPA (2004) draft, the Agency has developed a chronic criterion reflective of the effects of selenium concentrations in the reproductive tissues of fish species. The 2014 freshwater criterion for selenium is composed of four parts, or elements. The recommended elements are for (1) a fish egg/ovary element; (2) a fish whole-body and/or muscle element; (3) a water-column chronic element for lentic or lotic waterbody types; and (4) a water-column intermittent element for lentic or lotic waterbody types to account for potential chronic effects from repeated, short-term exposures to this bioaccumulative pollutant. All criterion elements are intended to protect aquatic life from the chronic effects of exposure to total selenium. The criterion is not intended to address concerns about selenium toxicity to aquatic-dependent wildlife such as aquatic bird species. Because the factors that control the bioaccumulation of selenium vary from location to location, a site-specific criterion for the protection of aquatic life can be developed as needed (Appendix I), when establishing allowable concentrations in water or resident fish.

The toxicity studies relevant to the derivation of the two 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 – tests measuring the survival and growth of organisms,

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for example, juvenile fish, exposed to elevated concentrations of selenium in their diet. Selenium in eggs or ovaries is measured in reproductive studies – tests measuring the health of the offspring of adult female fish exposed to elevated dietary selenium either in the lab or in the field. Selenium accumulation in the eggs of the exposed adult female prior to spawning has been shown to yield a statistically significant occurrence of deformities and reduced survival of the offspring.

The outcome of assessing both reproductive and non-reproductive studies under laboratory and field conditions ultimately led EPA to the conclusion, consistent with expert consensus (Chapman et al. 2009, 2010), that reproductive effects, linked to egg-ovary selenium concentrations, are of greater ecological concern and provide a more reliable basis for the criterion than 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). Applying the species sensitivity distribution concepts from the U.S. EPA (1985) Guidelines to the available data, the draft egg-ovary criterion element is 15.2 milligrams selenium per kilogram dry weight (mg Se/kg dw), based on 19 reproductive studies with nine fish genera.

The egg-ovary criterion element is expected to protect aquatic invertebrates and plants (in addition to fish) because field experience, corroborated by the available laboratory toxicity studies, indicates that these taxa are less sensitive than fish, based on the available data. Mechanism of action information suggests that amphibians would have sensitivity comparable to fish; however, EPA is not aware of existing amphibian studies of sufficient quality that can be used for selenium criteria derivation.

EPA is recommending one criterion with two fish tissue-based and two water-based criterion elements to protect against adverse effects of selenium on aquatic life. All four of these elements are based on the same assessment endpoint, reproductive effects in freshwater fish. EPA derived the values for the water-based criterion elements from the egg-ovary element by assessing food-chain bioaccumulation at representative field sites across the continental United States. EPA also used field observations to assess selenium enrichment in algae, detritus, and sediment relative to water, and used field observations and laboratory data to quantify trophic transfer functions from algae, detritus, and sediment into invertebrates, and from such prey into fish. EPA also used field observations to assess selenium partitioning between the whole-body

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and the eggs or ovaries of fish species. EPA tested and validated this approach using field data on existing conditions at 132 species-site combinations with a range of bioaccumulation potential due to different hydrologic and biotic characteristics. Two different, but related elements were developed for the water column portion of the selenium chronic criterion; a monthly average element and an element for intermittent exposures. Both water column elements are further refined into two values; one for lentic waters (e.g., lakes and impoundments) and one for lotic waters (e.g., rivers and streams). The lentic and lotic water values reflect the apparent difference in enrichment from water into algae, detritus, and sediment in these two types of aquatic systems. These lentic and lotic water concentrations were calculated based on the fish egg-ovary criterion element (15.2 mg Se/kg dw). This egg-ovary value was converted to estimated fish whole-body concentrations and fish muscle concentrations (for each species). Corresponding selenium concentrations were then predicted at each trophic level downward through the food chain, until arriving at predicted allowable water concentrations in lentic and lotic systems for the 132 species-site combinations. The 20th percentile of the distribution of predicted allowable site median selenium concentrations in water yields the national monthly water criterion element concentrations of 1.3 µg/L in lentic waters and 4.8 µg/L in lotic waters.

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Summary of the External Peer Review Draft Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life (See Section 5 for the complete criterion statement.)

Media Type	Fish Tissue		Water Column[3]	
Criterion Element	**Egg/Ovary[1]**	**Fish Whole Body or Muscle[2]**	**Monthly Average Exposure**	**Intermittent Exposure[4]**
Magnitude	15.2 mg/kg	8.1 mg/kg whole body or 11.8 mg/kg muscle (skinless, boneless filet)	1.3 µg/L in lentic aquatic systems 4.8 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[5]	Instantaneous measurement[5]	30 days	Number of days/month with an elevated concentration
Frequency	Never to be exceeded	Never to be exceeded	Not more than once in three years on average	Not more than once in three years on average

[1] Overrides any whole-body, muscle, or water column elements when fish egg/ovary concentrations are measured.
[2] Overrides any water column element when both fish tissue and water concentrations are measured.
[3] Water column values are based on dissolved total selenium in water.
[4] Where $WQC_{30\text{-day}}$ is the water column monthly element, for either a lentic or lotic system, as appropriate. C_{bkgrnd} is the average background selenium concentration, and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
[5] Instantaneous measurement. Fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish at a given site. Selenium concentrations in fish tissue are expected to change only gradually over time in response to environmental fluctuations.

EPA recommends that states and tribes adopt into their water quality standards a selenium criterion that includes all four elements, expressing 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

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any other element. Adoption of the fish whole-body or muscle elements into water quality standards ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and adoption of the water-column elements ensures protection when neither fish egg-ovary nor fish whole-body or muscle tissue measurements are available. (See Section 5.) EPA recommends that when states implement the criteria for selenium under the National Pollutant Discharge Elimination System (NPDES) permits program, states should establish additional procedures to facilitate translation of fish tissue criteria concentrations into water concentration permit limits.

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2 Introduction and Background

National Ambient Water Quality Criteria (AWQC) are established by the United States Environmental Protection Agency (EPA) under the Clean Water Act (CWA). As provided for by the Clean Water Act, EPA reviews and from time to time revises 304(a) AWQC to ensure the criteria are consistent with the latest scientific information. Section 304(a) aquatic life criteria serve as recommendations to states and tribes in 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 CWA goals of providing for the protection and propagation of fish and shellfish. When adopted into state water quality standards (WQS), these criteria can become a basis for establishing National Pollutant Discharge Elimination System (NPDES) program permit limits and Total Maximum Daily Loads (TMDLs).

2.1 History of the EPA 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 account for both the water-column and dietary uptake pathways manifested at Belew's Lake, North Carolina (USA), a cooling water reservoir that 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 Belew's 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 water-only exposure, neglecting the dietary uptake pathway. In addition, subsequent research has demonstrated that sulfate levels may affect selenate toxicity in water-only exposures.

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In 1998 EPA held a peer consultation workshop 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 better 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 a direct waterborne route.

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.

For this 2014 external peer review draft, EPA has conducted a new literature review and reanalyzed data considered in the 2004 and 2009 draft criteria documents. The 2014 criterion reflects the latest scientific consensus (e.g., Chapman et al. 2010) on the reproductive effects of selenium on aquatic life and their measure in aquatic systems. The criterion presented here supersedes all previous national aquatic life water quality criteria for selenium. Because of the bioaccumulative nature of selenium, EPA is recommending a national chronic criterion that is expressed as 4 elements. Two elements are fish tissue-based (concentrations in the egg-ovary and

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whole-body or muscle tissue). Two elements are water column based (a 30 day value and/or an intermittent value derived from equation). The 30-day average water concentration is protective against chronic effects of selenium derived from modeling selenium bioaccumulation via the food web in lotic and lentic waterbody types. To address intermittent exposures that could contribute to chronic effects from selenium bioaccumulation, EPA is also recommending an intermittent exposure water concentration element intended to limit cumulative exposure, based on the chronic 30-day water criterion. These water quality criterion elements apply to the total of all oxidation states (selenite, selenate, organic selenium, and any other forms).

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3 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. The goal of the Clean Water Act is to protect and restore the biological, chemical and physical integrity of waters of the U.S. 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 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).

States and authorized tribes may adopt EPA's recommended criteria into their water quality standards to protect designated uses of water bodies, or 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.

3.1 Overview of Selenium Sources and Occurrence

Selenium is a naturally occurring element present in sedimentary rocks and soils. It is also present in the environment as methyl derivatives of selenium (Ranjard et.al., 2003). 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). The distribution of organic-enriched sedimentary rocks, 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) (see Figures 1 and 2). Two major anthropogenic activities cause 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.

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Mining activities bring selenium deposits to the surface, where they are exposed to physical weathering processes (Figure 1). The release of selenium related to resource extraction activities is most common in the phosphate-rich beds 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). When selenium-containing minerals, rocks, and coal are mined, selenium can be mobilized when ore 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. When 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). 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 (Figure 2) can mobilize selenium and move off-site in surface water runoff or via leaching into ground water. Deposits of Cretaceous marine shales have weathered to produce high selenium soils in many areas of the western US (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 from the soil. The excess water (in tile drains or irrigation return flow) containing selenium can run off into nearby basins, ponds, or streams. For example, elevated selenium levels at the Kesterson Reservoir in California were reported to come from agricultural irrigation return flow collected in tile drains (Ohlendorf et al. 1986).

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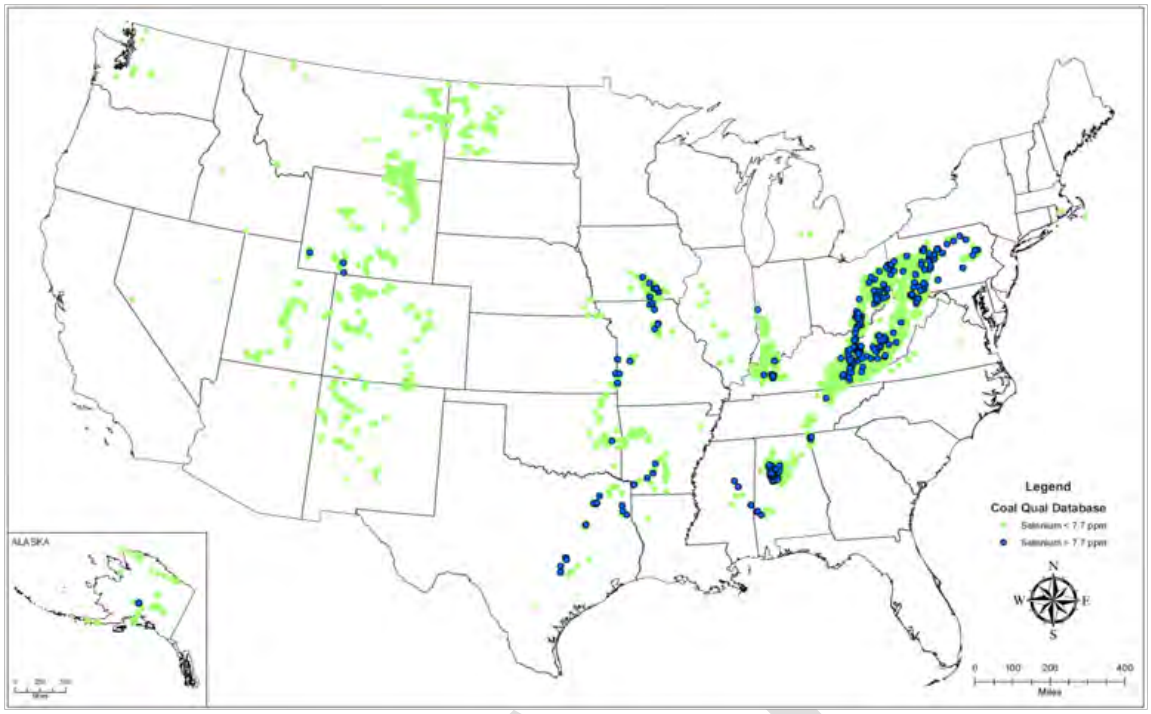

Figure 1. Map indicating deposits of selenium in mining regions.
Light green shading indicates lower selenium concentrations (≤ 7.2 mg/L), whereas darker green shading indicates higher selenium concentrations (> 7.2 mg/L) in underlying geology. Source of Map: SAIC, 2008.

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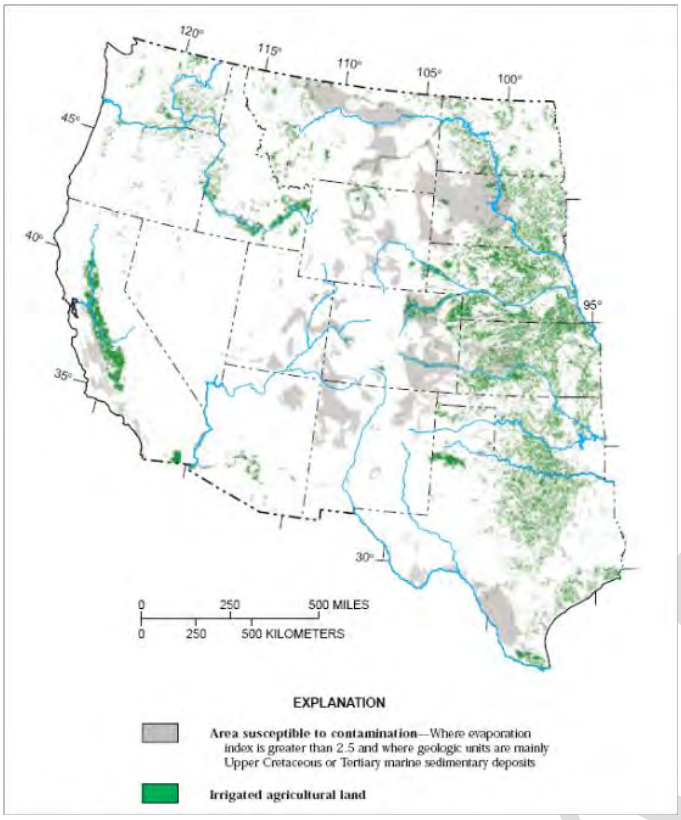

Figure 2. Areas of western U.S. susceptible to selenium contamination (gray) and where agricultural land is irrigated (green).
Overlap of gray and green show areas susceptible to selenium discharge from irrigation. Note: Eastern U.S. is not as susceptible since selenium does not occur at surface where agricultural practices can mobilize selenium. Source of Map: Seilor et al. 1999 page 31.

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 is transported and deposited into water bodies through 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. Table 1 shows the predominant form of selenium that is associated with different activities and industries.

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Table 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.

3.2 Environmental Fate and Transport of Selenium in the Aquatic Environment

As a member of Group 16 of the Periodic Table, selenium is a non-metallic chemical element with chemical activity and physical properties similar to sulfur and tellurium. Selenium speciation has important influences on the fate of the element, and therby occurrence. Because reproductive effects are based on selenium concentrations in fish egg/ovary tissue, the effects are integrated across forms of selenium; thus water column values are based on total selenium exposure.

3.2.1 Selenium Species in Aquatic Systems

The primary selenium species present in water are the anions selenate (SeO_4^{2-} or Se[VI]), selenite (SeO_3^{2-}, or Se[IV]) and organo-selenide (e.g., selenomethionine or org-Se[II]). Selenate usually predominates in well-aerated surface waters such as rivers and streams, especially under alkaline conditions, and is associated with calcareous soils. In water selenite tends to dominate in slow moving waters such as lakes and reservoirs. In soils, selenite is more typically found in acidic conditions (McLean and Bledsoe 1992). Organoselenium containing carbon-selenium chemical bonds is also found in water. The proportion of these different forms of selenium found in aquatic systems can vary. Factors that enhance selenium mobility in soils are; alkaline pH, high selenium concentration, oxidizing conditions, and high concentrations of other anions that strongly adsorb to soils, in particular phosphate (Balistrieri and Chao 1987).

The distribution of selenium among dissolved species cannot be predicted from thermodynamic equilibrium alone. Biological (kinetically driven) processes are just as important as geochemical processes in determining the forms of selenium that are present (Cutter and

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Bruland 1984). Biological processes are difficult to predict from environmental characteristics, so conventional speciation modeling is problematic for selenium. On the other hand, selenium is one of the few elements for which the different species can be directly measured at environmental concentrations (Cutter and Bruland 1984; Cutter and Cutter 2004). These data show that geologic and anthropogenic sources often release mostly selenate (U.S. EPA 1992), which is not reactive with particle surfaces, and is highly mobile in soils. Some types of bacteria convert selenate to elemental selenium in sediments (Oremland 1990). Selenate (SeO_4^{2-}) in the water column is taken up only slowly by bacteria, especially if competition with sulfate (SO_4^{2-}) is involved. Selenite is more reactive because of its more polar character, and tends to adsorb to soils and soil constituents (McLean and Bledsoe 1992). 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 (Riedel et al. 1991).

In nature, adverse effects from selenium are determined by a sequence of processes. The transformation of selenium to organic forms by organisms via methylation acts to increase the solubility and therefore bioavailability (Simmons and Wallschläger 2005). Generally, selenides (Se^{2-}) can be the precursors to organic selenides (e.g. methylated selenides, soluble seleno-amino acids), and also relatively insoluble inorganic selenides. When any form of selenium is taken up at the base of the food web by plants and microbes, it is converted to organo-selenide (Wrench, 1978). Organo-selenide is released back to the water column as these cells die or are consumed (Lee and Fisher, 1994), where some selenite is formed. In water, macrophytes and other plants (algae, phytoplankton) can readily take up selenite and selenate and incorporate selenium in the tissue as selenomethionine. In general, selenium concentrations in algae, microbes, sediments, or suspended particulates are 100−500 times higher than dissolved concentrations in selenate dominated environments such as streams and rivers. But when selenite or organo-selenide is proportionately more abundant, the ratio can be 1000−10,000, such as in wetlands, some estuaries, the oceans, and pure phytoplankton cultures. This variability of particulate concentrations relative to dissolved concentrations is a major cause of the variability in the relationship between selenium in water and selenium in organisms (Luoma and Presser 2009).

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Methylated selenides produced by biological reduction of selenite, usually occur at very low concentrations in water relative to the inorganic selenium species, and differ between flowing (lotic) and standing (lentic) fresh waters (Simmons and Wallschläger 2005). The accumulation patterns for selenium from water to sediment identify higher rates of methylation in lentic compared with lotic environments, and correspondingly higher accumulation rates of selenium in biota in lentic environments (USEPA, 2004). The result is a build-up of proportionately more organo-selenides and selenite as selenium is recycled through the base of food webs, and proportionately less selenate. This unidirectional build-up of potentially reactive forms, especially in environments where water residence times are extended (e.g., wetlands, estuaries, and lakes) is a key factor in the ecological risks posed by selenium. Anaerobic microbial reduction of selenate and selenite to insoluble elemental selenium can represent an important mechanism for removing selenium from water and transferring it to sediments (Lemly. 2004).

3.2.2 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; Ohlendorf et al. 1986; Saiki and Lowe 1987; Presser and Ohlendorf 1987; Luoma et al. 1992; Presser et al. 1994; Chapman et al. 2010). However, unlike other bioaccumulative contaminants such as mercury, 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 by factors of up to one million-fold (Orr et al. 2012; Stewart et al. 2010). Bioaccumulation and transfer through aquatic food webs constitute the major biogeochemical pathways of selenium in aquatic ecosystems. Dissolved selenium oxyanions are primarily absorbed by aquatic producers (trophic level 1 organisms), including phytoplankton and bacteria, and biotransformed into elemental selenium and organoselenium. These organisms, together with other particle-bound selenium sources, constitute the particulate selenium fraction in the water column. Selenium can then be transferred from these trophic level 1 organisms to aquatic primary

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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, selenium bioaccumulation 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; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005; EPRI 2006). 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. In addition, the biogeochemical processes that result in more bioavailable forms of selenium (selenite and organoselenium) occur to a greater extent in waterbodies with long residence times because sediments where these chemical reactions take place tend to settle in depositional areas rather than being transported downstream. As a result, selenium toxicity in flowing waters with short residence times may only be apparent far downstream of their selenium sources, whereas waters with long residence times are more likely to exhibit selenium toxicity near their sources (Presser & Luoma 2006).

Distribution of selenium between particulate and dissolved forms. Selenium is found in both particulate and dissolved forms in water. 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 (Ohlendorf et al. 1986; Saiki and Lowe 1987; Presser and Ohlendorf 1987; Luoma et al. 1992; Presser et al. 1994; Presser & Luoma 2006; Luoma and Rainbow 2005). Direct transfer of selenium from water to animals is a small proportion of total exposure.

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Bioaccumulation in prey. Trophic level 1 organisms such as algae and bacteria, detritus, and other forms of particulate material containing selenium 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 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 vertebrates (e.g., fish and birds) that prey upon them. Additionally, mollusks such as mussels and clams accumulate selenium to a much greater extent than planktonic crustaceans and insects (although not to toxic levels) due to higher ingestions 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). Because egg-laying vertebrates are the most sensitive groups to selenium (Janz et al. 2010), oviparous vertebrate consumers such as fish and birds are consequently the most vulnerable groups to selenium poisoning and the focal point of most 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 mollusks 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).

3.3 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

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exposure concentrations (Luckey and Venugopal 1977; USEPA 1987, 1998; Haygarth 1994; Chapman et al. 2009, 2010).

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 causing 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 and therefore its effect on the tertiary structure of the protein is insulated. 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

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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. 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. 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 against 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.

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3.4 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. Several of these proteins are enzymes that provide cellular antioxidant protection. Selenium is an essential element 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 concentrations of selenium that are only an order of magnitude greater than the required level have been shown to be toxic to fish due to generation of reactive oxidized species, resulting in oxidative stress. 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 are related to optimizing the health of test organisms cultured in the laboratory.

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3.5 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.

3.6 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 is aggregated into a sensitivity distribution that indicates the impact of the toxicant under study to a variety of genera representing the broader the aquatic community. Criteria are designed to be protective of the vast majority of aquatic animal species in an aquatic community (i.e., approximately 95th percentile of tested aquatic animals representing the aquatic community). As a result, health of the aquatic ecosystem may be considered as an assessment endpoint indicated by survival, growth, and reproduction.

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 Agency's 1985 Guidelines (Stephan et al. 1985):

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Acute toxicity test data (mortality, immobility, loss of equilibrium) for aquatic animals from a minimum of eight diverse taxonomic groups is required. 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 on the offspring of exposed fish, chronic effects are the focus of this selenium assessment. Shorter-term intermittent or pulsed exposure of selenium may result in bioaccumulation through the aquatic food web and consequently may adversely affect fish reproduction; such measures of effect are estimated from chronic assessment endpoints in the 2014 selenium criterion document. Available acute toxicity data based on acute water column-only exposure (i.e., $LC_{50's}$) are not used in this assessment to derive a traditional acute toxicity criterion because acute effects are not the effects of concern for the bioaccumulative chemical selenium.

Chronic toxicity test data (longer-term survival, growth, or reproduction) for aquatic animals are needed from a minimum of eight diverse taxonomic groups. 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 1985 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.),

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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 above eight MDRs of Stephan et al. (1985) (requirements 1, 2, 3, 6, 7, and 8). Acceptable information indicating relative insensitivity in accord with the approach of U.S. EPA (2008b) is available for the remaining two items (4 and 5). Consequently, the chronic selenium criterion was derived using the genus-level sensitivity distribution approach per the 1985 Guidelines (Stephan et al. 1985).

The 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 material, results of a plant in another phylum should also be available. No tests were conducted that evaluated a biologically relevant endpoint of an important aquatic plant species in which the concentrations of selenite or selenate were measured. Therefore, plant endpoints were not used in this criteria derivation, consistent with the relative sensitivity perspective of Chapman et al. 2010. A summary of studies investigating the toxicity of selenium on aquatic plants is provided in Appendix E.

The available scientific evidence indicates that for selenium, critical assessment endpoints 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) provide examples of adverse effects that occur through bioaccumulative processes at different levels of biological organization, and comprise integrated whole-ecosystem examples of trophic transfer resulting in population-level reductions of resident species.

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3.7 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 amount of toxicity testing data available for any given pollutant varies significantly, depending primarily on whether any major environmental issues are raised. An in-depth evaluation of available data on selenium has been performed by EPA to determine data acceptability (see Stephan et al. 1985 for additional detail).

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). Consquently, 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 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.

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3.7.1 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, and survival); the EC_{20} corresponds to 20 percent effect. The NOEC is the highest test 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. The MATC is the geometric mean of the NOEC and LOEC.

Wherever 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, Toxicity Relationship Analysis Program (U.S. EPA 2008, 2011). 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. Adverse effects were modeled as a sigmoid function of the logarithmic concentrations of the toxic substance. In most cases the following logistic equation was fit to concentration-response data using the TRAP software (U.S. EPA 2008a):

$$y = \frac{y_0}{1 + e^{(4S(x - x_{50}))}}$$

where y_0 is the background response level at a selenium concentration of zero and S is the slope at x_{50}, the selenium concentration associated with a 50% reduction in y, the response level, relative to y_0. In all analyses, selenium concentrations were log-transformed. In a few cases another mathematical function with a subtly different shape was used.

Only studies with a reference site (field surveys) or control treatment (experimental studies) were included in the analysis, because response levels at these low (background) 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

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criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually. Thus, where concentrations of selenium in fish tissue approach an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that are not as bioaccumulative. This calls for use of a lower level of effect to attain sufficient protection. Further, the EC_{10} was also preferred over the NOEC or LOEC as these measures of effect are highly influenced by study design, specifically the particular 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, are all based solely on EC_{10}s. NOECs contribute to some of the GMCVs for less sensitive species.

Comparing Effect Concentrations in Different Fish Tissues

In this document, chronic values are presented as tissue concentrations of 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 document's primary criterion, that for egg or ovary tissue, 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 (Osmudson et al. 2007; deBruyn et al. 2008). Such conversions were not needed for obtaining the egg-ovary chronic values. Tissue-to-tissue conversions were needed for calculating the reproductive toxicity-based whole-body and muscle chronic criterion element and water criteria concentration elements. Researchers often report concentrations of selenium in fish eggs or ovaries (Holm et al. 2005; Kennedy et al. 2000;

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Hermanutz et al. 1996). The selenium concentrations in eggs and ovaries are usually assumed to be approximately equal. 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. In this document, concentrations of selenium in ovaries are considered equivalent to concentrations of selenium in eggs because most studies measured selenium in the ovaries prior to spawning.

The overall assessment was structured to include both reproductive and non-reproductive studies. Selenium in eggs or ovaries is used in reproductive (maternal transfer) studies, and conversions to whole body or muscle tissue resulting in reproductive effects were estimated. Direct measurements of selenium in whole-body or muscle are used for non-reproductive studies to examine non-reproductive, chronic effects.

Selenium Fish Tissue Toxicity Data Fulfilling Minimum Data Needs

The toxicity data currently available for genera and species fulfilling the 1985 Guidelines recommendations for calculation of freshwater chronic criterion are described in Section 4.1.1 4.1.2 and Appendix C and summarized in Table 3.

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Table 3. 1985 Guidelines 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	5
2. Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	3	3
3. Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	3	4
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	**14**	**17**

The first three of these MDRs in Appendix C are easily fulfilled by the fish species represented in Section 4.1.1 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 invertebrates on chronic toxicity of dietary selenium have been very limited. The few dietary chronic toxicity studies that are available for invertebrate species indicate that they are among the more tolerant aquatic taxa, with available data indicating invertebrate mean chronic values ranging from approximately 3 to 12 times higher than the fish whole body criterion value recommended in this document (data provided in Section 4.1.3) The above invertebrate data address MDRs 6-8, leaving only MDRs 4 and 5, for the planktonic and benthic crustaceans, to be addressed. Because the 5th percentile calculation methods for the Final Chronic Value (FCV) per the Guidelines use actual numeric values for the GMCVs of only 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 low four. A recommendation in the draft white paper on Aquatic Life Criteria for Contaminants of Emerging Concern Part I (U.S. EPA

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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 criteria 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." If this concept is applied to the selenium criterion 5th percentile calculations, actual 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). It is thus concluded that there is adequate data to fulfill the data needs for developing a chronic selenium criterion.

The total number of GMCVs available to derive the chronic criterion is 14. These include nine fish genera from Section 4.1.1 (*Salmo, Lepomis, Micropterus, Oncorhynchus, Pimephales, Gambusia, Esox, Cyprinodon,* and *Salvelinus*) [Added to these are the tested invertebrate genera *Centroptilum, Brachionus,* and *Lumbriculus* from Section 4.1.3], and lastly the two waived genera for MDRs 4 and 5 (crustaceans).

3.7.2 Water

Because fish tissue measurements of selenium are not available for many waters, the EPA is estimating chronic measures of effect in the water-column using the chronic effect level measured by fish tissue. 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 in the water body type under consideration (lotic or lentic water bodies as described below in Section 4.2.4). 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.

The EPA collaborated with the United States Geological Survey to develop and peer-review (ERG 2008) a model relating 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. This model (which is a set of equations) is described in more detail in the Analysis Section 4.2.

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3.7.3 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 4.

Table 4. Summary of Assessment Endpoints and Measures of Effect Used in Criteria 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. 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.

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3.7.4 Conceptual Model of Selenium Effects on Aquatic Life

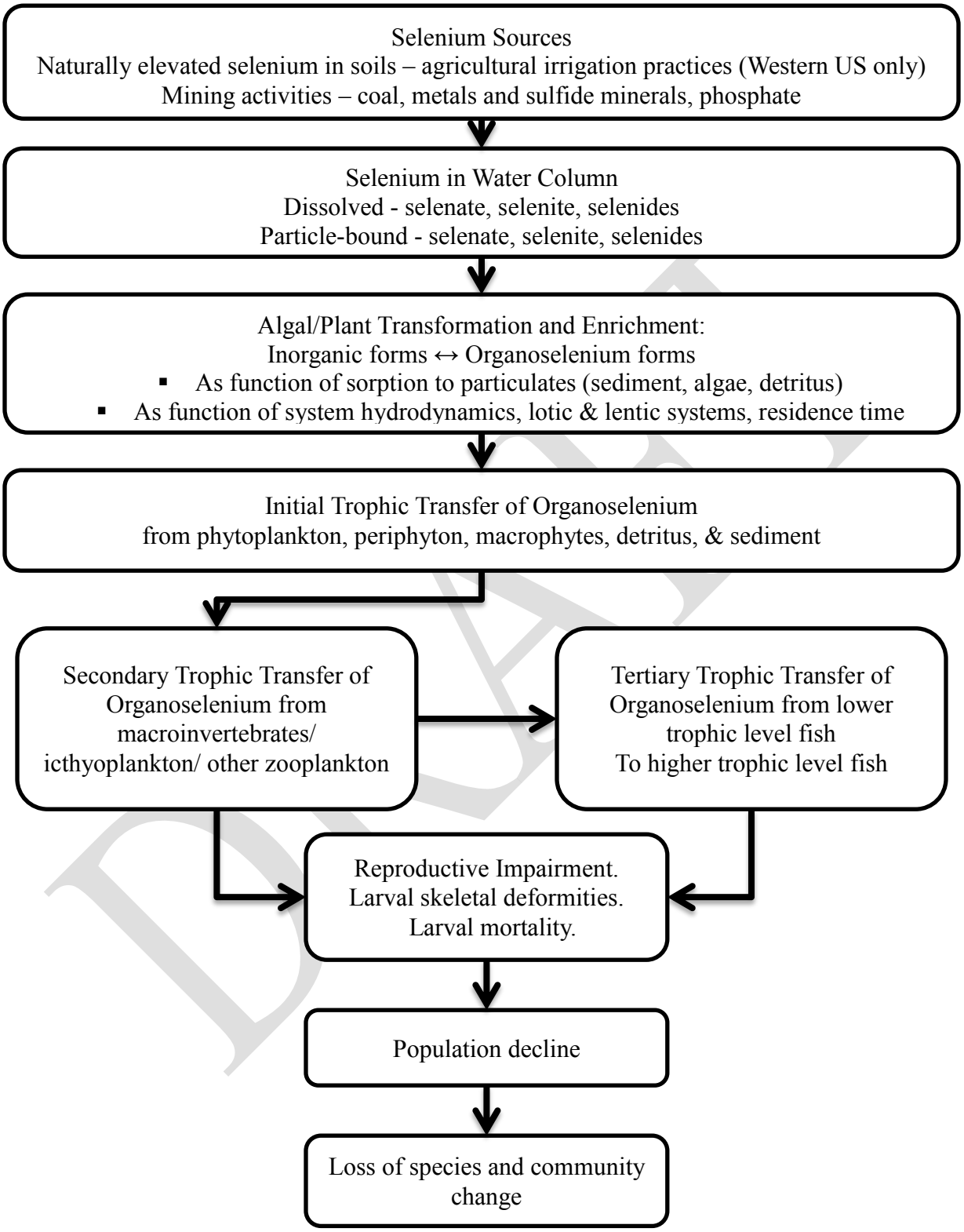

Figure 3. Diagram of selenium partitioning, bioaccumulation, and effects in the aquatic environment.

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The above conceptual model links sources, transformation and uptake through media phases, and consumer transfer and dynamics reflective of the movement of selenium through ecosystems (Figures 3). 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. Other important parameters include rates of input of selenium into the system, hydraulic residence time, and selenium speciation in water and particulates.

3.8 Analysis Plan

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 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. Available data indicate freshwater plants are not more sensitive to selenium than freshwater animals, thus, a plant criterion element was not developed.

3.8.1 Analysis Plan for Derivation of the Chronic Fish Tissue-Based Criterion Element

Data for possible inclusion in the selenium dataset were obtained primarily by search of published literature using EPA's public ECOTOX database and includes studies provided to EPA in public comments. These studies were screened for data quality as described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), but adjusted for factors related to dietary lab or field exposure, which were not considered at the time the Guidelines were written.

Chronic toxicity 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). It has been well established that diet is the primary route of exposure that controls

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selenium toxicity to fish, the taxonomic group considered to be the most sensitive to chronic selenium exposure (Coyle et al. 1993; Hamilton et al. 1990; Hermanutz et al. 1996, Chapman et al. 2010). Furthermore, the toxic potency of fish tissue residues acquired by routes other than dietary exposure do not appear to be equivalent to those acquired by through diet (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978). For example, Cleveland et al. (1993) used water-only and dietary exposure in separate tests with bluegill. Although water-only exposure required hundreds of µg/L to significantly elevate the tissue concentrations, those tissue concentrations began producing effects at 3-4 mg Se/kg dw WB. In contrast, through dietary exposure, no effects were observed at 14 mg Se/kg dw WB, the highest tissue concentration tested. This is hypothesized to be a result of the fact that the form or speciation of selenium differs among exposure routes (diet, water), and that the different forms have differing toxicities. Because water-only chronic exposure would rarely if ever occur in the environment at that magnitude, the criterion derivation uses only those studies in which test organisms were exposed to selenium in their diet, either via laboratory or field exposure, and either alone or in conjunction with elevated water exposure, 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 criteria 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 egg-ovary Final Chronic Value (FCV) was calculated from regression analysis of the four most sensitive GMCVs, in this case extrapolating to the 5th percentile of the

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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 1985 Guidelines).

For the whole-body and muscle criteria concentrations, the egg-ovary Genus Mean Chronic Values were converted to estimated equivalent whole-body or muscle GMCVs. The criterion concentration element expressed as whole-body or as muscle concentration was calculated in manner similar to the egg-ovary criterion element using conversion factors described below, from their respective genus-level sensitivity distributions.

3.8.2 Analysis Plan for Derivation of Duration of Fish Tissue Criterion Elements

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 at a given site. Selenium concentrations in fish tissue are expected to change only gradually over time (Section 4.2.6 and Appendix G) in response to environmental fluctuations, thus, there would be relatively little practical difference between outcomes with different possible specifications of the duration of an averaging period. When fish tissue concentrations are excessive, their duration can be assumed to occur for a sufficient length of time to elicit the effect associated with that concentration.

3.8.3 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. The EPA is using a method based on a peer-reviewed model to derive water concentrations from the egg-ovary criterion 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 the partitioning in fish whole-body and fish eggs and ovaries. The method is composed of four 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.

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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 organisms.
3. Account for bioaccumulation variability across aquatic sites by evaluating the bioaccumulation potential at the base of the aquatic food web, and classifying categories of aquatic systems where a single water column concentration would be adequately protective.
4. Apply a statistical threshold to the distribution of translated water column concentrations for each aquatic system category to derive a protective water column criterion for each aquatic system category.

The EPA worked with the United States Geological Survey to derive a translation equation to estimate the site-specific concentration of selenium in the water column corresponding to the egg-ovary criterion 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; Presser and Luoma 2010; Presser 2013). The equation uses site-specific food web models, species-specific bioaccumulation parameters (*CF* and *TTF*), and a site-specific enrichment factor (*EF*) 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 site-specific *EF* parameter. The EPA obtained these data by searching published literature using EPA's public ECOTOX database and other publication databases. Studies were screened using the same data quality guidelines as 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.

The EPA compiled a database 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

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was made, the location from where the sample was taken, and the date the sample was collected. The 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 level of site, although aggregate measurements was 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, the sample precision was noted, but a single effective collection date was assigned to all the samples.

The EPA also compiled a database of physiological coefficients for food ingestion rate (*IR*), selenium assimilation efficiency (*AE*), and rate of selenium loss (k_e). 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.

The EPA accounts 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). The EPA calculated *EF* values for a set of aquatic systems 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. The 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, the EPA selected the 20th percentile of the distribution of median water column values from 54 studies across the nation as the statistical cut-off. Using binary

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classification statistics, the EPA examined the the performance of the 20th percentile water column values by evaluating how likely it would be that meeting the the water column criterion element would achieve attainment of the fish tissue criteria element. In this analysis EPA used an independent data set composed of measured concentrations of selenium to complete a verification or "ground-truthing" of the selected 20th percentile water column values to evaluate their protectiveness, and found that the use of the 20th percentile water column would prevent potential exceedances of the fish tissue criterion value over 90% of the time, i.e., false negative conclusions regarding fish tissue exceedances would be minimized using the selected 20th percentile water column value for the water column criteria derivation. Figure 4 diagrams the conceptual framework the EPA used to derive water column criterion element from the egg-ovary criterion element.

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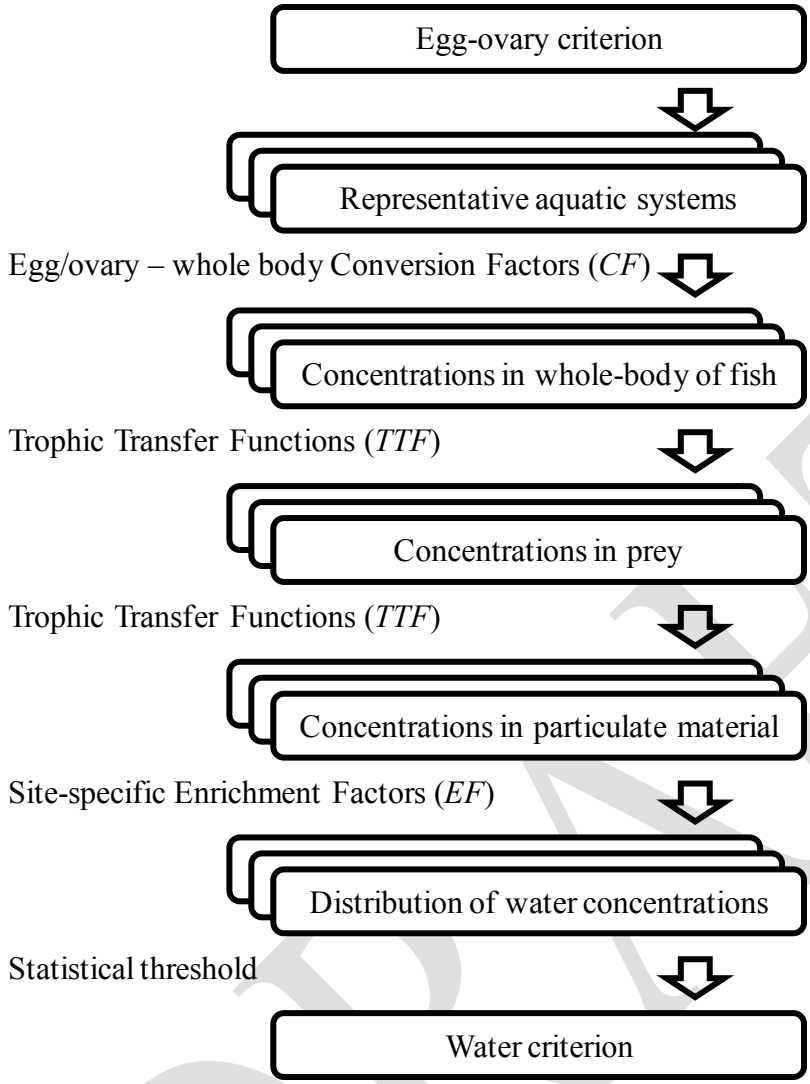

Figure 4. Conceptual model for translating the selenium egg-ovary concentration to a water-column concentration.

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

Like the chronic water criterion element, the intermittent-exposure criterion element is intended to protect against cumulative exposure that would cause exceedance of the fish tissue criterion element. Consequently, it is derived directly from the chronic water criterion element, algebraically rearranging it to establish a limit on an intermittent elevated concentration that occurs a specified percentage of time.

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4 Effects Analysis for Freshwater Aquatic Organisms

The following sections present the derivation of the freshwater aquatic life chronic tissue-based criterion elements (fish egg-ovary, whole-body and/or muscle), chronic water column-based criterion element, and intermittent water column-based criterion element for selenium. These criterion element concentrations are developed to protect against the same effect, reproductive impairment in fish due to maternal transfer to offspring, resulting in mortality and teratogenicity. The fish whole-body and muscle criterion element concentrations are derived by conversion of egg/ovary effect concentrations to whole-body and muscle effect concentrations. The water column-based criterion element concentrations are translated from the egg-ovary concentrations using food web modeling. The intermittent water column-based criterion element concentrations are derived by algebraic rearrangement of the 30-day chronic water criterion element concentrations to single day-steps.

4.1 Chronic Tissue-Based Selenium Criterion Element Concentration

Data were obtained primarily by search of published literature using EPA's public ECOTOX database. In addition, EPA considered studies submitted with comments during the review of the 2004 draft selenium criteria, and studies provided in response to an October 2008 Federal Register Notice of Data Availability. All available, relevant, and reliable chronic toxicity values were incorporated into the appropriate selenium AWQC tables and used to recalculate the Criterion Chronic Concentration (CCC) as outlined in detail in the 1985 Guidelines. The most recent literature search extended to July 2013.

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, survival and growth, the fundamental difference in these two categories of endpoints is exposure (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 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

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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, not directly applied to the criterion derivation are described in Section 7.1.3 and in Appendix D. Nonreproductive studies are described in Section 7.1.8.

4.1.1 Acceptable Studies of Reproductive Effects

Below is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding 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 5. Details of these studies are contained in Appendix C.

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 originally exposed to selenite that was added to artificial streams in a mesocosm study. The selenite entered the food web and contributed to exposure from the diet. Spawning platforms were submerged into treated and control streams. The embryo samples that were collected from the streams were brought into the laboratory and reared in incubation cups which received stream water dosed with sodium selenite via a proportional diluter. Edema and lordosis were observed in approximately 25 percent of the larvae spawned and reared in natural water spiked with 10 µg Se/L. Selenium residues in ovaries of females from the treated stream averaged 5.89 mg/kg ww

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or 23.85 mg/kg dw (applying 75.3 average percent moisture for fathead minnow eggs/ovaries from GEI Associates (2008) and Rickwood et al. (2008)).

The reproductive SMCV for fathead minnows is <23.85 mg Se/kg dw in ovary/eggs based on the Schultz and Hermanutz (1990) study. Because it is a "less than" value, the sensitivity of fathead minnows relative to the species having lower SMCVs is not certain. However, the Young et al. (2010) observation that fathead minnow populations remained after selenium contamination of Belews Lake had eliminated most other fish species, including bluegill and largemouth bass, indicates that fathead minnows at that site were not as sensitive as those species.

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. Milt and ova were stripped from gravid fish, eggs were fertilized in the field and then 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

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defining a threshold. That is, the NOEC and LOEC would be a "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.)

Salmonidae

Seven publications provide quantitative data on effects of selenium on salmonid embryo/larval survival and deformity used in calculating criteria values. All involve wild-caught adults from selenium contaminated streams, spawned for effects determination; exposure was through the parents. These data are for rainbow trout (*Oncorhynchus mykiss),* cutthroat trout *(Oncorhynchus clarki),* Dolly Varden *(Salvelinus malma)* and brown trout (*Salmo trutta*) and 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 until swim-up stage in the laboratory, for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. The temperature at which embryos were incubated was 8°C in 2000, with the exception of rainbow trout, which were incubated at 5°C in 2001 (Holm

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2002; Holm et al. 2005). 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 incidence 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 do not tend to start their lives in elevated exposure streams even though they may reside there later.

Estimates of effect concentrations for the combined data (i.e., 2000 through 2002) were derived with a fitted logistic equation (TRAP). Proportion of skeletal deformities in rainbow trout embryos was the most sensitive endpoint with an EC_{10} of 21.1 mg Se/kg dw and an EC_{20} of 28.4 mg Se/kg dw. Holm (2002) and Holm et al. (2005) reported egg selenium concentrations in wet weight. Egg wet weight was converted to dry weight assuming 61.2% moisture in rainbow trout eggs (Seilor and Skorupa 2001).

Oncorhynchus clarki lewisi (westslope cutthroat trout)

In a field study similar to those conducted by Holm et al. (2005) and Kennedy et al. (2000), 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 TRAP estimates of the EC_{10} and EC_{20} for survival in the eggs are 24.11 mg Se/kg dw and 28.73 mg Se/kg dw, respectively.

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

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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} determined by TRAP of 24.02 mg/kg egg dw. This result is very similar to the EC_{10} of 24.11 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.

Based on these two studies, for the EC_{10} level of effects, the SMCV for cutthroat trout, *Oncorhynchus clarki*, is 24.06 mg Se/kg dw in eggs derived from Rudolph et al. (2008) and Nautilus Environmental (2011) (24.11 and 24.02 mg Se/kg dw, respectively).

Salvelinus fontinalis (brook trout)

This data was not used directly in the criterion calculations See Section 7.1 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 5 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.

Salmo trutta (brown trout)

Formation Environmental (2011) collected adult female and male brown trout from sites with low and high selenium exposure in the proximity of a phosphate mine located in

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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 twelve 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. This document's analysis of the revised counts from AECOM (2012) builds upon but supersedes EPA's 2012 analysis (Taulbee et al. 2012), peer reviewed by ERG (2012). For the full test, hatch through 15-days post swim up, combining wild and hatchery fish, there are six EC_{10}s corresponding to survival, free of deformity, and combined survival and free of deformity, all for both the lab accident worst case assumption (fry lost in the lab accident during the 15-day portion of the study were assumed to have been dead or deformed) and the optimistic assumption (fry lost had the same rates of mortality and deformity as those not lost). These six EC_{10}s, shown in Section 7.1.4, fall in the range 15.91 – 21.16 mg/kg egg dw. Appendix C presents details of the study. The chronic value selected for the study was the low EC_{10} of 15.91 mg/kg egg dw, for total deformities, assuming the worst case scenario for fry lost in the lab accident.

Salmonidae SMCV and GMCV Summary

For the EC_{10} level of effects, the SMCV for cutthroat trout, *Oncorhynchus clarki*, is 24.06 mg Se/kg dw in eggs derived from Rudolph et al. (2008) and Nautilus Environmental (2011) (24.11 and 24.02 mg Se/kg dw respectively). The GMCV for the genus *Oncorhynchus* is 22.53 mg Se/kg dw in eggs, derived from the 21.1 mg Se/kg dw EC_{10} from the combined Holm (2002) and Holm et al. (2005) rainbow trout data, and the above mean of the Rudolph et al. (2008) and Nautilus Environmental (2011) Westslope cutthroat trout studies (24.06 mg Se/kg dw). 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, and the GMCV for the genus *Salmo* is the EC_{10} value of 15.91 mg Se/kg dw for brown trout (S. trutta) from the Formation Environmental (2011) study.

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Poeciliidae

Data are available for two species in this family. These studies are not represented in Table 5 because these species are live-bearing rather than egg-laying species, 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 two sites in the grassland water district, Merced County, California that were contaminated with selenium and two reference sites in the same area with relatively low selenium exposure (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 4 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

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accompanying egg-laying fish, by multiplying by the median fish egg-ovary to whole-body concentration ratio, 1.71. This yields >25.82 mg Se/kg dw equivalent egg-ovary.

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 7.1.1. It may be concluded that this genus is not among the most sensitive to selenium.

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 time, 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 suggests that the EC_{10} may be close to that concentration, as also noted by the authors.

Centrarchidae

Lepomis macrochirus (bluegill sunfish)

In a laboratory study, Doroshov et al. (1992) exposed adult bluegill for 140 days to three dietary concentrations of seleno-L-methionine 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

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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 5-day larval test, the average proportion of larvae with edema was 0% at an egg concentration of 8.33 mg Se/kg (the first treatment), 5% at an egg concentration of 19.46 mg Se/kg dw (the second treatment), and 95% at an egg concentration of 38.39 mg Se/kg dw (the highest treatment). The latter two were statistically different from the control (0% edema). All edematous larvae died in the high treatment. 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. The EC_{10} calculated with TRAP for the incidence of edema in the 5-day larval bioassay is 20.05 mg Se/kg dw in eggs.

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 after 60 days when spawning began to occur. There was no effect of the combination of the highest dietary selenium concentration (33.3 mg Se/kg dw) on *adult* growth, condition factor, gonadal somatic index, or the various reproductive endpoints (Appendix C). The survival of newly hatched larvae, however, was markedly reduced; only about 7 percent survived to 5 days post-hatch in the high dietary treatment. The TRAP-calculated EC_{10} value for larval survival is 24.55 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-type 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 criteria 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 to adult bluegill sunfish in the fall, and respective studies ended in the summer of the following year. Winter temperatures averaged

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4.6, 4.1 and 4.5°C and spawning months (June-July) averaged 26.4, 23.9 and 22.4°C, respectively for Studies I, II and III. 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). Embryo-larval effects were observed in the selenium-dosed streams in both Study I and Study II. 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 (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 above 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 ranged from 0.8 to 2.5 mg/kg dw in the control 7.6 to 10.9 mg/kg dw in the 2.5 µg/L treatment, and 17.7 to 30.0 mg/kg dw in the 10 µg Se/L treatment (values represent both Study I and II for the control and 10 µg/L treatments).

The embryo-larval data for continually exposed streams of Studies I and II of this experiment were combined and analyzed in response to measured selenium concentration in the maternal ovaries (mg/kg dw) using TRAP. In the recovering streams of Study II and Study III no effects were observed at concentrations that had previously caused effects in the continually exposed streams. Recovering streams were therefore excluded from this criteria document's analysis because they do not reflect the type of system that water quality criteria are most commonly applied to, those receiving existing waterborne pollutant discharges. That is, Study III consisted of the addition of new adult bluegill to the same streams that had received the 2.5, 10, and 30 µg/L sodium selenite during previous studies, but with all continued external dosing of selenite halted. The adult bluegills exposed only to dietary selenium present in the Study III food web accumulated selenium to levels very near to those accumulated during Study II in which aqueous selenium was also present demonstrating the importance of diet on selenium

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accumulation. There were no effects (no effect on larval survival, 0 percent deformities, 0 percent hemorrhaging), on the bluegill progeny in Study III even from fish that accumulated 26.04 and 10.17 mg/kg dw in the recovering 10 µg/L streams, and 15.92 mg/kg dw in the recovering 30 µg/L stream. The absence of effects at high tissue levels in the recovering streams of Study III provides experimental 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). Because Study III involved new (naïve) fish added to previously contaminated streams, neither acclimation nor adaptation would seem to explain this phenomenon. Overall, the implication is that for some period of time, recovering systems might possibly exceed tissue criteria concentrations even though the effects of selenium have been mitigated.

Of the three endpoints that attained model convergence when analyzed by TRAP (% edema, % lordosis, and % hemorrhage), % edema relative to ovary selenium concentration was the most sensitive yielding an EC_{10} of 12.68 mg Se/kg and an EC_{20} of 13.66 mg Se/kg dw ovary. The data for % lordosis indicate that it is a less sensitive endpoint. The data for % hemorrhage showed no conclusive concentration-response relationship: responses at concentrations 7.6 and 50.5 mg Se/kg dw varied widely without any relationship to selenium concentration, precluding its use for estimating an EC_{10}. The EC_{10} value of 12.68 mg/kg Se dw (larval edema in response to Se concentration in the parental ovaries) is considered an environmentally conservative chronic value for this bluegill study. (See Appendix C for more discussion of this study, and Section 7.1.5 for more discussion of this chronic value).

The SMCV for bluegill reproductive endpoints based on EC_{10} values is 18.41 mg Se/kg dw in egg/ovary, based on the EC_{10} values of Doroshov et al. (1992), Coyle et al. (1993), and Hermanutz et al. (1992, and 1996 as corrected by Tao et al. 1999)

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 for several months. Similar exposure studies were conducted in 1995 and 1996 resulting in successful spawning of adults. 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

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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). The percent survival of larval largemouth bass as a function of the selenium concentration in the parental ovary using TRAP produced an EC_{10} of 20.35 mg/kg dw and an EC_{20} of 23.60 mg/kg dw (Appendix C).

4.1.2 Summary of Acceptable Studies of Fish Reproductive Effects

Table 5 summarizes the effect concentrations obtained from reproductive studies with fish.

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Table 5. 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
Pimephales promelas fathead minnow	Schultz and Hermanutz 1990	dietary and waterborne (mesocosm: Monticello)	LOEC for larval edema and lordosis	<23.85 O[b]	<23.85 O	<23.85 O
Esox lucius northern pike	Muscatello et al. 2006	dietary and waterborne (field: Saskatoon, Sask.)	EC_{24} larval deformities	34.00 E	34.00 E	34.00 E
Oncorhynchus mykiss rainbow trout	Holm 2002; Holm et al. 2003; Holm et al. 2005	dietary and waterborne (field: Luscar River, Alberta)	EC_{10} for skeletal deformities	21.1 E[b]	21.1 E	22.53 E
Oncorhynchus clarki lewisi Westslope cutthroat trout	Rudolph et al. 2008	dietary and waterborne (field: Clode Pond, BC)	EC_{10} for alevin mortality	24.11 E	24.06 E	
Oncorhynchus clarki lewisi Westslope cutthroat trout	Nautilus Environmental 2011	dietary and waterborne (field: Clode Pond & Fording River, BC)	EC_{10} for survival at swim-up	24.02 E		
Salvelinus malma Dolly Varden	Golder 2009	dietary and waterborne (field: Kemess Mine NW British Columbia)	EC_{10} for total deformities	56.22 E	56.22 E	56.22 E

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Species	Reference	Exposure route	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
Salmo trutta brown trout	Formation Environmental 2011	dietary and waterborne (field: Lower Sage Creek & Crow Creek, ID)	EC_{10} for larval survival	15.91 E	15.91 E	15.91 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
Lepomis macrochirus bluegill	Doroshov et al. 1992	dietary (lab)	EC_{10} larval edema	20.05 E	18.41E	18.41E
Lepomis macrochirus bluegill	Coyle et al. 1993	dietary and waterborne (lab)	EC_{10} for larval survival	24.55 E		
Lepomis macrochirus bluegill	Hermanutz et al. 1992 Hermanutz et al. 1996	dietary and waterborne (mesocosm: Monticello)	EC_{10} for larval edema	12.68 O[b]		
Micropterus salmoides Largemouth bass	Carolina Power & Light 1997	dietary (lab)	EC_{10} for larval mortality & deformity	20.35 O	20.35 O	20.35 O

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

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

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Table 6a presents the Genus Mean Chronic Values from acceptable studies of reproductive effects in fish.

Table 6a. Ranked Genus Mean Chronic Values for Fish Reproductive Effects.

Rank	GMCV (mg Se/kg dw EO)	Species	SMCV (mg Se/kg dw EO)
9	56.22	Dolly Varden, *Salvelinus malma*	56.22
8	< 34	Northern pike, *Esox lucius*	< 34
7	27	Desert pupfish, *Cyprinodon macularius*	27
6	> 25.82 estim. EO* (> 15.1 meas. WB)	Eastern mosquitofish, *Gambusia holbrooki*	> 20.26 estim. EO* (> 11.85 meas. WB)
		Western mosquitofish, *Gambusia affinis*	> 25.82 estim. EO* (> 15.1 meas. WB)
5	< 23.85	Fathead minnow, *Pimephales promelas*	< 23.85
4	22.53	Cutthroat trout, *Oncorhynchus clarki*	24.06
		Rainbow trout, *Oncorhynchus mykiss*	21.1
3	20.35	Largemouth bass, *Micropterus salmoides*	20.35
2	18.41	Bluegill sunfish, *Lepomis macrochirus*	18.41
1	15.91	Brown trout, *Salmo trutta*	15.91

*For mosquitofish, a live bearer, the egg-ovary concentrations is that estimated to be typical for other fish species in an assemblage sharing the same exposure. Combining of its two "greater than" SMCVs into its GMCV follows the principles of Section 7.1.1.

4.1.3 Invertebrate Chronic Effects

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

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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 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 oligochate, *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.

Centroptilum triangulifer (mayfly)

Mayfly larvae (*Centroptilum triangulifer*) were exposed to dietary selenium contained in natural periphyton biofilms to eclosion (emergence) (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 Se an average of 1113-fold over the different aqueous selenium concentrations (see table below). 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 was measured in postpartum adults along with their dry weights and

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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.

4.1.4 Summary of Relevant Invertebrate Tests

The available measured invertebrate whole-body effect concentrations and their translation to fish egg-ovary concentrations are shown in Table 6b. Because the intent of this assessment is to derive a concentration expressed in terms of fish tissue, effect concentrations expressed as in terms of invertebrate tissue need to be translated across media in order to compare invertebrate effect concentrations to egg-ovary concentrations that would occur in a fish assemblage that would accompany the invertebrates. That is, using the bioaccumulation modeling approach of Section 4.2, invertebrate whole-body effect concentrations have been translated to fish egg-ovary concentrations using (a) the median trophic transfer factor of 1.27 from Table 10 and (b) the median whole-body to egg-ovary conversion factor of 1.71 from Table 11. This yields a combined conversion factor of 1.27 x 1.71 = 2.17. Mean whole body chronic values ranging from 24.2 mg/kg for the mayfly (*Centroptilum traingulifer*) to greater than 100 mg/kg for the oligochaete (*Lumbrilicus variegatus*),which is approximately 3 to 12 times higher than the fish whole body criterion.

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Table 6b. Ranked Invertebrate Whole-Body Chronic Values with Translation to Expected Accompanying Fish Egg-Ovary Concentrations.

SMCV & GMCV as measured (mg Se/kg dw WB)	SMCV & GMCV as estimated EO concentration in an accompanying fish assemblage (mg Se/kg dw EO)	Species
> 100	> 217	Oligochaete,black *Lumbriculus variegatus*
37.84	82.11	Rotifer, *Brachionus calyciflorus*
24.2	52.6	Mayfly, *Centroptilum triangulifer*

4.1.5 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 9 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 criteria because they were more variable and less reproducible than the data on reproductive effects. The SSD 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 6c, Section 4.1.2) have shown them to be more tolerant than most of the tested fish species. While potentially sensitive due to physiologic similarities to fish, amphibian 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

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Fish Egg-Ovary Concentration

The lowest four GMCVs from Table 6a are shown below in Table 6c.

Table 6c. Four lowest Genus Mean Chronic Values for Fish Reproductive Effects.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw egg-ovary)
4	*Oncorhynchus*	22.53
3	*Micropterus*	20.35
2	*Lepomis*	18.41
1	*Salmo*	15.91

With N=14 GMCVs, the 5th percentile projection yields an egg/ovary criterion of 15.2 mg Se/kg dw egg/ovary, lower than the most sensitive fish species tested, brown trout (*Salmo trutta*). The egg/ovary criterion element concentration is compared to the distribution of egg/ovary chronic values in Figure 5.

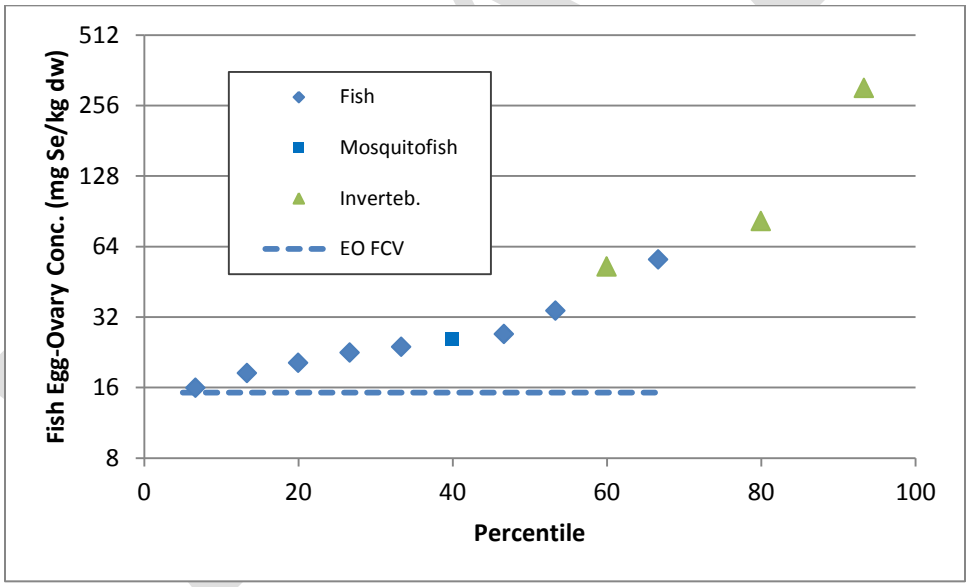

Figure 5. Distribution of (a) reproductive-effect GMCVs for fish measured as egg-ovary concentrations (diamond markers), (b) the reproductive-effect value for mosquito fish (square marker), a live-bearer measured as adult whole-body but translated to an equivalent egg-ovary concentration using the median conversion factor 1.71, and (c) invertebrate effect concentrations (triangle markers) measured as whole-body but translated to the equivalent fish egg-ovary concentrations expected in an accompanying fish assemblage, through the median trophic transfer factor of 1.27 from Table 10 and the median egg-ovary conversion factor of 1.71 from Table 11

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Fish Whole-Body Criterion Element Concentration

Using the egg-ovary to whole-body conversion factors of the bioaccumulation modeling approach presented subsequently in Section 4.2, Table 7a shows the conversion of reproductive-effect egg-ovary concentrations to whole-body concentrations. It can be seen that for some species, the conversion was done in a single step, using a measured egg-ovary (EO) to whole-body (WB) ratio specific to the taxon. But for other species, it was done in two steps, first converting to muscle (M) and then applying a generic M/WB ratio.

Table 7a. Tested Reproductive-Effect Egg-Ovary (EO) Concentrations Converted to Whole-Body (WB) Concentrations.

Taxon	EO Chronic Value	EO/WB Ratio	Calculated WB Repro Chronic Value	Basis for EO/WB Ratio (from Appendix B)
Salvelinus	56.22	1.61	34.90	Median Dolly Varden EO/M (1.264) x median fish M/WB (1.274)
Esox	34.00	2.39	14.23	Median northern pike EO/M (1.875) x median fish M/WB (1.274)
Cyprinodon	27.00	1.21	22.31	Median desert pupfish EO/WB
Pimephales	23.85	2.00	11.94	Median Cyprinidae EO/WB
O. mykiss	21.10	2.44	8.64	Median rainbow trout EO/M (1.916) x median fish M/WB (1.274)
O. clarkii	24.06	2.30	10.46	Median cutthroat trout EO/M (1.805) x median fish M/WB (1.274)
Onchyrhynchus	22.53	2.37	9.51	Using geomean of species ratios yields geomean of SMCVs
Micropterus	20.35	1.45	14.03	Median Centrarchidae EO/WB
Lepomis	18.41	2.13	8.63	Median bluegill EO/WB
Salmo	15.91	1.45	10.97	Median brown trout EO/WB

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Table 7b. The lowest four reproductive-effect whole-body GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw whole-body)
4	*Pimephales*	11.94
3	*Salmo*	10.97
2	*Oncorhynchus*	9.51
1	*Lepomis*	8.63

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=14 GMCVs, the 5th percentile projection yields a whole body criterion of 8.13 mg Se/kg dw whole-body, slightly lower than the most sensitive fish species tested, bluegill (*Leopmis machrochirus*) The fish whole body criterion is compared to the distribution of fish whole body chronic values in Figure 6.

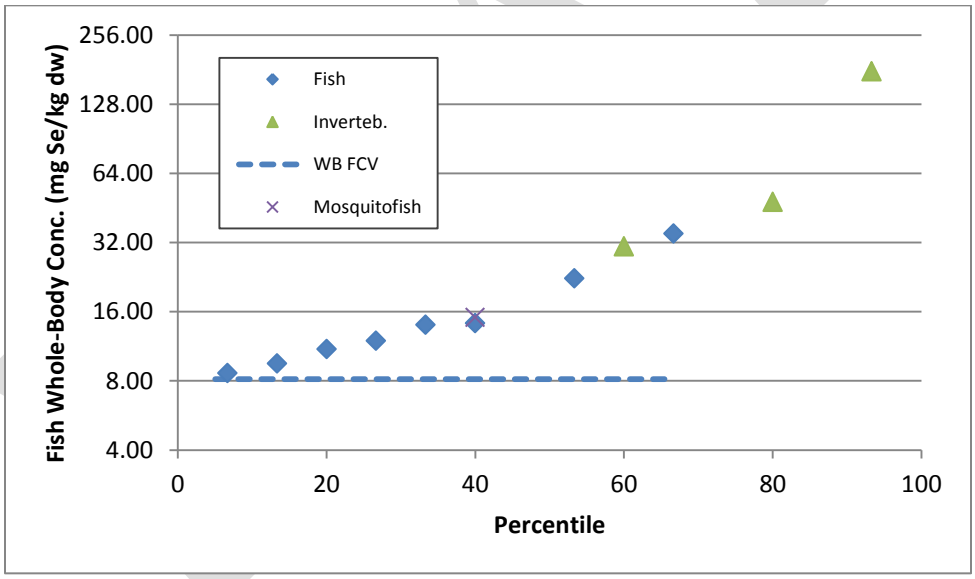

Figure 6. Distribution of (a) reproductive-effect GMCVs for fish, measured as egg-ovary concentrations but converted to whole-body concentrations as shown in Table 7, (b) the reproductive-effect value for mosquito fish, a live-bearer already measured as adult whole-body, and (c) invertebrate effect concentrations measured as whole-body and translated to equivalent fish whole-body concentrations through the median trophic transfer factor of 1.27 from Appendix B.

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Fish Muscle Criterion Element Concentration

Using the egg-ovary to muscle conversion factors of the bioaccumulation modeling approach presented subsequently in Section 4.2, Table 8a shows the conversion of reproductive-effect egg-ovary concentrations to whole-body concentrations. For all but *Cyprinodon* (desert pupfish), the conversion could be done in a single step, applying an EO/M ratio specific to the taxon.

Table 8a. Tested Reproductive-Effect Egg-Ovary (EO) Concentrations Converted to Muscle (M) Concentrations.

Taxon	EO Chronic Value	EO/M Ratio	Calculated Muscle Repro Chronic Value	Basis for EO/M Ratio (from Appendix B)
Salvelinus	56.22	1.264	44.48	Median Dolly Varden EO/M (1.264)
Esox	34	1.875	18.13	Median northern pike EO/M (1.875)
Cyprinodon	27	0.950	28.42	Median desert pupfish EO/WB divided by median fish M/WB (1.274)
Pimephales	23.85	1.590	15.00	Median *Cyprinidae* EO/M (1.590)
O. mykiss	21.1	1.916	11.01	Median rainbow trout EO/M (1.916)
O. clarkii	24.1	1.805	13.35	Median cutthroat trout EO/M (1.805)
Onchyrhynchus	22.56	1.860	12.124	Using geomean of species ratios yields geomean of SMCVs
Micropterus	20.35	1.187	17.15	Median *Micropterus* EO/M (1.187)
Lepomis	18.41	1.375	13.39	Median bluegill EO/M (1.375)
Salmo	15.91	1.135	14.02	Median brown trout EO/M (1.135)

Table 8b. The lowest four reproductive-effect fish muscle GMCVs.

Relative Sensitivity Rank	Genus	GMCV (mg Se/kg dw muscle)
4	*Pimephales*	15.00
3	*Salmo*	14.02
2	*Lepomis*	13.39
1	*Oncorhynchus*	12.12

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Because the factors used to convert egg-ovary to muscle concentrations vary across species, the whole-body rankings differ from both from the egg-ovary rankings and the muscle rankings. With N=14 GMCVs, the 5th percentile projection yields a muscle criterion of 11.8 mg Se/kg dw muscle, lower than muscle value for the most sensitive fish genus tested, *Oncorhyncus*.

4.2 Chronic Water Column-based Selenium Criterion Element

4.2.1 Translation from Fish Tissue Concentration to Water-Column Concentration

The chronic water column selenium criterion element is derived by translating the egg-ovary concentration to an equivalent water concentration. The EPA worked with the United States Geological Survey 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; Presser and Luoma 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 metal in water (µg/L)
C_{tissue}	=	Average concentration of metal 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)

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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) suggests 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.

The EPA evaluated the effect of removing the parameter g in the Equation 1 by performing a sensitivity analysis. The 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

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bioconcentration of selenium in their tissues. The 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 G.

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, 2010b, 2013) deemed removal of k_u from Equation 1 as an acceptable simplification.

The EPA evaluated the effect of removing the parameter k_u in the Equation 1 by performing a sensitivity analysis. The 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. The 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 G.

Derivation of the Translation Equation

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

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

or:

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

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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 Function (*TTF*) given as:

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

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:

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

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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_{food}^{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:

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

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 function 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 (USEPA 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

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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 function of trophic level 2 species
TTF^{TL3} = the trophic transfer function of the trophic level 3 species
TTF^{TL4} = the trophic transfer function of the trophic level 4 species
$TTF^{composite}$ = the product of all trophic transfer functions

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 function of the ith species at a particular trophic level
w_i = the proportion of the ith 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 7 describes four example food web scenarios and the formulation of $TTF^{composite}$ to model selenium bioaccumulation in each of them.

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A) Three trophic levels (simple):

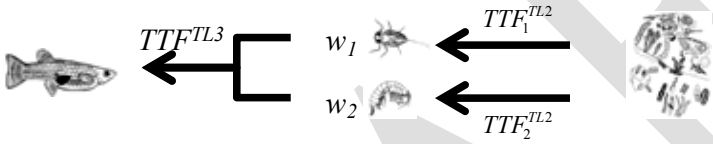
$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$

B) Four trophic levels (simple):

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

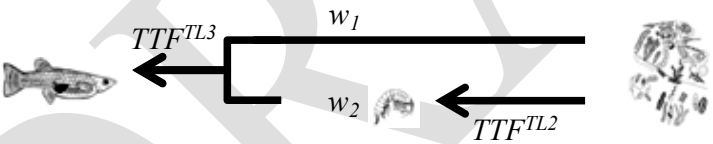
C) Three trophic levels (mix within trophic levels):

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



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):

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

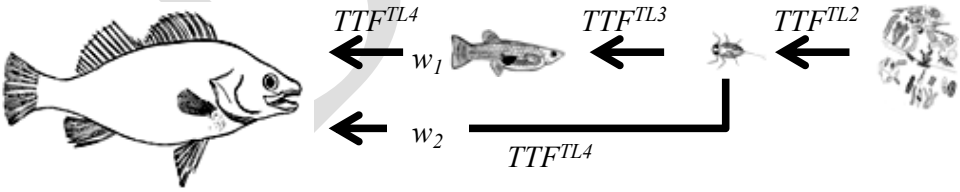


Figure 7. Example aquatic system scenarios and the derivation of the equation parameter TTFcomposite.
This parameter quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system. w_i, proportion of species consumed. See text for further explanation.

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Because the 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:

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

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

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

Because Equation 15 relates a concentration of selenium in water to the concentration of selenium throughout all tissues of the body, and the intention here is to relate the concentration of selenium in water to the concentration of selenium in the eggs or ovaries, $C_{whole\text{-}body}$ must be converted to an equivalent concentration in eggs or ovaries. The 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:

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

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_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 functions from trophic level 2 through the target fish species.

Equation 18 establishes 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, however, 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*.

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4.2.2 Equation Parameters

Empirical or laboratory data related to selenium bioaccumulation in aquatic organisms are needed to derive the equation parameters *EF*, *TTF*, and *CF*. The EPA obtained data from published literature as described above, using the EPA database ECOTOX. The search resulted in the retrieval of 54 acceptable studies containing a total of 6,838 selenium measurements at 610 aquatic sites (2,170 from water, 275 from algae, 30 from detritus, 780 from sediment, 1056 from various species of invertebrates, and 2,170 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*). The EPA used this database 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 the EPA calculates *EF* is described below. A more detailed description of how the EPA calculates *TTF* and *CF* is described in Appendix B.

Derivation of Trophic Transfer Function (TTF) Values

The 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, the EPA calculated a *TTF* value using the equation:

$$TTF = \frac{AE \times IR}{k_e}$$

Where:

k_e = Elimination rate constant (/d)

AE = Assimilation efficiency (%)

IR = Ingestion rate (g/g-d)

The EPA also derived *TTF* values using empirical measurements of selenium from field studies. The EPA searched its database of available selenium measurements and identified measurements taken from aquatic organisms. For each measurement from an aquatic organism,

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the EPA searched its selenium database again 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.

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. The 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 8 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 particulate and invertebrate measurements with a relative sample collection time of 30 to 60 days were from invertebrate and particulate samples collected at the same site, with the invertebrate samples collected 30 to 60 days after the particulate samples. Similarly, the correlation coefficient calculated from particulate and invertebrate measurements with a relative collection time of -60 to -30 days were from invertebrate and particulate samples that were collected at the same site, with the invertebrate samples collected 30 to 60 days before the particulate samples.

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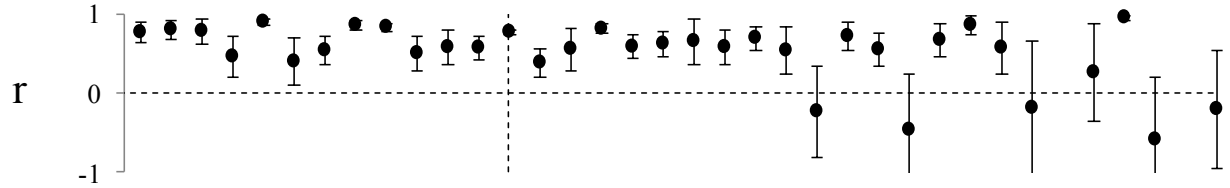
Particulate versus invertebrate



Invertebrate versus fish

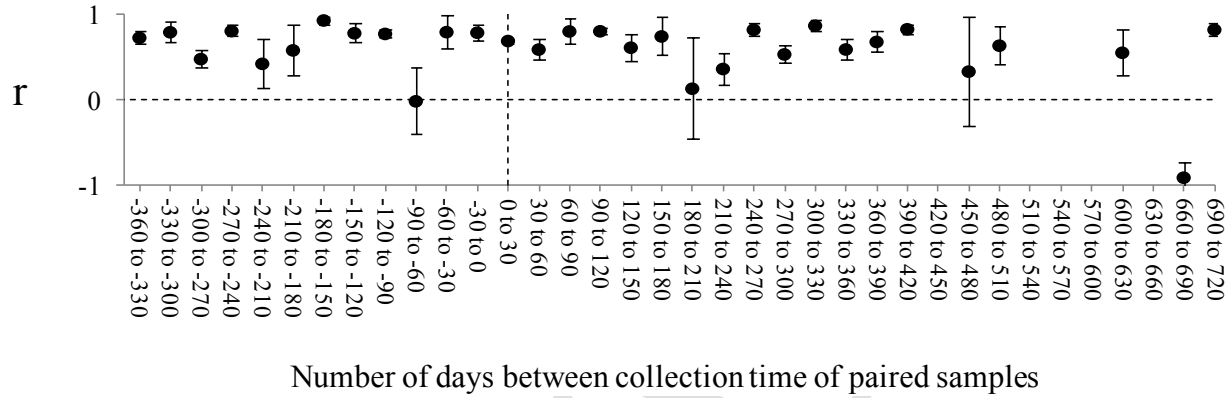


Figure 8. Effect of relative sample collection time on correlation coefficients of selenium measurements in particulate material, and invertebrate and fish tissue.
Error bars indicate the 95% confidence interval of r calculated using Fisher's r to z transformation. Horizontal dashed line indicates r = 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 somewhat 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 this analysis, the EPA concludes that selenium measurements from samples collected at the same aquatic site within one year of each other are reasonable acceptability criteria for matched pairs of measurements from the aquatic sites in the EPA database. Note that the 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

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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, the 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:

$$TTF = \frac{C_{tissue}}{C_{food}}$$

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. The 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 represents 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; USEPA 2007). Thus a distribution of ratios could be biased toward larger values if the data are obtained from aquatic systems with low selenium concentrations.

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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*. The 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 includes 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 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).

After evaluating both approaches, the 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, and using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias due to measurements taken from aquatic systems with very high or very low selenium concentrations.

The EPA calculated *TTF* values for 13 invertebrate species and 30 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 Tables 9 and 10. The presence of physiological coefficients for a taxon in Tables 9 and 10 indicates that the *TTF* values were calculated using those parameters. The absence of physiological coefficients for a taxon indicates the EPA derived the *TTF* value using field data. If a *TTF* value could be calculated from both

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physiological coefficients and field data, the EPA used the *TTF* value calculated from the substantially larger number of field measurements so as to minimize statistical uncertainty.

Table 9. EPA-derived Trophic Transfer Function (*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*	0.390	0.720	0.220	1.28
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 *Corbula amurensis*

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Table 10. EPA-Derived Trophic Transfer Function (*TTF*) Values for Freshwater Fish.

Common name	Scientific name	AE	IR	ke	TTF
Cypriniformes					
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
common carp	*Cyprinus carpio*	-	-	-	1.29
creek chub	*Semotilus atromaculatus*	-	-	-	1.12
fathead minnow	*Pimephales promelas*	-	-	-	1.57
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	1.06
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
sand shiner	*Notropis stramineus*	-	-	-	1.83
white sucker	*Catostomus commersonii*	-	-	-	1.18
Cyprinodontiformes					
mosquitofish	*Gambusia sp.*	-	-	-	0.97
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
western mosquitofish	*Gambusia affinis*	-	-	-	1.25
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.79
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.69
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.48
green sunfish	*Lepomis cyanellus*	-	-	-	1.27
largemouth bass	*Micropterus salmoides*	-	-	-	1.27
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.82
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.44
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.07
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.19
westslope cutthroat trout	*Oncorhynchus clarkii lewisi*	-	-	-	1.20
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.91
channel catfish	*Ictalurus punctatus*	-	-	-	0.73

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

The parameter *CF* (conversion factor) in Equation 18 represents the species-specific partitioning of selenium as measured in the whole-body and in egg-ovary tissue. The 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, 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. Similar to the procedure used to derive *TTF* values, the 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, the 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.

Some selenium measurements in eggs and/or ovaries were only available with corresponding measurements from muscle tissue. In those cases, the EPA estimated an equivalent whole body concentration from the muscle concentration by applying a muscle to whole-body conversion factor. The EPA derived the muscle to whole-body conversion factor using the same method that was used to derive *CF* values. Matched pairs of muscle concentration measurements were regressed on whole-body concentration, and if the regression resulted in a significant fit ($P \leq 0.05$) with a positive regression coefficient, the median of the whole-body to muscle concentration ratios was calculated for each species. Because data from only a few fish species were available, the EPA used the median of the resulting species-specific muscle to whole-body ratios as a single muscle to whole-body conversion factor of 1.27 for all fish species.

There were a sufficient number of egg-ovary and whole-body selenium measurements to directly derive *CF* values for 9 species of fish found in freshwater aquatic environments of North America. The EPA derived *CF* values for 7 additional species using muscle and egg-ovary selenium measurements and applying a muscle to whole-body conversion factor of 1.27. The

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data and methods used to derive the *CF* values are described in detail in Appendix B. The final *CF* values are listed below in Table 11.

Table 11. EPA-Derived Egg-Ovary to Whole-Body Conversion Factor (*CF*) Values.

Common name	Scientific name	CF
Cypriniformes		
bluehead sucker	*Catostomus discobolus*	1.82
common carp	*Cyprinus carpio*	1.92
flannelmouth sucker	*Catostomus latipinnis*	1.41
razorback sucker	*Xyrauchen texanus*	1.43
roundtail chub	*Gila robusta*	2.07
white sucker	*Catostomus commersonii*	1.41
Esociformes		
northen pike	*Esox lucius*	2.39
Perciformes		
bluegill	*Lepomis macrochirus*	2.13
green sunfish	*Lepomis cyanellus*	1.45
smallmouth bass	*Micropterus dolomieu*	1.42
Salmoniformes		
brook trout	*Salvelinus fontinalis*	1.38
brown trout	*Salmo trutta*	1.45
cutthroat trout	*Oncorhynchus clarkii*	2.30
Dolly Varden	*Salvelinus malma*	1.61
mountain whitefish	*Prosopium williamsoni*	7.39
rainbow trout	*Oncorhynchus mykiss*	2.44

Calculation of Site-Specific Enrichment Factor (EF) Values

The single 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). Uncertainty in translating a fish tissue concentration of selenium to a water column concentration using Equation 18 is minimized when site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity are used to accurately characterize *EF*. Thus the EPA only used aquatic sites with sufficient data to calculate a reliable *EF* value.

The EPA searched its database of selenium measurements to identify measurements from aquatic sites with sufficient particulate and water column data to calculate a reliable site-specific

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EF value. The EPA identified all the 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, the 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, the 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), the 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 ($r = 0.42$) compared to algae ($r = 0.65$; 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, the analysis was limited 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 69 individual aquatic sites.

4.2.3 Food-Web Models

For the aquatic sites with a calculated *EF* value, the EPA modeled the food webs as shown in Figure 9 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, the EPA used that site-specific information in the food web models. Most studies, however, did not provide site-specific food web information. In those cases, the food web 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 the food web models were developed, the EPA identified the appropriate species-specific *TTF* values for each model and calculated $TTF^{composite}$. Although *TTF* values were

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derived for several different taxa of invertebrates and fish (Tables 9 and 10), some of the food web models included one or more taxa for which no *TTF* value was available. The EPA assigned a *TTF* value to these taxa by sequentially considering 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, the EPA used the median *TTF* from the matching species.

For example, one study reported selenium concentrations in *Rhinichthys atratulus* (blacknose dace). Although the *TTF* value of *Rhinichthys atratulus* was not available, this species is in the family Cyprinidae, which also includes *Cyprinus carpio* (common carp) and *Gila robusta* (roundtail chub). Because Cyprinidae is the lowest taxonomic classification where the fish species being considered matches a species with an available *TTF* value, the EPA used the median of the common carp and roundtail chub *TTF* values for the *TTF* value of blacknose dace. In another example, a study reported selenium concentrations in *Sander vitreus*, walleye. Although the *TTF* value for *Sander vitreus* was not available, it is in the order Perciformes, which is common to *Pomoxis nigromaculatus* (black crappie), *Lepomis macrochirus* (bluegill), *Lepomis cyanellus* (green sunfish), and *Micropterus dolomieu* (smallmouth bass). Thus the EPA used the median *TTF* values of those four fish species for walleye. Substitution methods using other approaches such as feeding behavior yielded generally similar results.

For each food web model, a *CF* value was also identified for the targeted fish species using the list of available values in Table 11. Although the EPA had a relatively large amount of data to calculate a diverse set of species-specific *CF* values, some food web models included taxa for which no *CF* value was listed in Table 11. A *CF* value was assigned to these taxa using the same procedure that was used to assign *TTF* values by sequentially considering higher taxonomic classifications until one or more taxa for which a *CF* value was available matched the taxon of the targeted fish species.

4.2.4 Classifying Categories of Aquatic Systems.

Transformation reactions that convert dissolved selenium to particulate forms are the primary route of entry into aquatic system food webs and a critical step in selenium bioaccumulation and toxicity (Chapman et al. 2010). However, site-specific characteristics can result in substantial bioaccumulation variability and consequently different risks of selenium toxicity for a given concentration of dissolved selenium. One such site-specific characteristic is

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water residence time. Aquatic organisms living in waters with long residence times such as lakes, ponds, reservoirs, wetlands or estuaries tend to bioaccumulate more selenium than those living in waters with shorter residence times such as rivers and streams (ATSDR 2003; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005; EPRI 2006). Because water residence time is an almost universally described feature of aquatic systems, the EPA used this characteristic to evaluate potential categories of aquatic systems where a single water-column concentration value would be adequately protective.

The EPA evaluated potential categories of aquatic systems by analyzing the relationship between residence time and *EF*. From the studies contributing data to the EPA's database of available selenium measurements, the EPA identified the term used by the study authors to describe the waterbody with respect to residence time. Of the 69 aquatic systems with a calculated *EF* value, the study authors described them as either lakes (n = 7), reservoirs (n = 3), ponds (n = 16), rivers (n = 6), creeks (n = 27), drains (n = 3), marshes (n = 3), washes (n = 2), or streams (n = 2). Categories that did not have a sufficient number of values to perform a meaningful analysis were combined into single categories on the basis of residence time. Thus the EPA grouped aquatic sites into four categories: 1) lakes and reservoirs; 2) ponds and marshes; 3) rivers; and 4) streams, drains, washes and creeks. Figure 9 summarizes the distribution of *EF* values for these 69 aquatic sites when grouped into these 4 categories.

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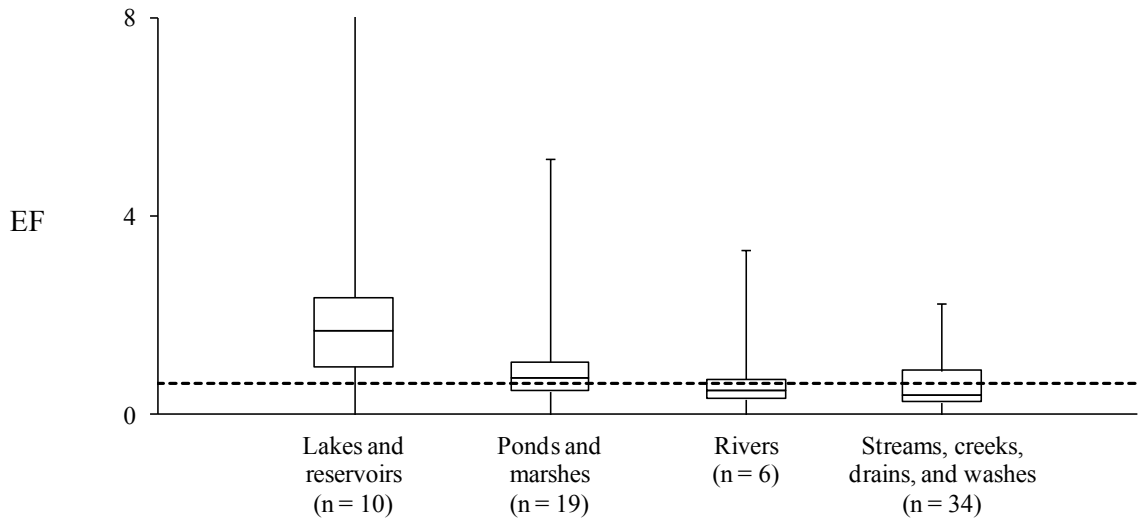

Figure 9. Distribution of EF values for 69 aquatic sites derived from published studies and grouped into 4 categories.
Boxes indicate first quartile, third quartile, and median; whiskers indicate minimum and maximum values (maximum value of the lakes and reservoirs category was allowed to exceed the y-axis to better compare medians, means, and quartiles). Dashed line shows the grand median of all data.

A Kolmogorov-Smirnov goodness of fit test indicated that the distribution of *EF* values within each category was not normally distributed (lakes and reservoirs: $P < 0.00001$; ponds and marshes: $P < 0.00001$; rivers: $P < 0.002$; streams, creeks, drains, and washes: $P < 10^{-8}$). Thus the EPA used nonparametric statistics to evaluate the central tendency of each group and test differences between them. A Kruskal-Wallis test indicated a significant difference among the medians of the 4 aquatic system categories ($P < 0.0005$). However, a multiple comparison of mean ranks using Scheffe's S procedure indicated a significant difference only between the mean ranks of the lakes and reservoirs category and the streams, creeks, drains, and washes category. There were no significant differences in mean ranks among the other categories. The EPA concludes from these data and analyses that currently available information does not adequately differentiate among these 4 categories of aquatic systems with respect to selenium bioaccumulation, and thus does not support the establishment of individual water concentration values for all four categories of water body.

The grand median of all *EF* values from all categories was calculated and compared to the median *EF* value of each category. The dashed line in Figure 11 shows the grand median *EF*

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value (0.62 L/g) for all 69 aquatic sites. All categories with a median greater than the grand median are lentic aquatic systems, and all categories with a median less than the grand median are lotic aquatic systems. The EPA evaluated the potential for differentiating aquatic system categories on the basis of whether they are lentic or lotic by grouping *EF* values from lakes, reservoirs, ponds, and marshes into the category lentic aquatic systems; and rivers, streams, creeks, drains, and washes into the category lotic aquatic systems. Figure 10 summarizes the *EF* values when grouped using these two categories.

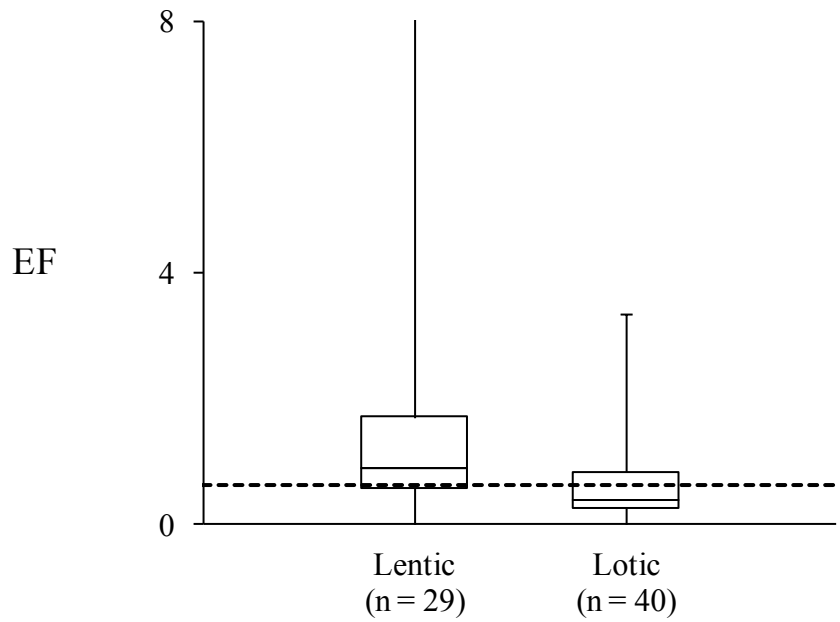

Figure 10. Distribution of EF values for the same 69 aquatic systems as shown in Figure 9 grouped into 2 categories (lentic and lotic).
Boxes depict first quartile, third quartile, and median; whiskers depict minimum and maximum values (maximum value of lentic aquatic systems was allowed to exceed the y-axis to better compare medians, means, and quartiles).

A Kolmogorov-Smirnov goodness of fit test indicates that the distribution of *EF* values in the lentic ($P < 10^{-8}$) and lotic ($P < 10^{-10}$) categories are not normally distributed. Thus the EPA again used nonparametric statistics to characterize the central tendency of each group and to test differences between them. A Mann-Whitney U test indicates *EF* values the from lentic and lotic categories are significantly different from each other ($P < 0.001$). The EPA concludes from these

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data and analyses that selenium bioaccumulation tends to be greater in lentic systems compared to lotic systems. This result is also consistent with other studies (Hamilton and Palace 2001; Brix et al. 2005; Orr et al. 2006). The EPA further concludes from this analysis that currently available data and information support the establishment of separate water quality concentration values for lentic and lotic systems.

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

To derive ambient water quality concentration values appropriate for lentic and lotic aquatic systems, the EPA translated the egg-ovary criterion element to a set of water concentration values on the basis of the *EF* values calculated for 69 aquatic sites and food web models of the fish present in the aquatic systems. Because more than one fish species were often present, many sites provided more than one translated water column concentration (one for each species at each site). Thus the egg-ovary criterion element was translated into a total of 132 water column concentration values (50 values from 29 lentic aquatic systems and 82 values from 40 lotic aquatic systems).

The EPA used the distribution 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 12 shows the model parameters used to translate the egg-ovary criterion element to site-specific water concentrations and Figure 11 shows distribution of the translated values.

Table 12. Site-Specific Data for the 132 Species-Site Combinations and Translation of the Egg-Ovary Criterion Concentraiton Element to a Water Column Concentration.[a]

Identification				Model Parameters			Translated chronic water criterion concentration element
Ref[a]	**Site[a]**	**Species[a]**	**Type**	**EF[b]**	**CF[c]**	**TTF[composite-d]**	**C_{water}[e]**
Bi	22	FM	lentic	2.37	2.00	2.35	1.37
Bi	27	FM	lentic	0.87	2.00	2.35	3.71
Bi	23	FM	lentic	1.21	2.00	2.35	2.68
Bi	20	ID	lentic	2.31	1.45	2.56	1.77
Bi	7	ID	lentic	0.88	1.45	2.56	4.67
Bi	22	ID	lentic	2.37	1.45	2.56	1.72
Bi	23	ID	lentic	1.21	1.45	2.56	3.38
Bi	30	NPK	lentic	1.70	1.71	1.98	2.63

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Identification				Model Parameters			Translated chronic water criterion concentration element
Ref[a]	Site[a]	Species[a]	Type	EF[b]	CF[c]	TTF[composite-d]	C_{water}[e]
Bi	3	NPK	lentic	0.58	1.71	1.98	7.76
Bi	27	NPK	lentic	0.87	1.71	1.98	5.13
Bi	23	NPK	lentic	1.21	1.71	1.98	3.71
Bu91	4	BhS	lotic	0.63	1.82	1.21	10.94
Bu91	4	BnT	lotic	0.63	1.45	2.42	6.91
Bu91	4	FS	lotic	0.63	1.41	1.47	11.68
Bu91	4	MS	lotic	0.63	1.71	2.21	6.36
Bu91	4	RT	lotic	0.63	2.44	2.04	4.85
Bu91	4	WS	lotic	0.63	1.41	1.53	11.23
Bu93	SP2	BhS	lotic	0.18	1.82	1.21	38.53
Bu93	N2	BT	lentic	1.26	1.45	2.42	3.45
Bu93	SP2	BT	lotic	0.18	1.45	2.42	24.32
Bu93	N2	BB	lentic	1.26	1.71	1.19	4.92
Bu93	N2	ChC	lentic	1.26	1.71	1.19	5.91
Bu93	N2	CC	lentic	1.26	1.92	1.63	3.86
Bu93	SP2	FM	lotic	0.18	2.00	2.35	18.12
Bu93	SP2	SD	lotic	0.18	2.00	2.23	19.10
Bu93	SP2	WS	lotic	0.18	1.41	1.53	39.54
Bu95	ME2	BhS	lotic	0.37	1.82	1.21	18.75
Bu95	ME3	BhS	lotic	0.10	1.82	1.21	72.21
Bu95	NW	BhS	lotic	0.20	1.82	1.21	35.20
Bu95	SJ1	BhS	lotic	0.26	1.82	1.21	26.30
Bu95	SJ3	BhS	lotic	0.29	1.82	1.21	23.84
Bu95	ME3	BB	lotic	0.10	1.71	1.43	64.94
Bu95	SJ1	ChC	lotic	0.26	1.71	1.19	28.43
Bu95	SJ3	ChC	lotic	0.29	1.71	1.19	25.77
Bu95	ME4	CC	lotic	0.12	1.92	1.63	40.56
Bu95	ME3	CC	lotic	0.10	1.92	1.63	50.95
Bu95	SJ1	CC	lotic	0.26	1.92	1.63	18.56
Bu95	SJ3	CC	lotic	0.29	1.92	1.63	16.82
Bu95	HD2	FM	lotic	0.15	2.00	2.35	21.73
Bu95	ME1	FM	lotic	0.90	2.00	2.35	3.60
Bu95	ME2	FM	lotic	0.37	2.00	2.35	8.82
Bu95	ME4	FM	lotic	0.12	2.00	2.35	27.03
Bu95	ME3	FM	lotic	0.10	2.00	2.35	33.96
Bu95	WC	FM	lotic	0.40	2.00	2.35	8.01
Bu95	SJ1	FS	lotic	0.26	1.41	1.47	28.09
Bu95	HD2	FS	lotic	0.15	1.41	1.47	49.34
Bu95	ME2	FS	lotic	0.37	1.41	1.47	20.03

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Identification				Model Parameters			Translated chronic water criterion concentration element
Ref[a]	Site[a]	Species[a]	Type	EF[b]	CF[c]	TTF[composite-d]	C_{water}[e]
Bu95	ME4	FS	lotic	0.12	1.41	1.47	61.38
Bu95	ME3	FS	lotic	0.10	1.41	1.47	77.10
Bu95	SJ3	FS	lotic	0.29	1.41	1.47	25.46
Bu95	ME3	GnS	lotic	0.10	1.45	2.11	51.93
Bu95	ME4	RSh	lotic	0.12	2.00	2.16	29.38
Bu95	ME3	RSh	lotic	0.10	2.00	2.16	36.90
Bu95	SJ1	RSh	lotic	0.26	2.00	2.16	13.44
Bu95	ME1	SD	lotic	0.90	2.00	2.23	3.80
Bu95	ME2	SD	lotic	0.37	2.00	2.23	9.30
Bu95	ME3	SD	lotic	0.10	2.00	2.23	35.79
Bu95	NW	SD	lotic	0.20	2.00	2.23	17.45
Bu95	SJ1	SD	lotic	0.26	2.00	2.23	13.04
Bu95	HD2	Su	lotic	0.15	1.41	1.27	57.17
Bu97	MUD2	BhS	lotic	0.07	1.82	1.21	98.08
Bu97	MNP2	FM	lentic	2.00	2.00	2.35	1.62
Bu97	MUD2	FM	lotic	0.07	2.00	2.35	46.12
Bu97	WCP	FM	lentic	0.90	2.00	2.35	3.58
Bu97	CH1	GnS	lotic	0.20	1.45	2.11	25.39
Bu97	MUD2	GnS	lotic	0.07	1.45	2.11	70.54
Bu97	MNP3	SB	lentic	5.15	1.42	2.61	0.80
Ca	DC	RT	lotic	2.24	2.44	2.04	1.37
Ca	LC	RT	lotic	0.33	2.44	2.04	9.39
Fo	CC-1A	BnT	lotic	0.80	1.45	2.54	5.18
Fo	CC-3A	BnT	lotic	0.81	1.45	2.56	5.09
Fo	CC-150	BnT	lotic	1.04	1.45	2.52	4.00
Fo	CC-350	BnT	lotic	1.16	1.45	2.46	3.67
Fo	CC-75	BnT	lotic	1.19	1.45	2.54	3.48
Fo	DC	BnT	lotic	1.55	1.45	2.49	2.72
Fo	HS	BnT	lotic	0.24	1.45	3.60	11.96
Fo	HS-3	BnT	lotic	0.54	1.45	2.31	8.48
Fo	LSV-2C	BnT	lotic	0.45	1.45	2.57	9.16
Fo	LSV-4	BnT	lotic	0.69	1.45	2.56	5.92
Fo	SFTC	BnT	lotic	1.32	1.45	2.55	3.11
Fo	CC-1A	Sc	lotic	0.80	1.71	2.28	4.87
Fo	CC-3A	Sc	lotic	0.81	1.71	2.29	4.79
Fo	CC-150	Sc	lotic	1.04	1.71	2.26	3.77
Fo	CC-350	Sc	lotic	1.16	1.71	2.20	3.46
Fo	CC-75	Sc	lotic	1.19	1.71	2.28	3.28
Fo	DC	Sc	lotic	1.55	1.71	2.23	2.56

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Identification				Model Parameters			Translated chronic water criterion concentration element
Ref[a]	Site[a]	Species[a]	Type	EF[b]	CF[c]	TTF[composite-d]	C_{water}[e]
Fo	HS	Sc	lotic	0.24	1.71	3.22	11.25
Fo	HS-3	Sc	lotic	0.54	1.71	2.07	7.98
Fo	LSV-2C	Sc	lotic	0.45	1.71	2.30	8.62
Fo	LSV-4	Sc	lotic	0.69	1.71	2.29	5.57
Fo	SFTC	Sc	lotic	1.32	1.71	2.29	2.93
Gr	17	FM	lentic	0.86	2.00	2.35	3.75
Gr	17	WS	lentic	0.86	1.41	1.53	8.18
HB	LEMC	CT	lotic	1.32	2.30	1.78	2.83
Le	BA	BB	lentic	8.54	1.71	1.66	0.62
Le	BE	BB	lentic	2.09	1.71	1.66	2.55
Le	HR	BB	lentic	3.81	1.71	1.66	1.40
Le	BA	CC	lentic	8.54	1.92	1.63	0.57
Le	BE	CC	lentic	2.09	1.92	1.63	2.33
Le	HR	CC	lentic	3.81	1.92	1.63	1.28
Le	BA	FM	lentic	8.54	2.00	2.35	0.38
Le	BE	FM	lentic	2.09	2.00	2.35	1.55
Le	HR	FM	lentic	3.81	2.00	2.35	0.85
Le	BA	GnS	lentic	8.54	1.45	2.11	0.58
Le	BE	GnS	lentic	2.09	1.45	2.11	2.38
Le	HR	GnS	lentic	3.81	1.45	2.11	1.30
Le	BA	WM	lentic	8.54	1.71	1.57	0.66
Le	BE	WM	lentic	2.09	1.71	1.57	2.70
Le	HR	WM	lentic	3.81	1.71	1.57	1.48
Le	BA	RSh	lentic	8.54	2.00	2.16	0.41
Le	BE	RSh	lentic	2.09	2.00	2.16	1.69
Le	HR	RSh	lentic	3.81	2.00	2.16	0.92
Sa87	KP11	WM	lentic	0.51	1.71	2.03	8.62
Sa87	KP2	WM	lentic	0.32	1.71	2.03	13.79
Sa87	KP8	WM	lentic	0.60	1.71	2.03	7.24
Sa87	SLD	WM	lentic	0.36	1.71	2.03	12.13
Sa87	VP26	WM	lentic	0.93	1.71	2.03	4.66
Sa87	VW	WM	lotic	1.03	1.71	2.03	4.24
Sa93	GT4	BgS	lentic	0.43	2.13	2.11	7.90
Sa93	GT5	BgS	lentic	1.37	2.13	2.11	2.47
Sa93	SJR2	BgS	lotic	0.36	2.13	2.11	9.44
Sa93	SJR3	BgS	lotic	0.75	2.13	2.11	4.52
Sa93	GT4	LMB	lentic	0.43	1.42	1.86	13.47

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Identification				Model Parameters			Translated chronic water criterion concentration element
Ref[a]	**Site[a]**	**Species[a]**	**Type**	**EF[b]**	**CF[c]**	**TTF[composite-d]**	**C_{water}[e]**
Sa93	GT5	LMB	lentic	1.37	1.42	1.86	4.21
Sa93	SJR2	LMB	lotic	0.36	2.13	1.86	16.11
Sa93	SJR3	LMB	lotic	0.75	1.42	1.86	7.71
Sa93	GT4	WM	lentic	0.43	1.71	1.93	10.78
Sa93	GT5	WM	lentic	1.37	1.71	1.93	3.37
Sa93	SJR2	WM	lotic	0.36	1.71	1.93	12.89
Sa93	SJR3	WM	lotic	0.75	1.71	1.93	6.17
St	M4720	BB	lentic	0.10	1.71	1.66	55.63
St	M4720	CC	lentic	0.10	1.92	1.63	50.76

a- See Appendix L for description of abbreviations.
b- Geometric mean of the median enrichments functions (EF) for all available food types (algae, detritus, and sediment). EF (L/g) = C_{food}/C_{water}.
c- Taxa-specific conversion whole-body to egg ovary conversion factor (CF; dimensionless ratio).
d- Composite trophic transfer factor ($TTF^{composite}$). Product of TTF valuess for all trophic levels.
e- Translated water concentration corresponding to an egg-ovary criterion element of 15.2 mg Se/kg dw, calculated by Equation 18.

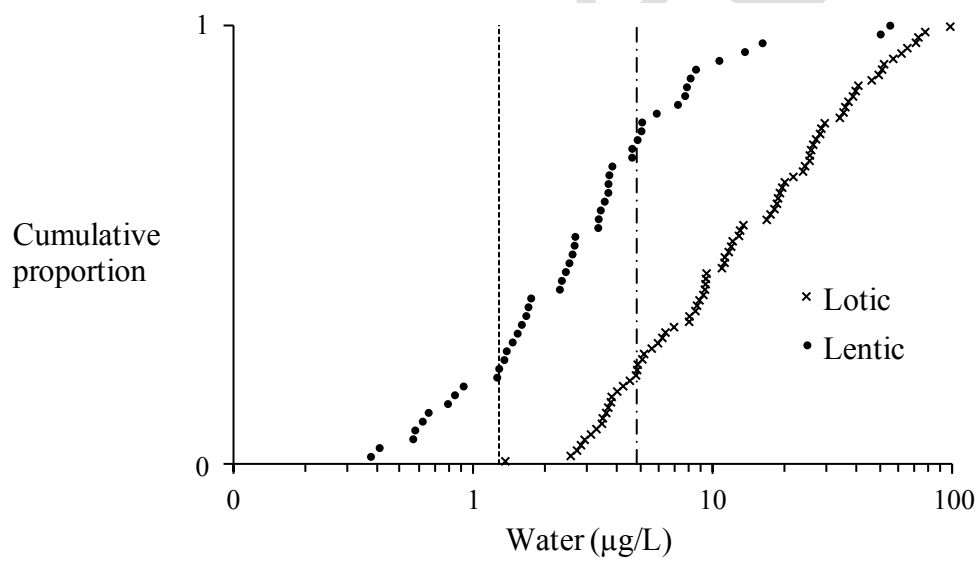

Figure 11. Probability distribution of the water-column concentrations translated from the egg-ovary criterion at lentic and lotic aquatic sites.
Dashed and dash-dot lines show the 20th percentiles of the lentic and lotic distributions, respectively.

Table 13 summarizes the distributions of translated water concentration values. As discussed in the Introduction and Background, the bioaccumulation potential of selenium

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depends on many different biogeochemical factors that characterize a particular aquatic system. Uncertainty in the translation of the egg-ovary criterion concentration element to a water column concentration element can be reduced by using site-specific data and information such as an *EF* value calculated from site-specific measurements and a food-web model derived from a biological assessment of the aquatic system. The basis for national water column criterion element values, however, is constrained by the need to apply a single value to a large number of aquatic systems, the available data used to derive the values, and the need to implement a variety of simplifying assumptions which introduces uncertainty. Although the EPA utilized medians as the statistical method of choice to characterize multiple measurements (except when estimating the selenium concentration in particulate material from algae, detritus, and sediment for the reasons described above), the EPA selected the 20th percentile of the translated water column values of each category (lotic and lentic) as the final water column criterion concentration element. The EPA selected the 20th percentile to ensure adequate protection of the aquatic species. The EPA examined the the performance of the 20th percentile water column values by evaluating the protectiveness of the water column criterion element using binary classification statistics. In this analysis EPA used an independent data set composed of measured concentrations of selenium from 2,588 lotic and 596 lentic sites to complete a verification or "ground-truthing" of the selected 20th percentile water column values to evaluate their protectiveness, and found that the use of the 20th percentile water column values would prevent potential exceedances of the fish tissue egg-ovary criterion element over 90% of the time, i.e., false negative conclusions regarding fish tissue exceedances would be minimized using the selected 20th percentile water column value for the water column criterion element derivation. However, states and tribes may choose to adopt a lower percentile value if there is reason to believe that selenium bioaccumulation is greater and/or more variable in their waters or other site-specific considerations. For this reason, the 10th and 5th percentile of the distribution of translated water column concentrations are also presented in Table 13. States and tribes may also chose to translate the egg-ovary criterion element to a water column concentration on a site-specific basis.

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Table 13. Summary of water column criterion element concentration values translated from the egg-ovary criterion element.
The 20th percentile values are the water column criterion element concentration values for the national selenium criterion. All units µg/L. This analysis is based on 50 lentic sites and 82 lotic sites.

	Lentic	Lotic
Median	2.7	12.0
20th percentile (final water-column criteria)	1.3	4.8
10th percentile	0.7	3.5
5th percentile	0.6	2.9

The distributions of translated chronic water column concentrations to which the 20th percentile applies were derived using the medians of site-specific measurements. However, a site would not attain its chronic water criterion element if only its median concentration attained; rather its high-end concentrations would need to attain. Consequently, it cannot be inferred that as many as 20% of sites that are at their tissue concentration would attain their chronic water criterion element. Rather, the protectiveness of the water criteria are further described in Section 7.2.2.

4.2.6 Derivation of Averaging Period for Chronic Water Criterion Element

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 waterborne direct toxicity of metals, 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.

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 two trophic levels sequentially, invertebrates and fish, the characteristic time is likewise the time needed for c/c_o reach a value of 1/e.

For the first trophic level, the kinetics for algal bioaccumulation and depuration were assumed to be rapid compared to the larger organisms as 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 in elsewhere in the document. A value of 0.1/day appears to be environmentally conservative, considerably

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higher than those for *Lumbriculus*, Asian clam, zebra mussel, but a bit lower than that for 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 G, 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 G. That analysis demonstrated that a 30-day averaging period for the chronic water criterion affords protection under all conditions, and is therefore the duration recommended for the chronic water column criteria.

4.3 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. Consequently, the available database of acute toxicity LC_{50}s for selenite and selenate are not useful for criteria purposes. As demonstrated in Appendix G, the kinetics of selenium accumulation and depuration are sufficiently slow that attainment of the water criterion concentration element by ambient 30-day averages will protect sensitive aquatic life species even where concentrations exhibit a high degree of variability.

To address intermittent exposures that could contribute to chronic effects of selenium due to its bioaccumulative nature, EPA is providing an intermittent exposure water criterion concentration element intended to limit cumulative exposure to selenium, which is derived from

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the chronic 30-day water criterion. To illustrate the concept, Figure 12 shows a possible sequence of exposures over a 30-day period.

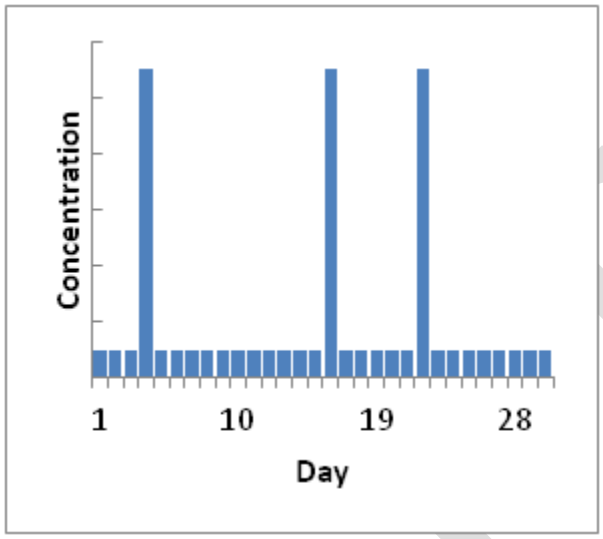

Figure 12. Illustration of intermittent spike exposure occurring for a certain percentage of time (e.g., 10%) over a 30-day period, and background exposure occurring for the remaining percentage of time (e.g., 90%).

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

$$C_{30\,day} = C_{int} f_{int} + C_{bkgrnd}(1 - f_{int})$$

where C_{int} is the intermittent spike concentration, f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, and C_{bkgrnd} is the background concentration occurring during the remaining time. $C_{30\,day}$ is not to exceed the chronic criterion, $WQC_{30\,day}$. If the intent is to apply a criterion element, WQC_{int} to the intermittent spike concentrations, then replacing C_{int} with WQC_{int} and $C_{30\,day}$ with $WQC_{30\,day}$ in the above equation, and then solving for WQC_{int} yields:

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

The above 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-

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case assumption inherent in this approach is that selenium bioaccumulation is linear over a very wide range of concentrations: that is, EFs and TTFs do not decrease significantly as concentrations increase.

If the heights of three spikes in Figure 14 were to differ somewhat between each other, then the intermittent criterion would apply to the arithmetic mean of the three. If the background concentrations were to vary somewhat, then the arithmetic mean background would be input to the equation. Nevertheless, the above approach is not intended for application to ordinary smoothly varying concentrations. That situation is better addressed simply by applying the chronic water criterion as a 30-day average for a lentic or lotic system, as appropriate.

Table 14 illustrates example values for the intermittent water criterion concentration element. The bottom row of the lotic and lentic values and the right column are to emphasize that WQC_{int} is not an independent criterion element but a re-expression of the 30-day average water criterion concentration element. WQC_{int} converges to $WQC_{30\ day}$ when the background concentration is already at $WQC_{30\ day}$ or when the intermittent exposure is said to occur throughout the 30-day period.

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Table 14. Representative Values of the Intermittent Water Criterion Concentration Element.

Bkgrnd Conc, C_{bkgrnd} (µg/L)		Fraction of Time, f_{int} in a 30-day period				
	0.033 (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, WQC_{int} (µg/L)					
0	145.5	96	48	24	9.6	4.8
1	116.2	77	39	20	8.6	4.8
2	86.8	58	30	16	7.6	4.8
3	57.5	39	21	12	6.6	4.8
4.8	4.8	4.8	4.8	4.8	4.8	4.8
	Lentic Intermittent Criterion, WQC_{int} (µg/L)					
0	39.4	26	13	6.5	2.6	1.3
0.5	24.7	16.5	8.5	4.5	2.1	1.3
0.7	18.9	12.7	6.7	3.7	1.9	1.3
1	10.1	7.0	4.0	2.5	1.6	1.3
1.3	1.3	1.3	1.3	1.3	1.3	1.3

If the value of f_{int}, the intermittent exposure fraction of the month, is assigned a value less than 1 day, then 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 1 day might not be much greater than those observed to be toxic in 2-4 day exposures, the intermittent fraction of the month should *not* be assigned a value less than 0.033, corresponding to 1 day.

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5 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:

1. The concentration of selenium in the eggs or ovaries of fish does not exceed 15.2 mg/kg, dry weight; [1]
2. The concentration of selenium (a) in whole-body of fish does not exceed 8.1 mg/kg dry weight, or (b) in muscle tissue of fish (skinless, boneless fillet) does not exceed 11.8 mg/kg dry weight; [2]
3. The 30-day average concentration of selenium in water does not exceed 4.8 µg/L in lotic (flowing) waters and 1.3 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]

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Table 15. 2014 External Peer Review Draft Freshwater Selenium Ambient Water Quality Chronic Criterion for Aquatic Life.

Media Type	Fish Tissue		Water Column[3]	
Criterion Element	**Egg/Ovary[1]**	**Fish Whole Body or Muscle[2]**	**Monthly Average Exposure**	**Intermittent Exposure[4]**
Magnitude	15.2 mg/kg	8.1 mg/kg whole body or 11.8 mg/kg muscle (skinless, boneless filet)	1.3 µg/L in lentic aquatic systems 4.8 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[5]	Instantaneous measurement[5]	30 days	Number of days/month with an elevated concentration
Frequency	Never to be exceeded	Never to be exceeded	Not more than once in three years on average	Not more than once in three years on average

[1] Overrides any whole-body, muscle, or water column elements when fish egg/ovary concentrations are measured.
[2] Overrides any water column element when both fish tissue and water concentrations are measured.
[3] Water column values are based on dissolved total selenium in water.
[4] Where $WQC_{30\text{-day}}$ is the water column monthly element, for either a lentic or lotic system, as appropriate. C_{bkgrnd} is the average background selenium concentration, and f_{int} is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
[5] Instantaneous measurement. Fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish at a given site. Selenium concentrations in fish tissue are expected to change only gradually over time in response to environmental fluctuations.

EPA recommends that states and tribes adopt into their water quality standards, is a selenium criterion that includes all four elements, and expressing 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 elements over the water column element, and the egg-ovary element over

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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 intothe selenium criterion ensures protection when neither fish egg-ovary nor fish whole-body or muscle tissue measurements are available.

To assure that the contribution of short-term exposures to the bioaccumulation risks is accounted for in all situations, EPA is also recommending that the selenium intermittent exposure element be included in the selenium criterion, as noted above. However, 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. If there are rare instances where selenium sources could cause acute effects without also exceeding the selenium chronic criterion outlined above, a pollution control authority could establish a site-specific criterion to protect from those effects.

EPA recommends that when states implement the water quality criterion for selenium under the NPDES permits program, states should establish additional procedures due to the unique components of the selenium criterion expressions. Where states adopt the selenium water column concentration criterion element values only 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 would be appropriate. However, if states also decide to adopt 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 attainment of the fish tissue criterion element(s).

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

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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 in order to insure that water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

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6 Site-specific Criteria

All four elements of the selenium criterion can 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 modification of that element can be done by applying the Recalculation Procedure (U.S. EPA 2013a) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while recognizing tested surrogates for untested resident species.

However, species in the national data set that are not present at a site should not be deleted from the data set if the species serves as a surrogate for other species known or expected to be present at a site. Confidence in the applied tissue criterion element can be improved by further toxicity 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 water concentration can be performed in a manner that accounts for site-specific conditions. Appendix I provides a step-wise process for deriving each parameter used in Equation 18 to perform a site-specific translation. These steps include:

1. selection of a target fish species,
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 I also provides information on how to obtain the site-specific information for each step in the process. Other scientifically defensible translations, including traditional Bioaccumulation Factors (BAFs), may also be appropriate.

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Where sensitive aquatic-dependent (e.g., bird) species are known to exist, states should consider developing site-specific criteria based on data on such species.

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7 Effects Characterization

7.1 Fish

7.1.1 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}. When data did not allow calculation of ECs but did allow calculation of closely spaced NOECs and LOECs, then the NOEC was used to approximate the EC_{10}. No NOEC values were used in calculating the numeric value of the criterion.

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 4.1.1 and 7.1.3 (reproductive effects) and in Section 7.1.8 (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 criteria values.

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 criteria values.

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

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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.

7.1.2 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 below levels observed in other studies in Section 4.1.2 and Appendix C. These data were not included in derivation of the criteria because the injection route of exposure is not an acceptable experimental protocol for studies used in criteria 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

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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 16. 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 16. 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. Observations of selenium contaminated Belews Lake accord with the above; Young et al. (2010) indicate that out of as many as 29 resident species documented prior to contamination, only

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common carp, catfish, and fathead minnows remained after contamination. Based on field observations, catfish and bullheads (Ictaluridae) thus appear to be less vulnerable than the fish taxa most at risk (e.g., Centrarchidae, Salmonidae) in selenium-contaminated water bodies, contrary to what might be suggested by the Doroshov et al. (1992) injection study.

7.1.3 Reproductive Studies Not Used in the Numeric Criterion Derivation

Pimephales promelas (fathead minnow)

GEI Associates (2008) collected fathead minnows from three sites in Colorado of moderate to high selenium exposure and transported them to the laboratory for spawning and subsequent assessment of embryo and larval development. Egg production, fertilization success, embryo mortality and larval deformities from the offspring of the wild caught fish were compared to offspring spawned from fathead minnows obtained from a commercial fish supplier. Mean selenium concentrations in field-collected adult females ranged from 9.17 to 44.53 mg/kg dw whole body; the mean selenium concentration in control females from the commercial supplier was 2.86 mg/kg dw whole body. The response measurements for the embryo assessment endpoints were variable and lacked a relationship with selenium exposure. Consequently, the results of this study could not support a reliable estimate of an effect concentration, and chronic values are not given in this document. A detailed summary of the GEI Associates (2008) study is given in Appendix D. These values were not used in the criterion derivation.Salmonidae

Oncorhynchus clarki (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

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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 EC10 available for the related species, *Salvelinus malma* (Dolly Varden, Section 4.1.1), the "greater than" chronic value for brook trout are not used to obtain the GMCV, in accordance with the principles of Section 7.1.1.

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Lepomis machrochirus (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 ug/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 fish 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, 1985b) 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.

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

The lowest GMCV in the reproductive effects dataset is for *Salmo*; the calculated egg-ovary criterion element is more sensitive to this value than to any other. However, several reasonable EC_{10}s can be calculated from the Formation Environmental (2011) data for brown trout. Because of the importance of this data for the numeric criterion calculation, EPA conducted a careful and thorough reanalysis of the study data and subjected its 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 are superseded by this document's reanalysis of a more complete enumeration of the

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deformity counts provided by AECOM (2012), provided in Appendix C. Below is a summary of key issues considered in the EPA reanalysis of the data and EPA's conclusions regarding the appropriate endpoint and effect concentration to use in the criteria derivation.

As described in detail in Appendix C, Formation Environmental (2011) evaluated survival and deformities in the offspring of wild-caught brown trout having a range of exposure levels. The evaluation of offspring had two key phases, hatch to swim-up, and swim-up to 15-days post-swim-up. Based on its reanalysis of the data, EPA concludes that the best-supported EC_{10}s fall into the range, 15.91 – 21.16 mg Se/kg (dw egg). Uncertainties in the EC_{10} appropriate for this species stem from the observed high background deformity rates and by a lab accident causing overflow loss of some organisms from several aquaria during the post-swim-up portion of the test. This accident occurred when aquaria drainpipe filters became clogged with uneaten food.

Deformities for the full test (hatch to 15-days post swim-up), calculated assuming all overflow-missing individuals were deformed, yield the lowest of the above mentioned EC_{10}s, 15.91 mg Se/kg from Figure 13b, but the following factors may be noted:

a) High background deformity rates in unexposed, hatchery-reared fish (points with log concentration <0.2) as shown in Figure 15a and b, increase the uncertainty in the 15.91 mg Se/kg EC_{10} of Figure 13b.
b) The worst-case assumption that all individuals lost in the overflow were deformed depresses the above EC_{10}. An assumption that the lost individuals had the same frequency of deformity as the others yields an EC_{10} of 18.36 mg Se/kg (Figure 13a).
c) In Figure 13b, the fitted line's under-prediction of the fraction empirically observed to be normal at 17.7 and 20.5 mg Se/kg (log ≈ 1.3) suggests that the 15.91 mg Se/kg EC_{10} may be a low estimate. The under-prediction of the fraction normal in that key exposure range is caused by TRAP applying a shallower slope so as to reduce its error in predicting the fraction normal in the exposure range of ≥36 mg Se/kg, a range of less interest for deformities because of the failure of all individuals to reach the swim-up stage at such high exposures. When the combined survival and deformities are considered, and failing to swim-up by the end of the test is equated with failing to survive (while applying the same worst-case assumption that individuals lost in the overflow were dead, dying, or deformed), the EC_{10} rises to 20.65 mg Se/kg, as shown in Figure 15f. Having no partial

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effects at exposures ≥26.8 mg Se/kg, allows a steep slope, which then allows an exact fit to the responses at 17.7 and 20.5 mg Se/kg. It is for this reason that combining observed effects, survival and deformities, has the unexpected result of increasing rather than decreasing the EC_{10}.

d) Survival and deformity EC_{10}s are very close in magnitude. Assuming individuals lost in the overflow were dead or dying, the survival EC_{10} for the full test is 16.79 mg Se/kg (Figure 13d), only 6% higher than the corresponding deformity EC_{10} of 15.91 mg Se/kg.

e) The assumption about organisms lost to the overflow affects the survival EC_{10} as it did the deformity EC_{10}. If the health of missing organisms were assumed to be the same as those remaining, the survival EC_{10} would be 20.40 mg Se/kg (Figure 13c). The peer review conducted by ERG (2012) did not provide a consensus on expectations of whether less healthy organisms were more likely to have been lost in the overflow.

f) For combined survival and deformities, assuming the health of overflow-missing organisms to be the same as those remaining, the EC_{10} is 21.16 mg Se/kg (Figure 13e). The use of the lowest of the above values (15.91 mg Se/kg) for setting the chronic value for brown trout provides a greater margin of protection than would one of the higher values. Were the Salmo GMCV set at the geometric mean of the above six values for the test, 18.77 mg Se/kg, the FCV would be 17.3 mg Se/kg.

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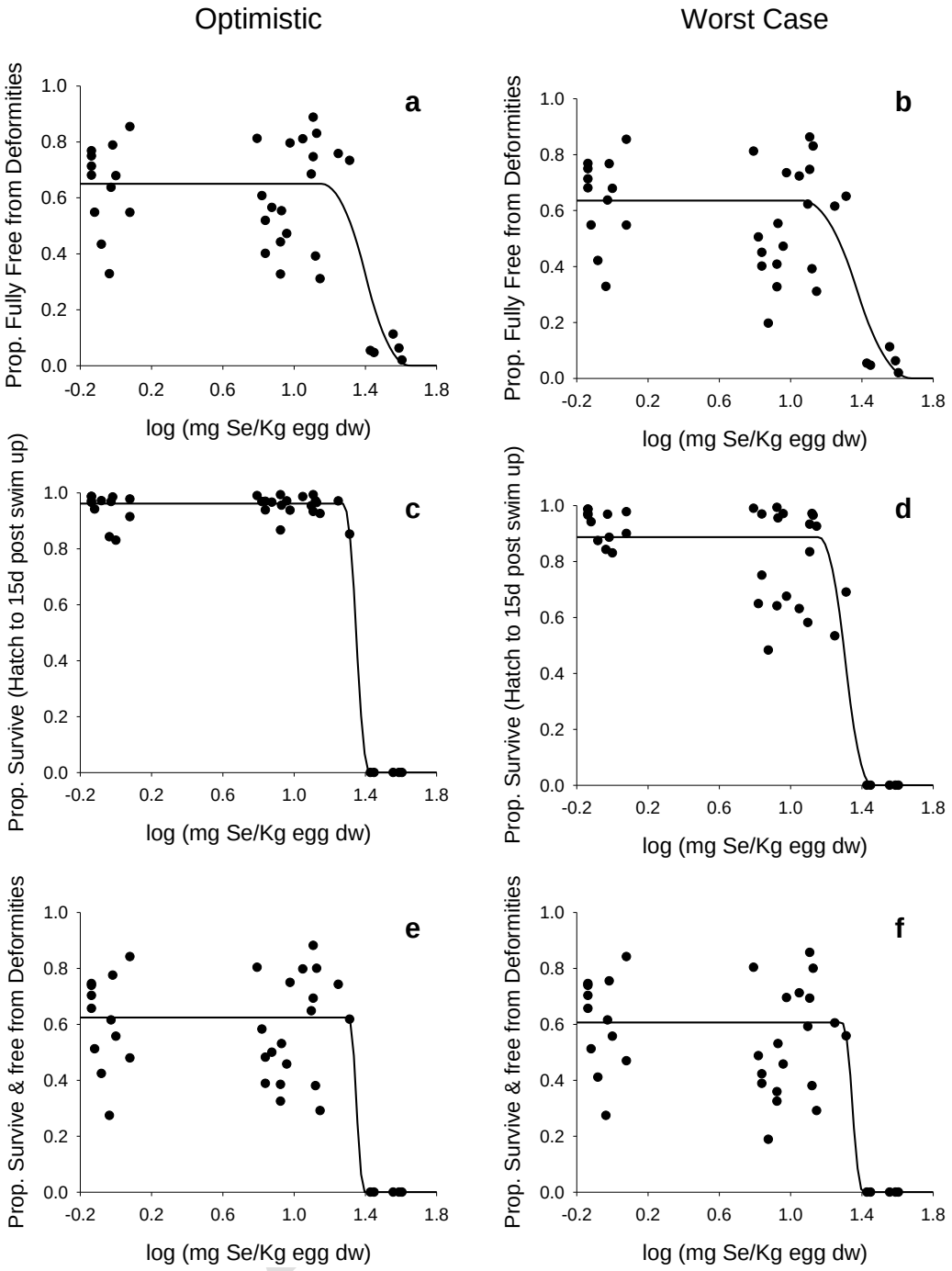

Figure 13. Concentration-response relationships of brown trout deformities (a-b), survival (c-d), and deformities+survival (e-f) in response to selenium concentrations in eggs. Each endpoint was evaluated under optimistic and worst-case scenarios with respect to larval fry lost during the 15-day post swim up test. EC_{10}s (in mg Se/kg egg dw) for each endpoint-scenario combination were as follows: Deformities (18.36 – optimistic (a); 15.91 – worst case (b)); Survival (20.40 – optimistic (c); 16.79 – worst case (d)); Combined (21.16 – optimistic (e); 20.65 – worst case (f)).

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7.1.5 Influence of Curve-fitting on Calculation of *Lepomis* GMCV

The *Lepomis* GMCV, the second lowest in the Species Sensitivity Distribution, has been calculated to be 18.41 mg Se/kg, based on EC_{10}s from three studies with bluegill, 20.05 mg Se/kg from Doroshov et al. (1992), 24.55 mg Se/kg from Coyle et al. (1993), and 12.68 mg Se/kg from Hermanutz et al. (1992, 1996). The 12.68 value is low compared to the other studies with bluegill or any other species. It stems from an environmentally conservative fitting of a sigmoid curve to the data, as illustrated in Figure 14. This was the model fit having lowest error, when measured vertically.

The dashed line of Figure 14 mimics an alternate orthogonal regression approach effectively reduceing combined vertical and horizontal errors. It results in an EC_{10} of 18.40 mg Se/kg. Replacing 12.68 with 18.40 mg Se/kg in calculating the *Lepomis* GMCV would only change the *Lepomis* chronic value from 18.41 to 20.84 mg Se/kg. This would change the FCV from 15.2 to 15.6 mg Se/kg. The recommended FCV of 12.68 is based on the more conservative curve-fitting approach.

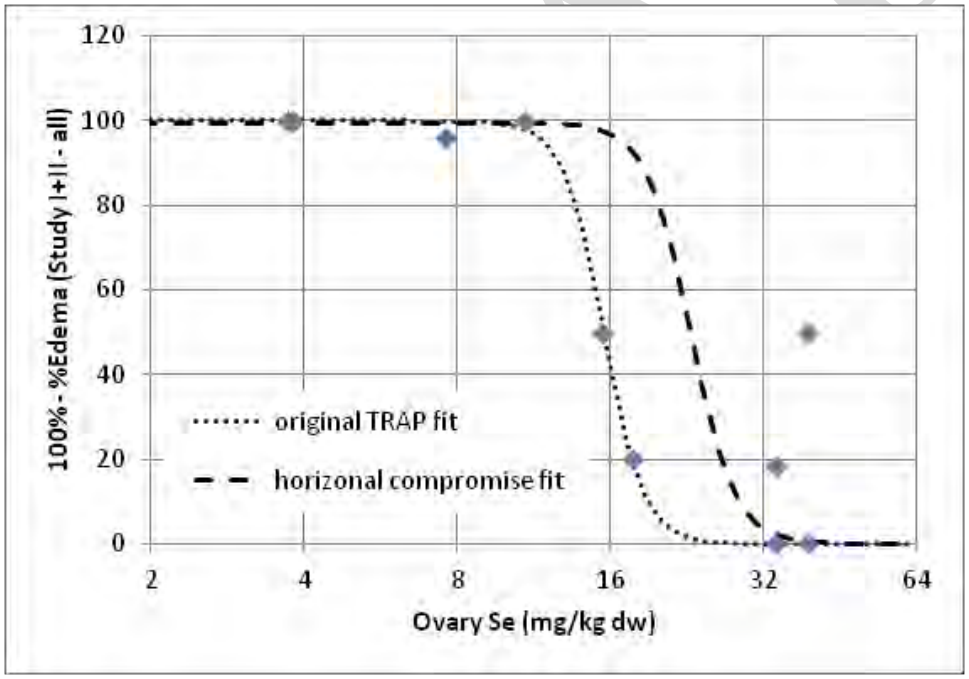

Figure 14. Fitting the Hermanutz et al. (1992, 1996) data to yield (a) the 12.68 mg Se/kg EC_{10} (dotted line) with TRAP measuring error vertically, and (b) a possible alternative 18.40 mg Se/kg EC_{10} (dashed line), reducing horizontal error by running TRAP after combining two points averaging 49.85% normal (absence of edema) into one point at their geometric mean exposure of 24.56 mg Se/kg, and combining the two points averaging 19.3% normal into one at their geometric mean exposure, 24.45 mg Se/kg.

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7.1.6 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 8 minimum data requirements were met, and the other 2 (for planktonic and benthic crustaceans) were waived (see section 3.7.1.2. Of the N=14 genera used for the calculation of the criterion, nine are fish, which in general are more sensitive than invertebrates. Of the nine fish genera, five are either salmonids or centrarchids. Had a broader array of expected insensitive taxa been included, better reflecting the taxonomic composition of real-world sites, then the four most sensitive genera would not likely change, but N would increase.

Nevertheless, the criterion calculation for selenium is not sensitive to the value of N. Setting N=20 would only raise the criterion from 15.2 mg Se/kg to 16.0 mg Se/kg. Setting N=25 would raise the egg- ovary criterion element to 16.7 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.

7.1.7 Conversions between Concentrations in Different Tissues

Chapman et al. (2009, 2010) note that risk characterization may start with selenium concentrations in any environmental compartment, but uncertainty about potential adverse effects is lowest when the concentrations in reproductive tissue are known. Because the egg-ovary (EO) criterion element is derived from studies measuring the reproductive tissue concentrations, it has greater certainty than the muscle (M) and whole-body (WB) criteria element concentrations. As indicated by the information tabulated in Section 4.1.5, to obtain the muscle criterion element concentration, conversion from EO to M involved dividing by the EO/M ratios measured in each species except desert pupfish, where the EO/M ratio was the measured desert pupfish EO/WB divided by the median generic fish M/WB ratio. As indicated in Section 4.1.5, to obtain the whole-body criterion element value, conversion from EO to WB involved dividing by EO/WB ratios measured in each taxa except *Esox*, *Salvelinus*, and *Oncorhynchus*, where the EO/WB ratios were each of their own EO/M ratios times the median generic fish M/WB ratio.

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7.1.8 Studies of Non-Reproductive Effects

This section presents laboratory-based dietary chronic exposure studies involving non-reproductive endpoints. These 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 and muscle effect concentrations cannot be directly compared to egg-ovary effect concentrations*. Non-reproductive effects were ultimately determined to provide a less reliable basis for a criterion, in part because comparatively few of such studies provided sigmoidal concentration-response curves.

Acipenseridae

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.

Cyprinidae

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

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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 criteirion values derived based on reproductive endpoints are protective of the endpoint measured in this non-reproductive study.

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

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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 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

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Se/kg dw as fathead minnow whole-body tissue. The concentration-response relationship, as indicated by the study data presented in Appendix D, 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.

Catostomidae

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

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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 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). Summaries of each of these two studies as well as a third study with razorback suckers (Hamilton et al. 2005d) are presented in Appendix D.

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 D. 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.

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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.

Salmonidae

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.

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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.

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

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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 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.

Moronidae

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

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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.

Centrarchidae

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

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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 4EC 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 a 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

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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. As these two cold-temperature juvenile-survival lab studies are far more similar than they are different, their results were combined per the standard Guidelines procedure to determine the non-reproductive SMCVs for bluegill. These SMCVs were determined separately for two temperature conditions for bluegill, 4°C and 9°C. The SMCV for 4°C is 8.15 mg Se/kg dw whole body, based on three 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), and (c) the McIntyre et al. (2008) ES2 NOEC (>9.992 mg Se/kg dw whole body). This value is not less than the reproductive endpoint-based whole-body criterion concentration of 8.13 mg Se/kg dw. The SMCV for 9°C is 14.00 mg Se/kg dw whole body, based on the McIntyre et al. (2008) ES3

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EC_{10}. The studies of Bryson et al (1985b) and Cleveland et al. (1993) were not conducted at cold temperature and were thus not used for these SMCV calculations.

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Summary of Studies with Non-Reproductive Effects.

Table 17. 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 EC_{20} juvenile growth	15.08 WB 27.76 M 17.82 WB 32.53 M	EC_{10} 15.1 WB 27.8 M EC_{20} 17.8 WB 32.5 M	15.1 WB 27.8 M
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	dietary (lab) 9 months	selenized-yeast	NOEC LOEC MATC juvenile deformities (juvenile exposure only)	10.1 M 15.1 M 12.34 M	10.1 M 15.1 M 12.3 M	10.1 M 15.1 M 12.3 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	51.40 WB

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Species	Reference	Exposure route and duration	Selenium form	Toxicological endpoint	Chronic value, mg/kg dw[a]	SMCV mg/kg dw	GMCV mg/kg dw
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 WB[b]	69.83 M	69.83 M
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 WB[b]	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 WB[b]		
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

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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 EC_{20} for juvenile growth	7.355 WB 10.47 WB	EC_{10} 9.052 WB EC_{20} 12.83 WB	EC_{10} 9.052 WB
		dietary (lab) 60 days	mosquitofish spiked with SLD diet	EC_{10} for juvenile growth EC_{20} for juvenile growth	11.14 WB 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 LOEC MATC	21 Liver 71.7 Liver 38.80 Liver	NOAEC 28.98 L LOAEC 84.68 L MATC 49.52 L	
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab) 20 weeks	sodium selenite in food preparation	juv. survival & growth NOEC LOEC MATC	40 Liver 100 Liver 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

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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	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 Threshold prior to "winter stress"	<7.91 WB 5.85 WB	4°C EC_{10}-NOAEC 8.15 WB	8.15 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	4°C EC_{20}-LOAEC 8.80 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 EC_{20} juv. survival ES1	9.27 WB 9.78 WB	9°C EC_{10} 14.0 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 EC_{20} juv. survival ES3	14.00 WB 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		

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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

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

A chonic criterion element of 15.2 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, and is expected to be protective of non-reproductive endpoints such as juvenile survival and growth. The egg/ovary chronic criterion element can be compared to the most sensitive non-reproductive SMCV, cold-stressed (4°C) bluegill, using a conversion from selenium in egg/ovary to whole body tissue. For bluegill the EO/WB ratio of 2.13 is based on n=27 observations compiled from four different sources (Coyle et al. 1993; Doroshov et al. 1992; Hermanutz et al. 1996; and Osmundson et al. 2007) and discussed in Section 4.1.5. These yielded a good correlation between the concentrations of selenium in egg/ovary relative to whole body. Using an egg-ovary to whole-body ratio of 2.13, a concentration of 15.2 mg/kg dw in the egg-ovary of bluegills converts to a whole-body selenium concentration of 7.14 mg/kg dw, which is below the 4°C non-reproductive SMCV for bluegill of 8.15 mg/kg dw. Equivalently, the same whole-body to ovary-egg conversion factor of 2.13 can be used to convert the non-reproductive bluegill SMCV of 8.15 mg/kg Se whole-body to an ovary/egg concentration of 17.36 mg Se/kg dw, which would be protected by the reproductive egg/ovary criterion of 15.2 mg/kg.

The bluegill cold-stressed non-reproductive SMCV is also protected by the reproductive effect-based whole-body criterion of 8.13 mg Se/kg. Figure 15 shows the non-reproductive effect whole-body GMCVs compared to the whole-body criterion.

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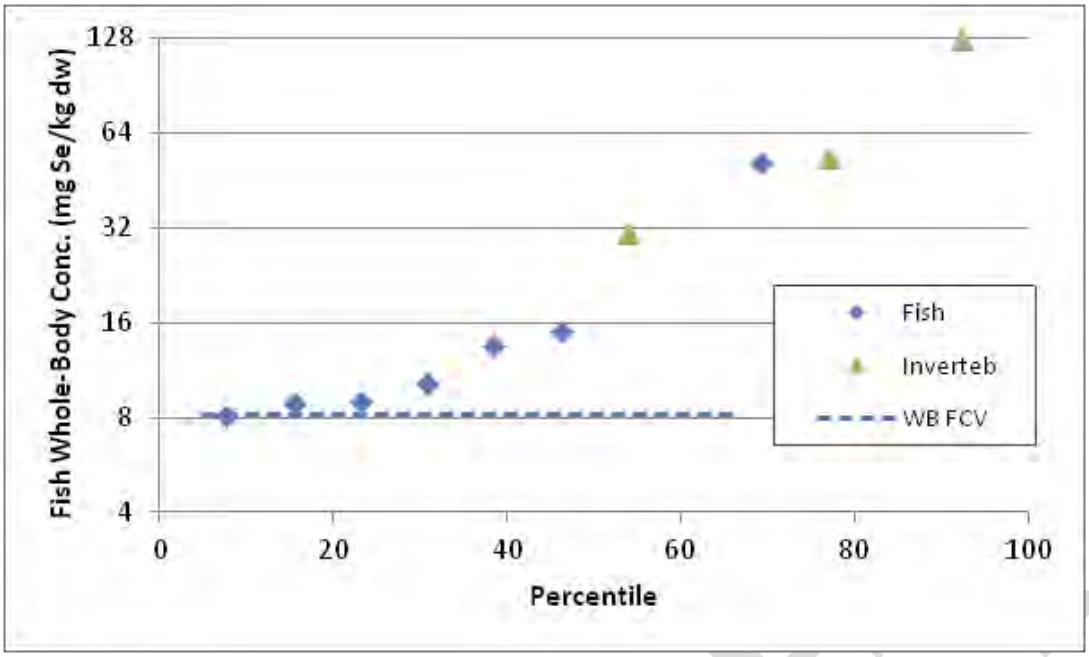

Figure 15. Distribution of (a) non-reproductive effect genus mean values for fish measured as whole-body concentrations or muscle concentrations converted to whole body, and (b) invertebrate effect concentrations converted to equivalent fish whole-body concentrations using a trophic transfer factor of 1.27, both compared to the reproductive effect whole-body FCV.

For establishing a reliable criterion, the sufficiency of and consistency among the data underlying the Section 4.1.14.1.1 and Appendix C reproductive-endpoint GMCVs favor their use over any non-reproductive endpoint data. Most of the reproductive studies involved examining the offspring of wild-caught females, exposed under real-world conditions. Most had unambiguous 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). Being a laboratory investigation of acceptable quality, the Lemly (1993a) 4°C chronic value of 5.85 mg/kg dw was included in the determination of the 8.15 mg/kg dw whole-body non-reproductive SMCV for bluegill. Nevertheless, the mortality observed in that 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 a field study, Lemly (1993b) found larval centrarchid deformities at concentrations ranging from

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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.

In contrast to the non-reproductive effects, the reproductive effects show more clear-cut concentration-response relationships (11 of the 19 reproductive chronic values are specific ECs, whereas only 5 of the 19 non-reproductive chronic values), are more readily reproducible, and are better corroborated by field observations. Reproductive effects represent the endpoint of

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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 criteria are designed to protect 95% of the genera, the criterion of 15.2 mg/kg dw ovary/egg should be protective of aquatic populations of fish and invertebrates.

7.2 Water

7.2.1 Validation of Translation Equation for Developing Water Column Concentrations

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

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

Because fish bioaccumulate selenium over a relatively long time period, 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 the EPA used a validation approach based on fish tissue measurements rather than single water measurements.

The EPA solved Equation 18 for egg-ovary concentration yielding:

$$C_{egg-ovary} = C_{water} \times TTF^{composite} \times EF \times CF \qquad \text{(Equation 19)}$$

The EPA then used Equation 19 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. The EPA then compared those predicted values to the measured concentration in the fish.

The EPA searched its selenium database for measurements of selenium 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, the EPA searched its selenium database again for water column measurements that were taken from

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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, the EPA identified appropriate species-specific *TTF* and *CF* values as described previously, and the *EF* value from the site the samples were taken. The EPA then used Equation 19 to calculate the predicted egg-ovary concentration from the observed water column concentration. Finally, the EPA compared the predicted egg-ovary concentrations with the observed egg-ovary concentrations.

The EPA identified 300 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 16 shows all 300 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 is highly correlated ($r = 0.84$, $t_{(298)} = 26.28$, $P < 10^{-80}$).

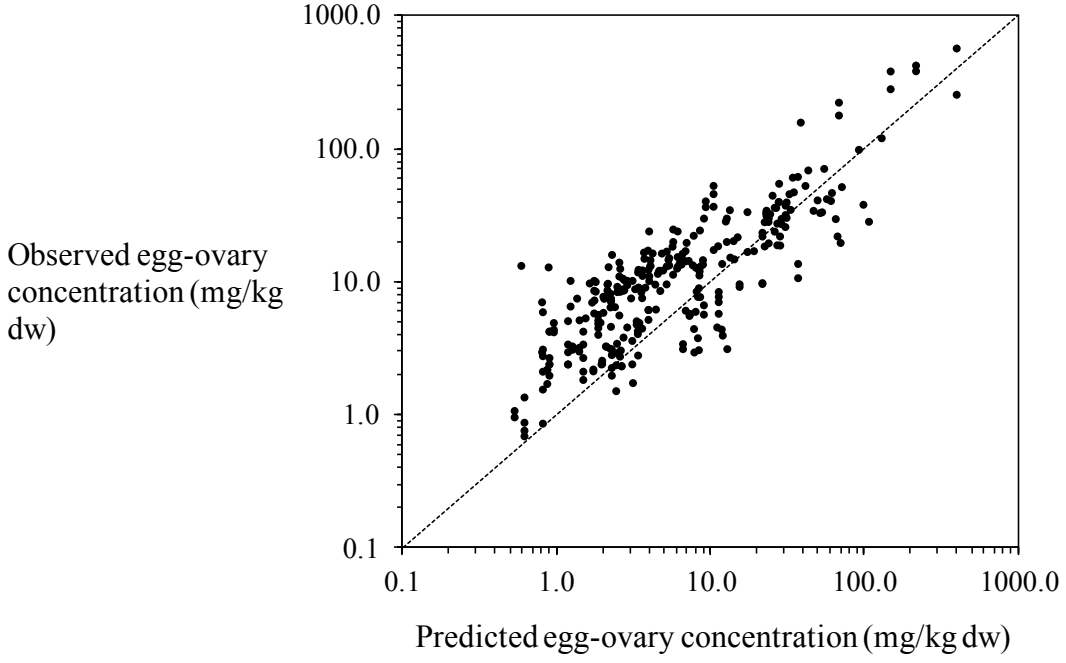

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Figure 16. Scatter plot of predicted versus measured concentrations of selenium in fish. Dashed line shows unity y = x line.

Although there is a strong correlation between predicted and observed water concentration values, there is some scatter around the hypothesized y = x unity line. Dispersion around the unity line is likely attributable to several sources of uncertainty. Potential sources of uncertainty include small sample sizes, temporal or spatial variability in selenium exposure, and local variability in aquatic food webs. The EPA limited its analysis to only those aquatic sites with at least two particulate measurements available to calculate its *EF* value and with at least one of them from algae or detritus. Nevertheless, only one or two measurements of algae and/or detritus were available for 41 of the 69 aquatic sites evaluated. Although such a restriction reduces uncertainty when applying Equation 19 to available data, the 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, the 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 to a site-specific water concentration.

7.2.2 Evaluating the Protectiveness of the Final Water-Column Criterion Values

To evaluate the protectiveness of the water column criterion values, the EPA used field data to compare attainment or exceedance of the egg-ovary criterion element to attainment or exceedance of the water column criterion element values at aquatic sites with measurements of selenium in both tissue and water. The EPA identified fish tissue measurements in its database of available selenium measurements, and then searched for matched measurements in water defined as those measurements collected at the same aquatic site within one year of the tissue measurement. Because water measurements represent the concentration of selenium at a specific location and point in time whereas tissue measurements represent the accumulated exposure to selenium over a larger geographic area and time period, the EPA assessed tissue attainment or exceedance by comparing the egg-ovary criterion element to each single tissue sample, and assessed water column attainment or exceedance by comparing the water column criterion element value to a water column concentration that was estimated from at least 3 matched water measurements. Measurements of selenium in tissue other than eggs or ovaries were converted to

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an equivalent egg-ovary concentration using the same method that was used for validation of Equation 18 described above.

The final selenium water criterion values are expressed as a 30-day average not to exceed more than once in 3 years on average. To assess attainment or exceedance using water concentration measurements that were randomly sampled at a site over two years (between one year before and one year after the site-matched tissue sample was collected), the EPA adjusted the water concentrations matched to each tissue measurement by assuming a lognormal distribution and calculating the 95th percentile. For tissue measurements with 6 or more matched water-column measurements, the EPA calculated the 95th percentile using the standard deviation of the measurements matched with the tissue measurement. For tissue measurements with fewer than 6 matching water measurements, the EPA calculated the water column standard deviation for each matched tissue measurement, calculated the average standard deviation, and then used the average standard deviation to calculate the 95th percentile water column concentration for each tissue measurement. Because the average water column standard deviation for lentic aquatic systems was significantly smaller than for lotic aquatic systems ($t_{(117)} = 2.32$, $P < 0.05$), the EPA applied the average standard deviation from lentic aquatic systems to water measurements from lentic aquatic systems, and applied the average standard deviation from lotic aquatic systems to water measurements taken from lotic aquatic systems.

The EPA used these data to assess attainment or exceedance of 140 instances in lentic aquatic systems and 688 instances in lotic aquatic systems. Although such a binary classification scheme does not consider the degree to which measurements are above or below a criterion, water quality standards are usually implemented as a binary decision (a water body either attains or exceeds criteria) and thus is a useful tool to evaluate the performance of the water column criterion element concentrations. Table 18 and Table 19 summarize the results of this binary classification.

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Table 18. Comparison of criterion attainment using the water column and egg-ovary concentration values in lentic aquatic systems.

	Tissue concentration greater than tissue criterion element	Tissue concentration less than tissue criterion element
Water concentration greater than water criterion element	96 (69%)	16 (11%)
Water concentration less than water criterion element	10 (7%)	13 (15%)

Table 19. Comparison of selenium criterion attainment using the water column and egg-ovary concentration values in lotic aquatic systems.

	Tissue concentration greater than tissue criterion element	Tissue concentration less than tissue criterion element
Water concentration greater than water criterion element	248 (36%)	206 (30%)
Water concentration less than water criterion element	52 (8%)	182 (26%)

The EPA used these binary classifications tables to calculate the binary classification statistics specificity, sensitivity, positive prediction value, negative prediction value, and accuracy. Sensitivity is the probability that the water column concentration will exceed the water column criterion element when the egg-ovary concentration is exceeding the egg-ovary citerion element. Specificity is the probability that the water column concentration will attain (be equal to or less than) the water column criterion element when the egg-ovary concentration is attaining (equal to or less than) the egg-ovary criterion element. Positive prediction value is the probability that the egg-ovary concentration will exceed the egg-ovary criterion element when the water column concentration is exceeding the water column criterion element. Negative prediction value is the probability that the egg-ovary concentration will attain the egg-ovary criterion element when the water concentration is attaining the water criterion element. Accuracy is the probability that any assessment decision will be correctly categorized. Finally, environmental protectiveness indictates the percent of time/measurements that meeting the 30-day water column criterion element would be expected to protect against any fish egg-ovary

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criterion element exceeedances, which are 93 and 92% for lentic and lotic systems, respectively. The environmental protectiveness value indicates that false negative conclusions regarding fish tissue exceedances would be minimized, and would occur less than 10% of the time, if the selected 20th percentile water column value for the water column criterion element is not exceeded. The binary classification statistics are shown in Table 20.

Table 20. Binary classification statistics for lentic and lotic aquatic systems.

	Lentic	Lotic
Sensitivity	0.91	0.83
Specificity	0.53	0.47
Positive prediction value	0.86	0.55
Negative prediction value	0.64	0.78
Accuracy	0.81	0.62
Environmental protectiveness	0.93	0.92

These binary classification statistics indicate that the chronic water criterion element values are highly sensitive to exceedance of the egg-ovary criterion element (that is, when the egg-ovary criterion element is exceeded, it is highly likely that the water column criterion element will also be exceeded). Specificity, positive prediction value, negative prediction value, and accuracy are all within a reasonable range of values (see appendix H for further explanation and details on the binary classification statistics). The EPA concludes from these analyses that the lentic and lotic water column criterion element values are adequately protective of aquatic life.

7.2.3 Uncertainty in Bioaccumulation of Total Dissolved Selenium

Geochemical form of selenium. The form of selenium in water (selenate, selenite, or organoselenium) determines how readily selenium enters aquatic food webs and cycles through particulate matter, consumer organisms, and predators. Inorganic selenium forms (e.g., selenate and selenite) have relatively limited bioavailability compared with organo-selenium forms. Typically, inorganic selenium released to aquatic systems is reduced and biotransformed to organo-selenium compounds (Bowie et al. 1996). Organoselenium and selenite are more bioavailable than selenate and thus may bioaccumulate to a greater extent (Besser et al. 1993;

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Rosetta and Knight 1995). Thus variability and uncertainty in the form of selenium released to a waterbody contributes to uncertainty in anticipated effects for a given water body.

7.3 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).

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 of 15.2 mg Se/kg dw.

Oncorhynchus mykiss has a SMCV of 21.1 mg Se/kg dw egg, and is in the fourth most sensitive genus. The dataset contains multiple studies with cutthroat trout (*Oncorhynchus clarki*) some subspecies of which are Federally listed as threatened. The SMCV for cutthroat trout is 24.06 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 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 Section 7.1.3 conservatively sets the NOEC at >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 criterion of 15.2 mg Se/kg (dw) egg-ovary element is below all of the above mentioned chronic values for threatened and endangered (or closely related) species. However, because other threatened or endangered species might be more sensitive, if relevant new

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information becomes available in the future, it should be considered in state- or site-specific criteria calculations.

7.4 Aquatic-Dependent Wildlife is Beyond the Scope of this Aquatic Criteria Derivation.

AWQC that are developed by the 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 science evidence. EPA plans, in the future, 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 possibly less stringent, than the values provided for aquatic life in this 2014 criteria document. This is likely because data indicate that selenium does not significantly biomagnify moving up the food chain except in specific ecosystems with mollusk-based food-webs, unlike bioaccumulative chemicals such as mercury. 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, as well as the lower rate at which they eliminate selenium (Luoma and Rainbow 2005). Thus, aquatic-dependent wildlife criteria for species that feed primarily on mollusks would be expected to have lower values than the 2014 selenium criterion 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.

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4304T

EPA 822-P-14-001
May 2014

External Peer Review Draft

Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014 (Appendices A-K)

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

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APPENDIX A: Selenium Chemistry

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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)].

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.

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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 ± 1.7 × 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

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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 prevelant 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.

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.

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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).

Departure from Thermodynamic Equilibrium

In the highly dynamic natural waters, there is often a departure from thermodynamic equilibrium. In the thermodymanic 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.

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 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

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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. Particulate and dissolved selenium as a function of total selenium in freshwater and marine aquatic ecoystems

Reference	Waterbody	Particulate Se (% of Total)	Fraction dissolved, fd
Cutter 1989	Carquinezitist, 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 Oufall Drain, CA	0	1
Welsh and Maughan 1994	Pretty Water Lake, CA	21	0.79

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APPENDIX B: Conversions

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Conversion of wet to dry tissue weight

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-1 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-1. 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

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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

Derivation of tissue conversion factors

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:

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

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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. EPA obtained matched pairs of selenium measurements in the whole-body and eggs and/or ovaries of fish from published scientific literature. When both egg and ovary measurements were reported, EPA used the average. 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 the bottom portion of Table B-1. Studies that reported selenium measurements in egg-ovary tissue did not always report matched pairs of selenium measurements in the whole-body. However, several of these studies did report selenium measurements in muscle tissue. When matched pairs of selenium concentrations from egg-ovary and muscle tissue were available, EPA calculated a species specific egg-ovary to muscle conversion factor following the procedures used to calculate the egg-ovary to whole-body conversion factor. The species-specific egg-ovary to muscle conversion factors were then converted to egg-ovary to whole-body conversion factors by calculating and applying a single muscle to whole-body conversion factor.

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

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of each matched pair of measurements and then calculated the median ratio. Because the number of matched pairs of selenium measurements from muscle and whole-body were limited, the EPA took the median of all the species-specific muscle to whole-body conversion factors to derive a single muscle to whole-body conversion factor, and applied that conversion factor to the species-specific muscle to egg-ovary conversion factors to derive species specific egg-ovary to whole-body conversion factors.The EPA then used the average of the species-specific median ratios as the correction factor for all uncorrected *CF* values.

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 $\left(\frac{C_{egg}+C_{ovary}}{2}\right)$

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

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

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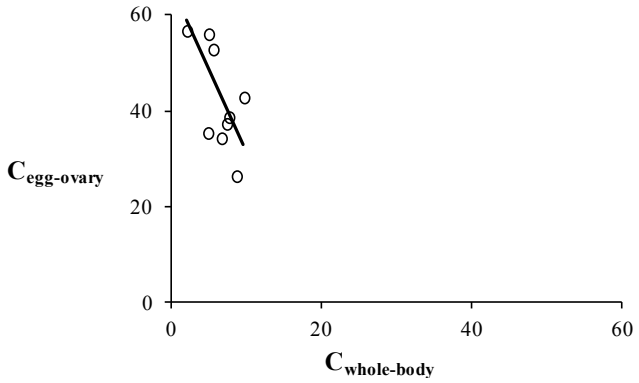

Median ratio: 6.29

R^2: 0.37
F: 4.67
df: 8
P: 0.063

Not used because P > 0.05 and negative slope.

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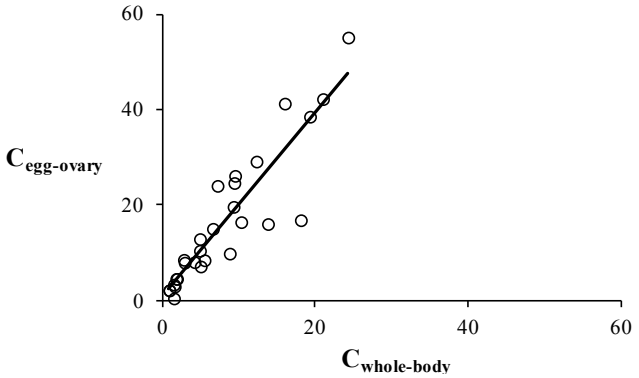
Bluegill (*Lepomis macrochirus*)

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

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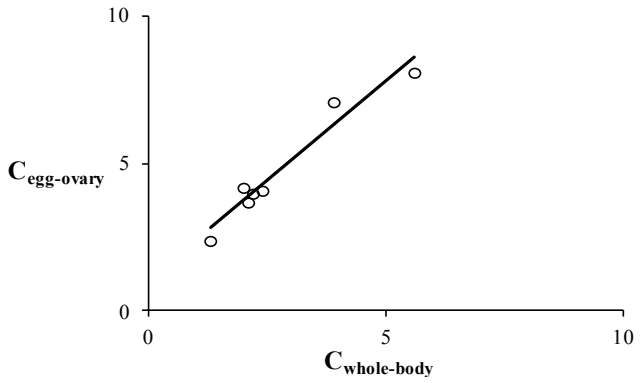
Bluehead sucker (*Catostomus discobolus*)

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
NewFields 2009	3.60	0.80	-	0.80	0.22
NewFields 2009	4.10	0.90	-	0.90	0.22
NewFields 2009	3.70	0.80	-	0.80	0.22
NewFields 2009	4.30	0.90	-	0.90	0.21
NewFields 2009	3.00	1.20	-	1.20	0.40
NewFields 2009	3.10	1.20	-	1.20	0.39
NewFields 2009	2.70	1.00	-	1.00	0.37
NewFields 2009	2.50	1.00	-	1.00	0.40
NewFields 2009	8.90	12.80	-	12.80	1.44
NewFields 2009	13.80	40.30	-	40.30	2.92
NewFields 2009	17.90	36.00	-	36.00	2.01
NewFields 2009	13.60	26.80	-	26.80	1.97
NewFields 2009	17.20	26.90	-	26.90	1.56
NewFields 2009	6.70	18.60	-	18.60	2.78
NewFields 2009	9.60	17.70	-	17.70	1.84
NewFields 2009	22.60	38.80	-	38.80	1.72
NewFields 2009	7.20	13.20	-	13.20	1.83

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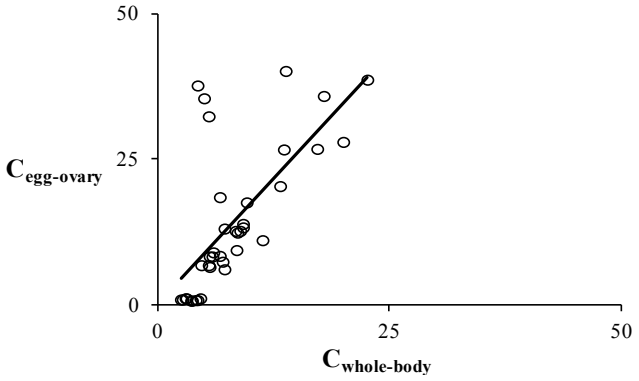
Brown trout (*Salmo trutta*)					
NewFields 2009	9.20	13.40	-	13.40	1.46
NewFields 2009	13.20	20.50	-	20.50	1.55
NewFields 2009	8.60	12.50	-	12.50	1.45
NewFields 2009	11.30	11.20	-	11.20	0.99
NewFields 2009	20.00	28.10	-	28.10	1.41
NewFields 2009	8.40	12.80	-	12.80	1.52
NewFields 2009	5.60	8.40	-	8.40	1.50
NewFields 2009	6.70	8.50	-	8.50	1.27
NewFields 2009	5.90	8.40	-	8.40	1.42
NewFields 2009	6.00	9.10	-	9.10	1.52
NewFields 2009	7.00	7.50	-	7.50	1.07
NewFields 2009	5.60	6.60	-	6.60	1.18
NewFields 2009	4.70	6.90	-	6.90	1.47
NewFields 2009	7.20	6.20	-	6.20	0.86
NewFields 2009	9.20	14.00	-	14.00	1.52
NewFields 2009	5.50	6.90	-	6.90	1.25
NewFields 2009	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

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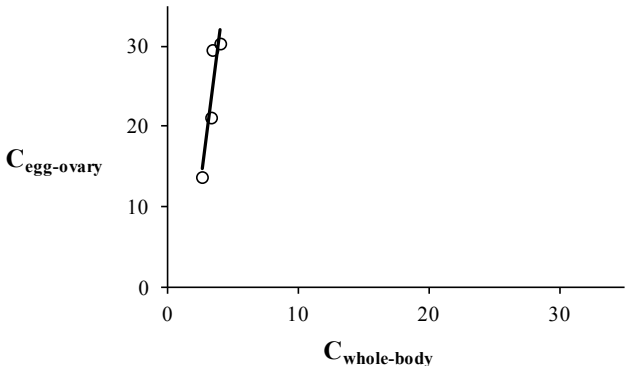
Channel catfish (*Ictalurus punctatus*)

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



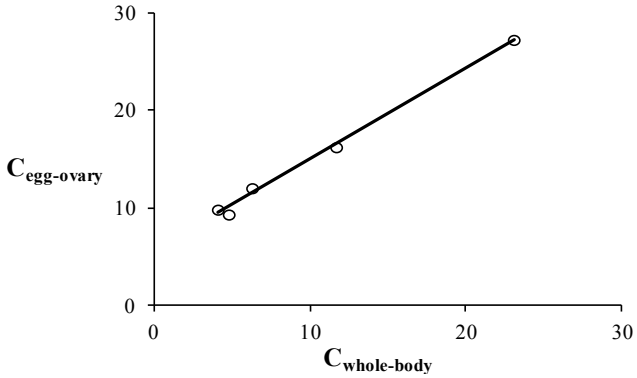
Median ratio: 6.98

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

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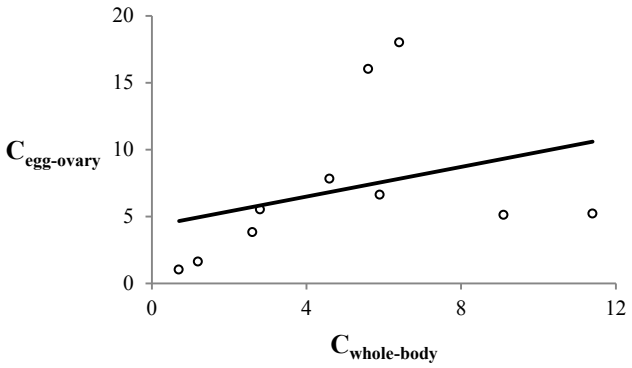
Cutthroat trout (*Oncorhynchus clarkii*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	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



Median ratio: 1.45

R^2: 0.11
F: 1.01
df: 8
P: 0.406

Not used because P > 0.05.

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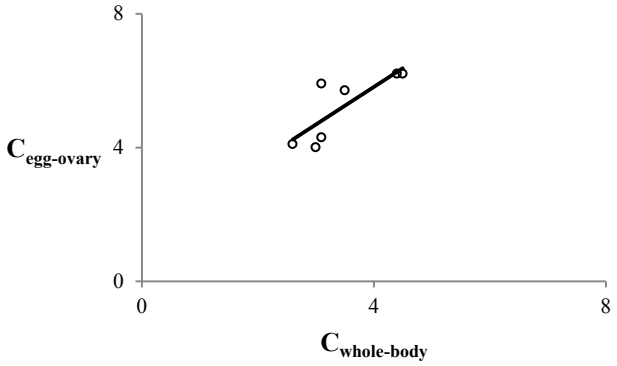
Flannelmouth sucker (*Catostomus latipinnis*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	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

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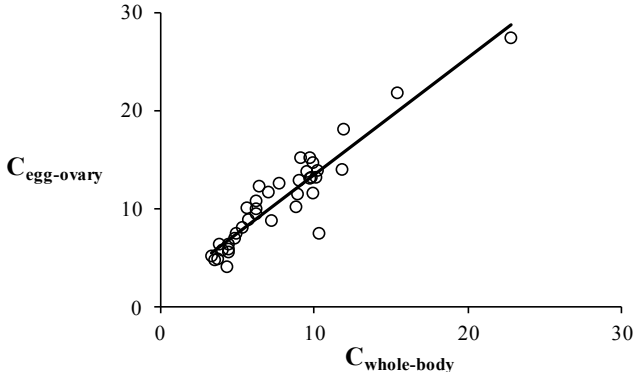
Green sunfish (*Lepomis cyanellus*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
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



Median ratio: 1.45

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

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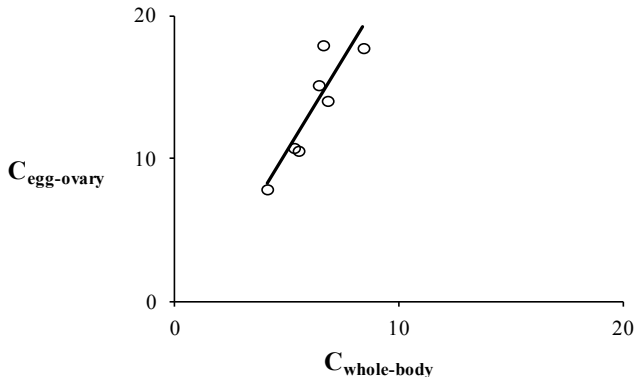
Roundtail chub (*Gila robusta*)

Study	$C_{whole-body}$	C_{egg}	C_{ovary}	$C_{egg-ovary}$	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

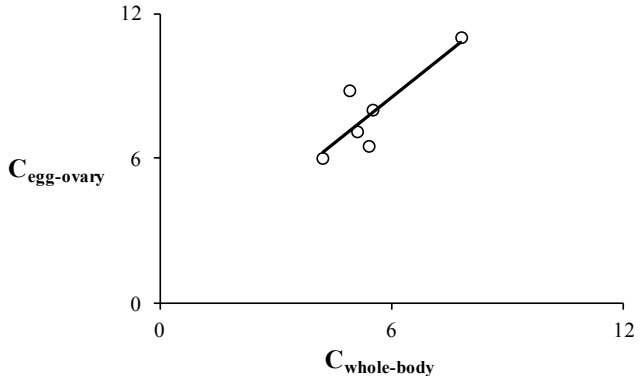


Median ratio: 2.07

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

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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
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

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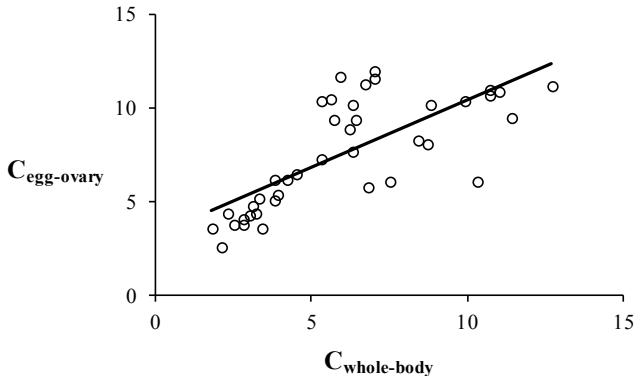

Median ratio: 1.41

R^2: 0.54
F: 45.4
df: 38
P: < 0.001

Table B-2. Summary of whole-body to egg-ovary 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
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*	1.41

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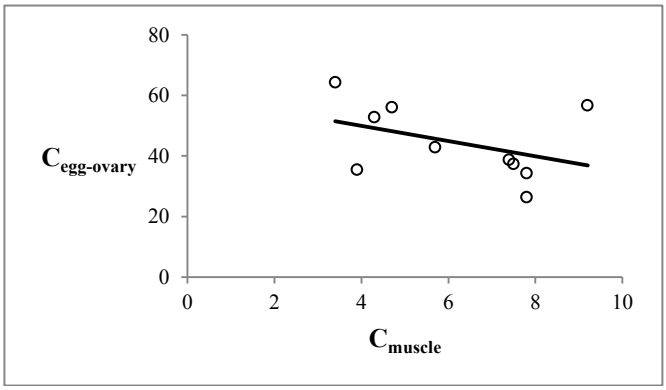
Muscle to egg-ovary conversion factors

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 $\left(\frac{C_{egg} + C_{ovary}}{2}\right)$

$$\text{Ratio} = \frac{C_{egg-ovary}}{C_{muscle}}$$

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.

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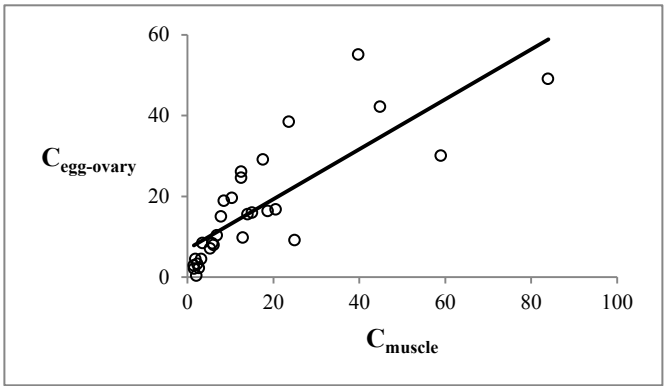
Bluegill (*Lepomis macrochirus*)

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

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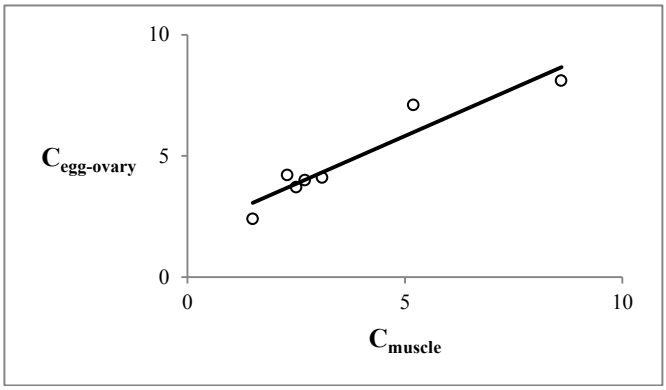
Bluehead sucker (*Catostomus discobolus*)

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

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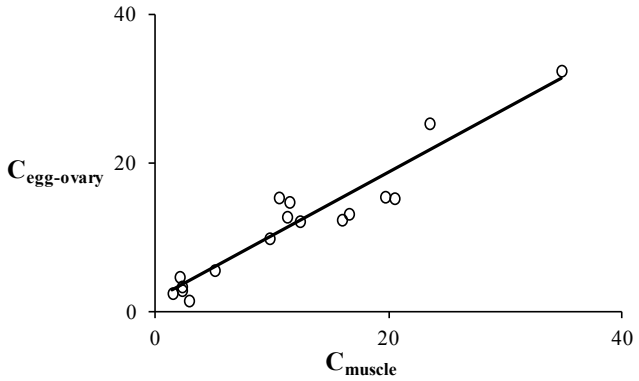
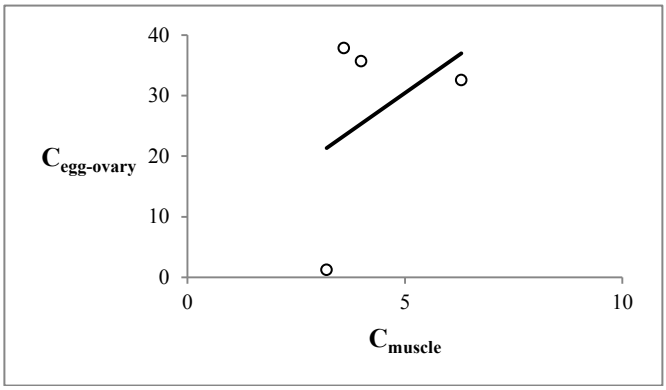


Median ratio: 1.09

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

Brown trout (*Salmo trutta*)

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



Median ratio: 7.03

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

Not used because P > 0.05.

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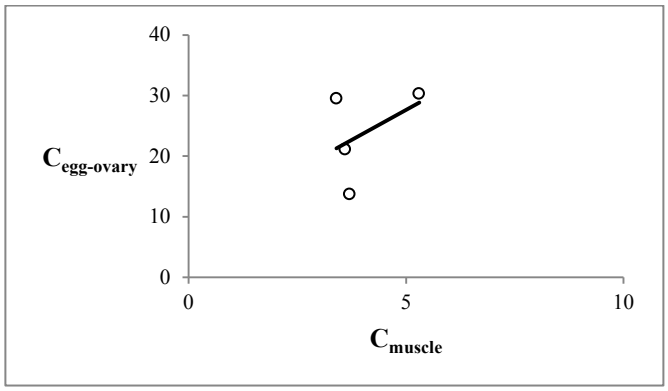
Channel catfish (*Ictaluris punctatus*)

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



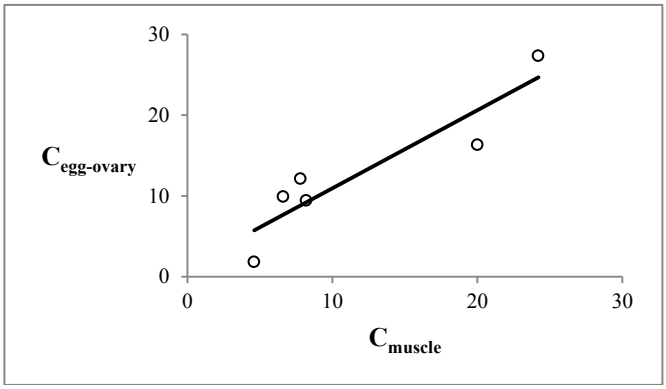
Median ratio: 5.79

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

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Cutthroat trout (*Oncorhynchus clarkii*)

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

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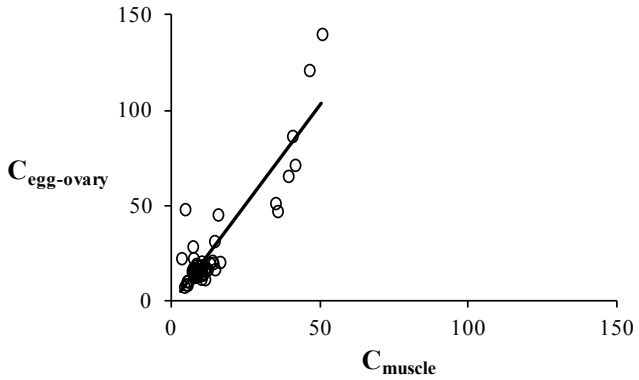
Cutthroat trout (*Oncorhynchus clarkii*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Kennedy et al. 2000	10.20	12.30	14.50	13.40	1.31
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

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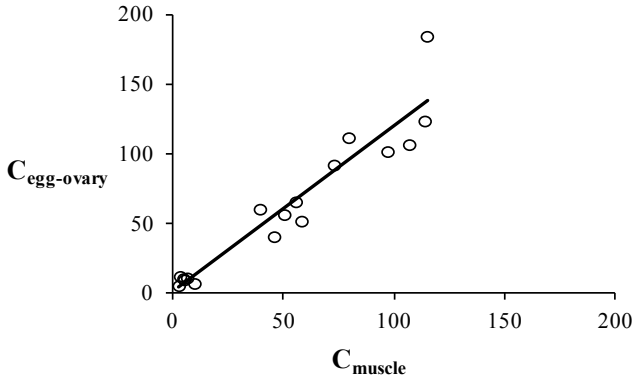
Dolly varden (*Salvelinus malma*)

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



Median ratio: 1.26

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

Flannelmouth sucker (*Catostomus latipinnis*)

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

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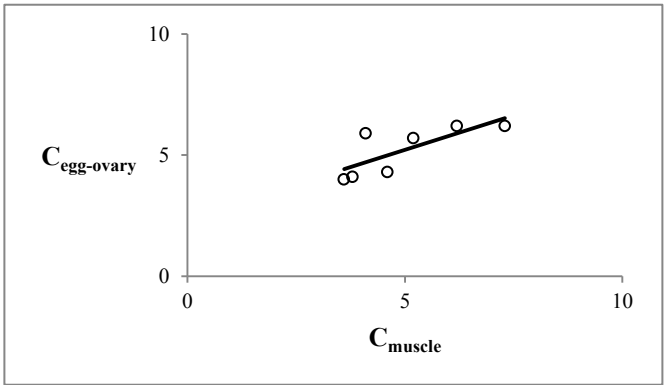

Median ratio: 1.08

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
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

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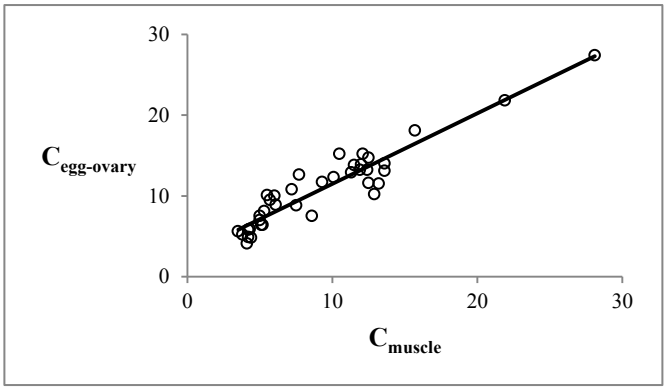
Green sunfish (*Lepomis cyanellus*)					
Osmundson et al. 2007	3.8	-	5.2	5.2	1.37
Osmundson et al. 2007	4.2	-	5.8	5.8	1.38
Osmundson et al. 2007	4.1	-	4.1	4.1	1.00
Osmundson et al. 2007	4.2	-	4.9	4.9	1.17
Osmundson et al. 2007	5.7	-	9.5	9.5	1.67
Osmundson et al. 2007	4.4	-	4.8	4.8	1.09
Osmundson et al. 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

Mountain whitefish (*Prosopium williamsoni*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	**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

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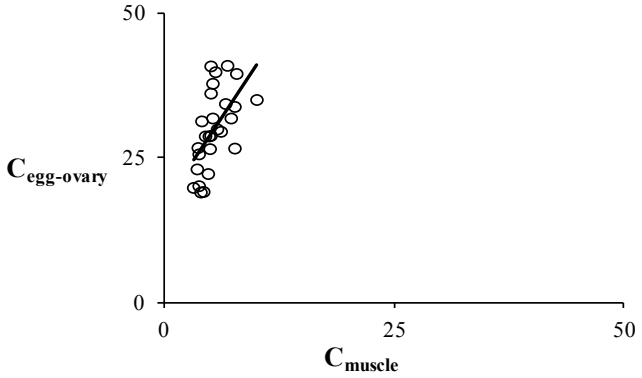
Mountain whitefish (*Prosopium williamsoni*)					
Golder 2005	4.00	-	31.50	31.50	7.88
Golder 2005	10.00	-	35.20	35.20	3.52
Golder 2005	4.90	-	26.70	26.70	5.45
Golder 2005	7.60	-	26.80	26.80	3.53
Golder 2005	6.10	-	29.70	29.70	4.87
Golder 2005	6.80	-	41.10	41.10	6.04
Golder 2005	5.00	-	29.00	29.00	5.80
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}	$C_{egg\text{-}ovary}$	**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

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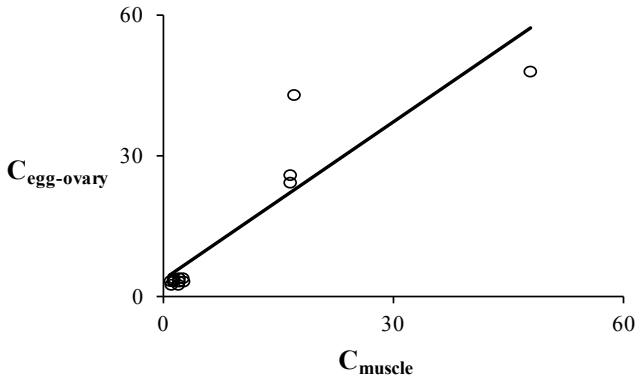

Median ratio: 1.88

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

Rainbow trout (*Oncorhynchus mykiss*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg\text{-}ovary}$	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
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

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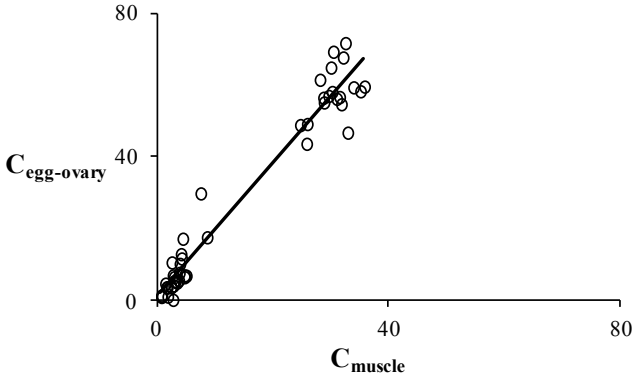
Rainbow trout (*Oncorhynchus mykiss*)					
Casey and Siwik 2000	29.90	64.70	-	64.70	2.16
Casey and Siwik 2000	32.80	46.60	-	46.60	1.42
Casey and Siwik 2000	31.40	56.50	-	56.50	1.80
Casey and Siwik 2000	32.00	67.50	-	67.50	2.11
Casey and Siwik 2000	35.70	59.40	-	59.40	1.66
Casey and Siwik 2000	24.60	48.70	-	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



Median ratio: 1.92

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

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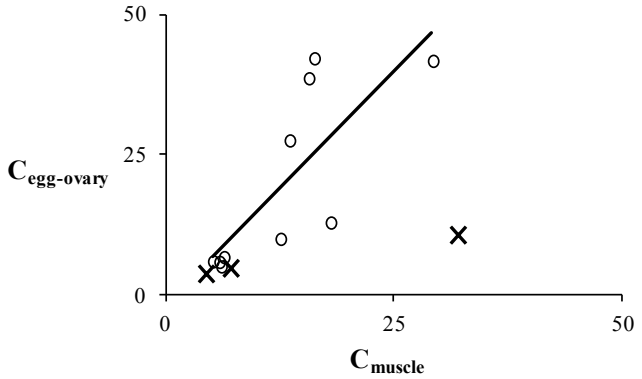
Razorback sucker (*Xyrauchen texanus*)

Study	C_{muscle}	C_{egg}	C_{ovary}	$C_{egg-ovary}$	Ratio
Hamilton et al. 2005 a,b,c	6.30	6.50	7.00	6.75	1.07
Hamilton et al. 2005 a,b,c	15.60	46.50	30.60	38.55	2.47
Hamilton et al. 2005 a,b,c	29.20	37.80	45.50	41.65	1.43
Hamilton et al. 2005 a,b,c	5.10	6.00	-	6.00	1.18
Hamilton et al. 2005 a,b,c	5.80	-	5.90	5.90	1.02
Hamilton et al. 2005 a,b,c	13.50	-	27.50	27.50	2.04
Hamilton et al. 2005 a,b,c	16.20	-	42.10	42.10	2.60
Hamilton et al. 2005 a,b,c	6.00	-	5.10	5.10	0.85
Hamilton et al. 2005 a,b,c	12.50	-	10.00	10.00	0.80
Hamilton et al. 2005 a,b,c	18.00	-	12.90	12.90	0.72
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: 1.12

R^2: 0.61
F: 12.5
df: 8
P: 0.008

[a] Data from this study labeled above with 'x's' were excluded because results appeared 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

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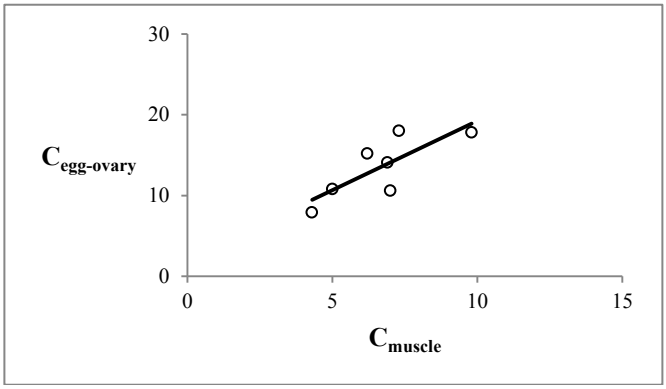
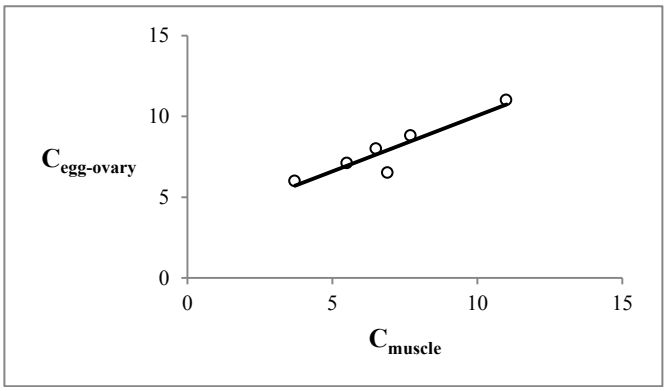


Median ratio: 2.04

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

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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
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

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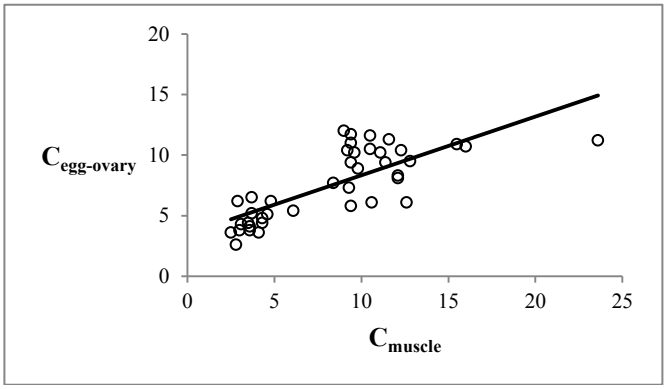

Median ratio: 1.00

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

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

Common name	Scientific name	Median ratio
bluegill	*Lepomis macrochirus*	1.38
bluehead sucker	*Catostomus discobolus*	1.48
brook trout		1.09
common carp	*Cyprinus carpio*	1.14
cutthroat trout	*Oncorhynchus clarkii*	1.81
dolly varden		1.26
flannelmouth sucker	*Catostomus latipinnis*	1.08
green sunfish	*Lepomis cyanellus*	1.21
mountain whitefish		5.80
northern pike		1.88
rainbow trout	*Oncorhynchus mykiss*	1.92
razorback sucker		1.12
roundtail chub	*Gila robusta*	2.04
smallmouth bass	*Micropterus dolomieu*	1.19
white sucker	*Catostomus commersonii*	1.00

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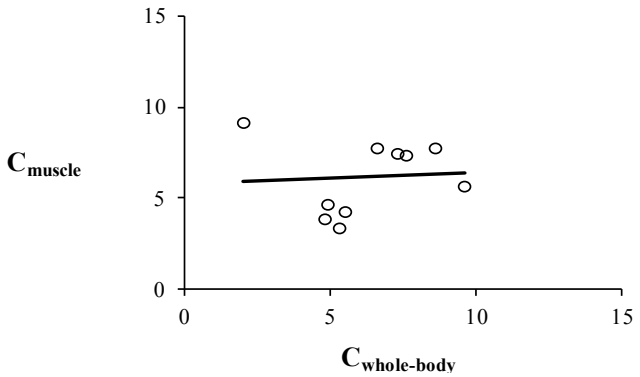
Muscle to whole-body correction factor

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

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

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

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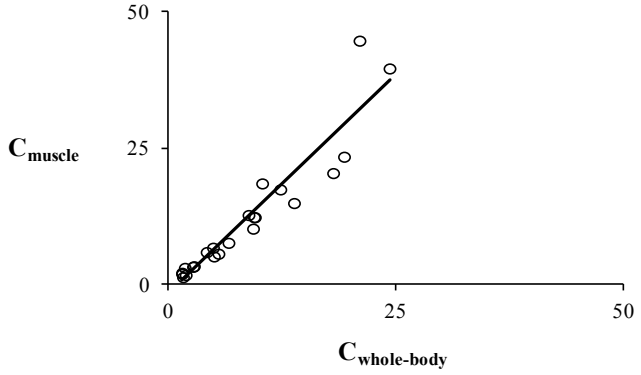
Bluegill (*Lepomis macrochirus*)			
Hermanutz et al. 1996	2.80	3.50	1.25
Hermanutz et al. 1996	12.30	17.60	1.43
Hermanutz et al. 1996	9.40	12.50	1.33
Hermanutz et al. 1996	1.50	2.30	1.53
Hermanutz et al. 1996	4.90	6.90	1.41
Hermanutz et al. 1996	21.00	44.90	2.14
Hermanutz et al. 1996	24.30	39.80	1.64
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

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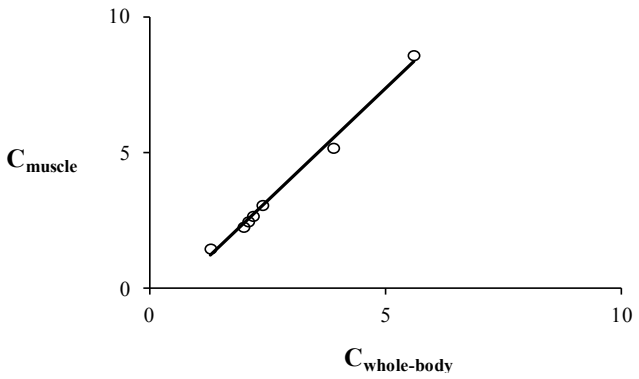
Bluehead sucker (*Catostomus discobolus*)

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

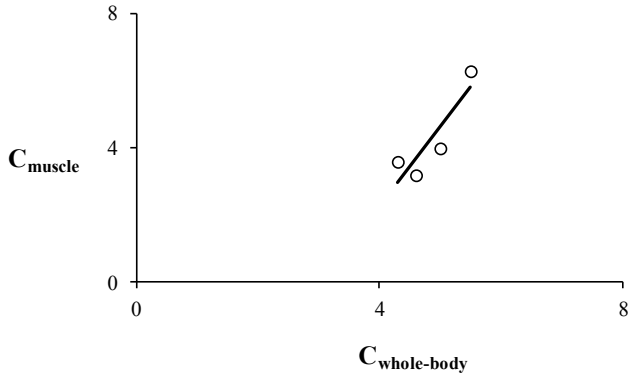


Median ratio: 1.23

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.

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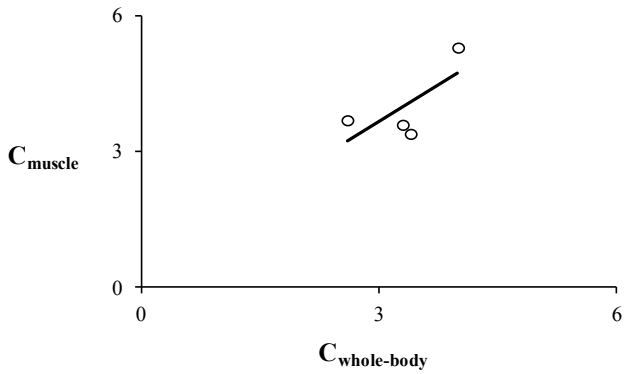
Channel catfish (*Ictalurus punctatus*)

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



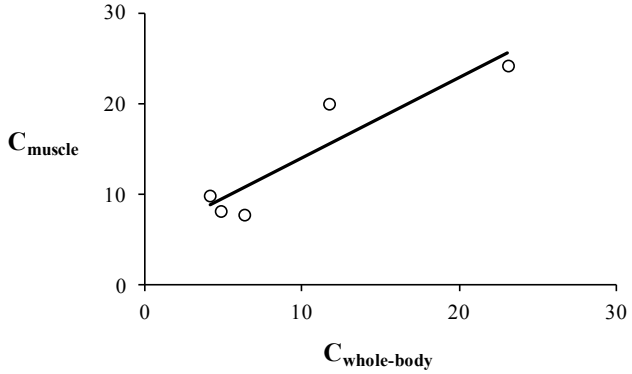
Median ratio: 1.21

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

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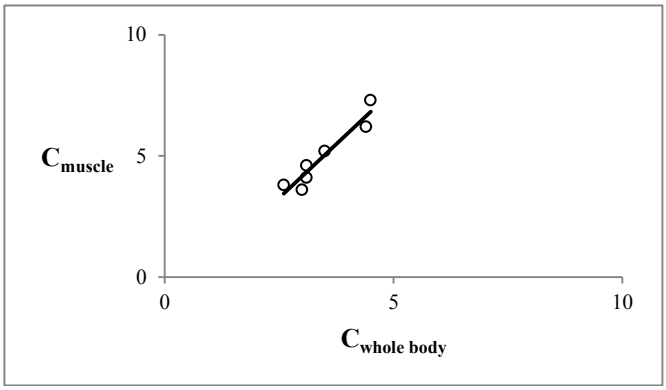
Flannelmouth sucker (*Catostomus latipinnis*)

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

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Green sunfish (*Lepomis cyanellus*)			
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
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

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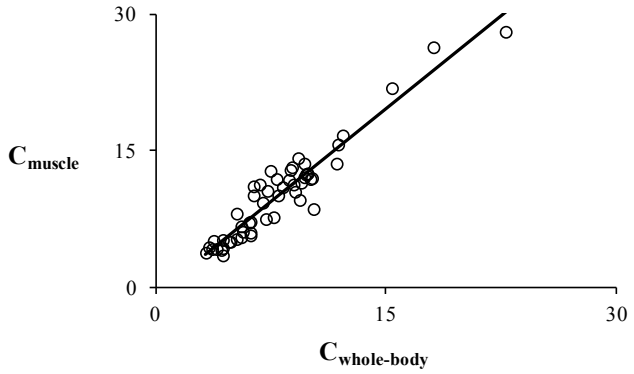
Green sunfish (*Lepomis cyanellus*)

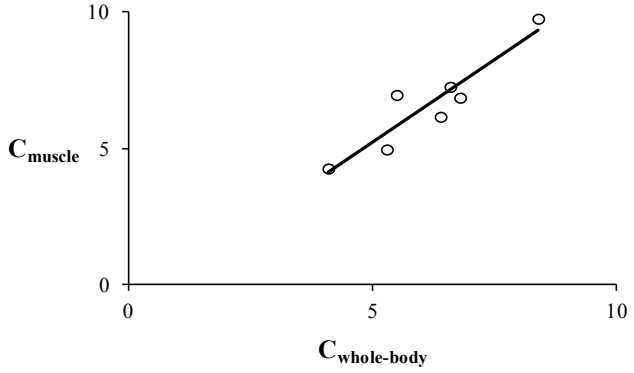


Median ratio: 1.23

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

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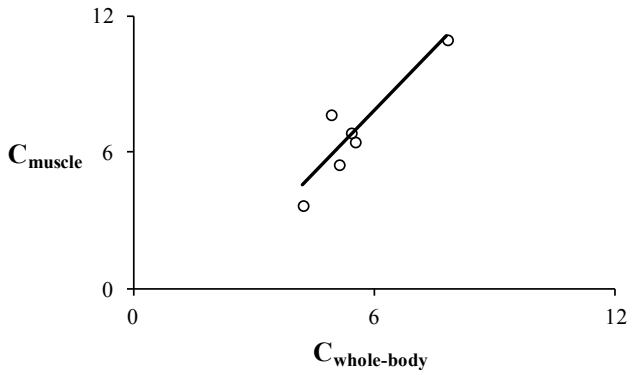
Smallmouth bass (*Micropterus dolomieu*)

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: 1.23

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

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White sucker (*Catostomus commersonii*)

Study	$C_{whole\text{-}body}$	C_{muscle}	Ratio
Osmundson et al. 2007	5.30	9.30	1.75
Osmundson et al. 2007	6.20	9.80	1.58
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

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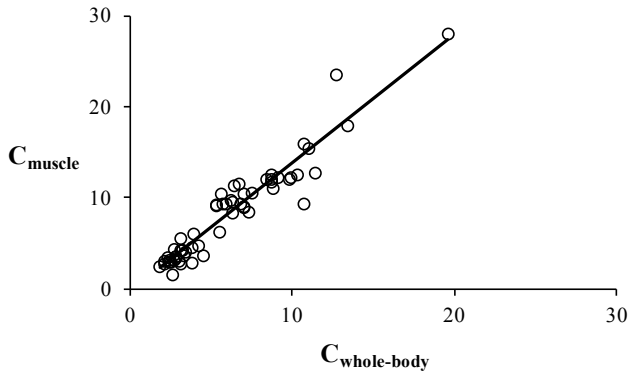
White sucker (*Catostomus commersonii*)

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
Median muscle to whole-body correction factor		1.27

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Table B-5. Final whole-body to egg-ovary 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
Cutthroat trout		1.81	1.27	2.30
Dolly varden		1.26	1.27	1.61
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		1.12	1.27	1.42
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sucker	1.41			1.41
Genus				
Catostomus				1.41
Esox				2.39
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				2.37
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				2.00
Salmonidae				1.96

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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
Order				
Perciformes				1.45
Class				
Actinopterygii				1.71

Derivation of Trophic Transfer Function values

Methodology

Taxa specific trophic transfer functions (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.

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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} equaled 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 were 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.

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TTF values from physiological coefficients

AE (%) = Assimilation efficiency
IR ($g\ g^{-1}\ d^{-1}$) = Ingestion rate
k_e (d^{-1}) = Efflux rate constant
$TTF = \frac{AE\ x\ IR}{K_e}$

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*

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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
63.0		0.037		Wang and Fisher 1996
61.5		0.05		Wang and Fisher 1996

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Physiological Parameters				
AE (%)	**IR(g g^{-1} d^{-1})**	**k_e (d^{-1})**	**TTF**	**Study**
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

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		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	

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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*)

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	

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

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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

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	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 values from field data

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

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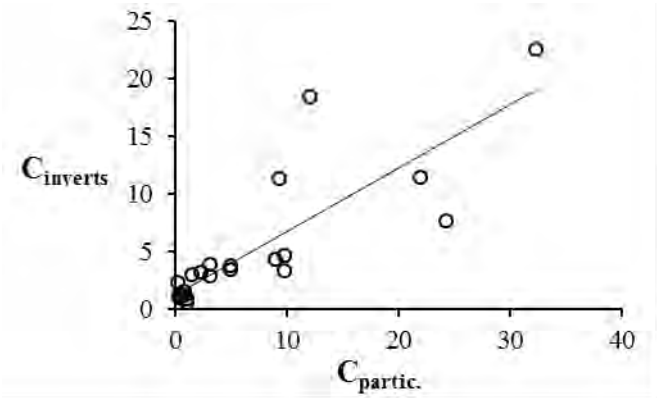
Saiki et al. 1993	ET7	0.16	0.76		0.46	1.10	2.42

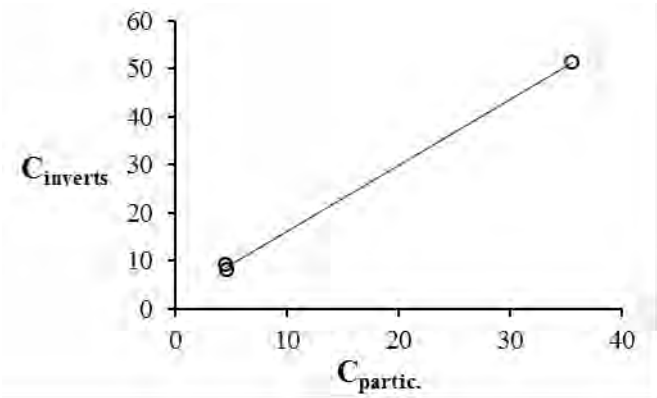


Median ratio: 1.22

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

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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

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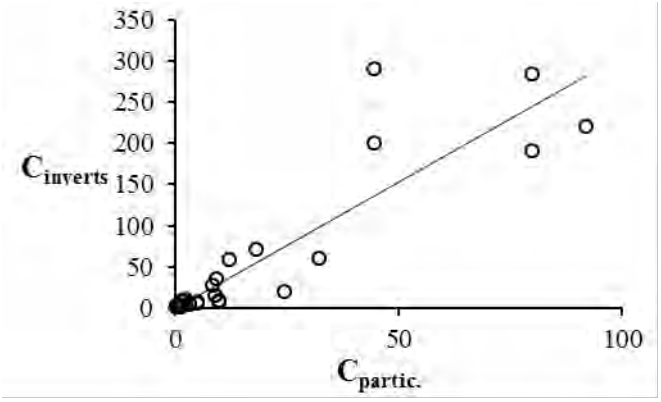
Saiki et al. 1993	ET7	0.16	0.76		0.46	0.84	1.85

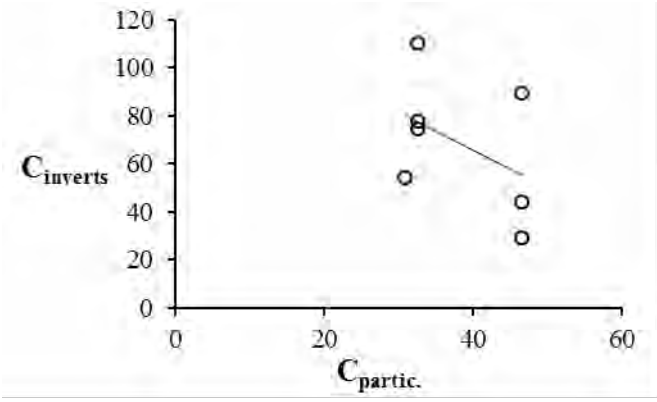


Median ratio: 1.90

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.

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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
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

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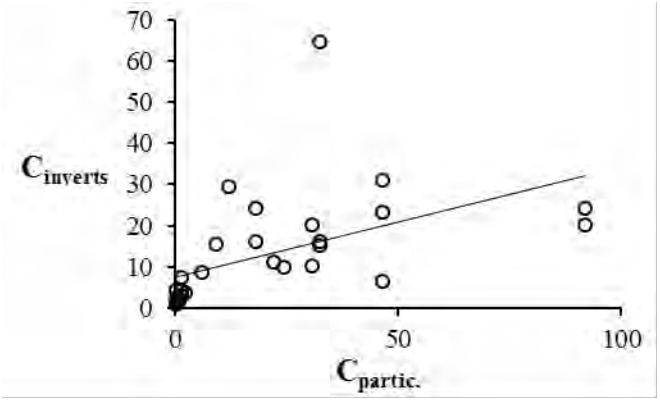
Rinella and Schuler 1992	18	0.59		0.59	2.70	4.58



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

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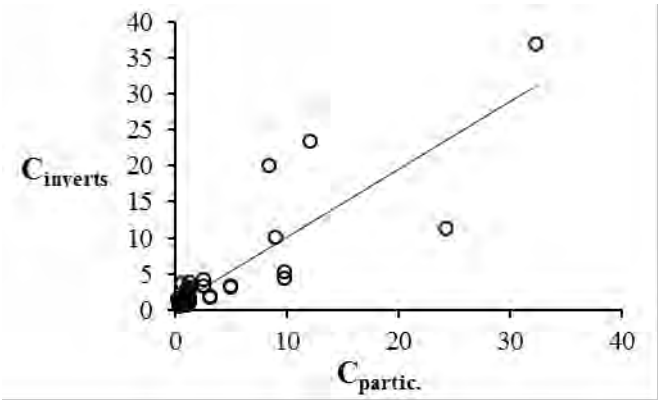
Crayfish (*Astacidae*)							
Butler et al. 1995	SD	0.77		0.50	0.64	1.40	2.20
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.40	6.83
Butler et al. 1995	YJ2	0.31		0.10	0.21	1.50	7.32
Butler et al. 1997	CHK	1.19			1.19	0.90	0.76
Butler et al. 1997	MN2	0.79			0.79	0.83	1.06
Butler et al. 1997	MUD2	1.30			1.30	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
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

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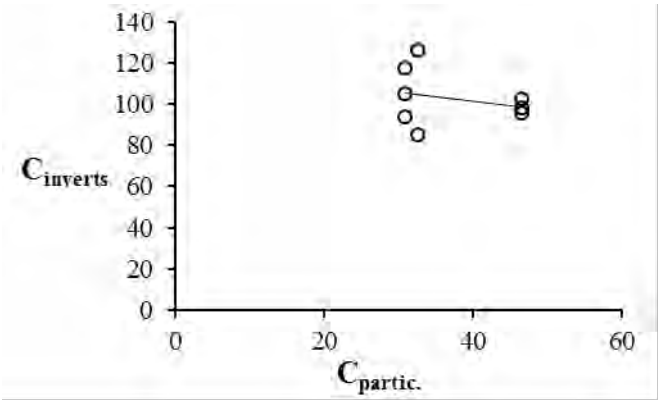
True flies (*Diptera*)

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.

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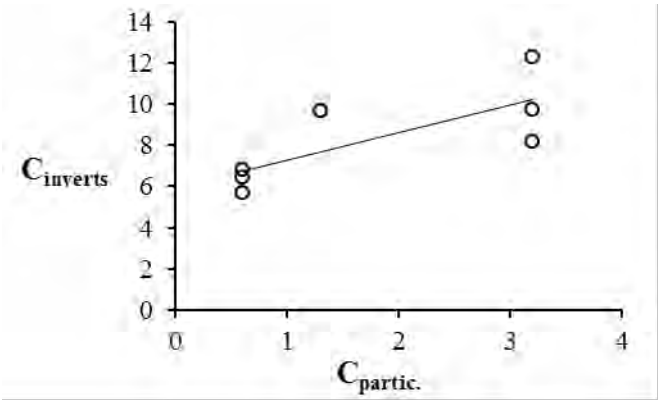
Mayflies (*Ephemeroptera*)

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



Median ratio: 7.39

R^2: 0.58
F: 6.77
df: 5
P: 0.04
Not used because of anomalously high ratio. TTF from physiological data used instead.

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

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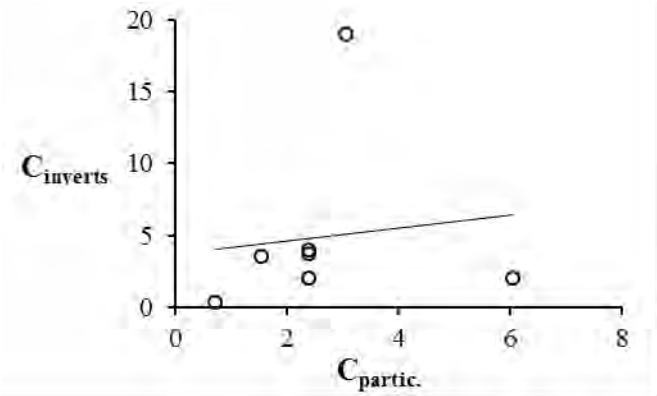

Median ratio: 1.54

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
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
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

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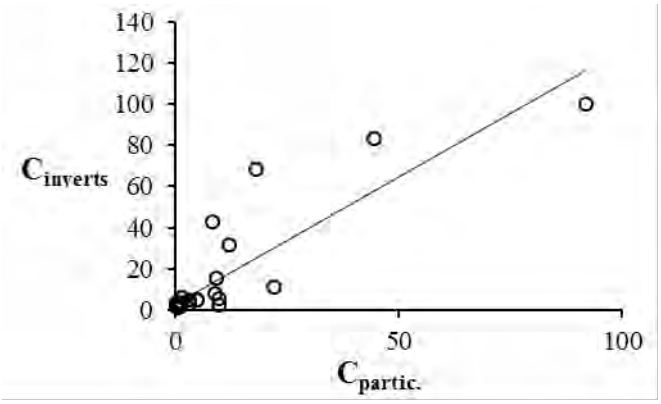
Zooplankton						
Saiki et al. 1993	SJR1	0.22	0.50	0.36	1.40	3.89
Saiki et al. 1993	SJR1	0.22	0.50	0.36	1.30	3.61
Saiki et al. 1993	ET7	0.16	0.76	0.46	0.63	1.38
Saiki et al. 1993	ET7	0.16	0.76	0.46	1.40	3.08



Median ratio: 1.89

R^2: 0.76
F: 85.7
df: 27
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 $\frac{C_{dragon}}{C_{food}}$

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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
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

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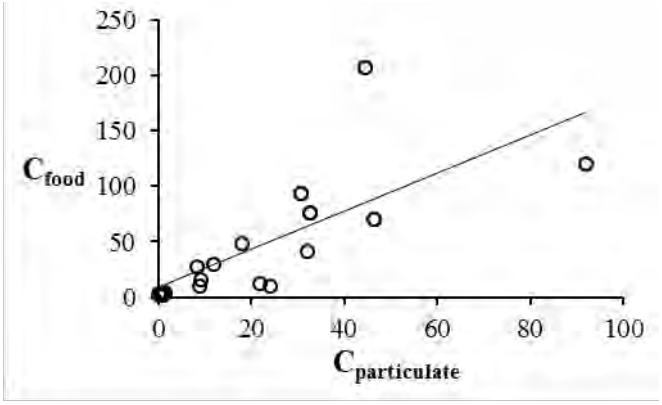
Co-occurring potential food species of damselflies and dragonflies (*Odonata*)

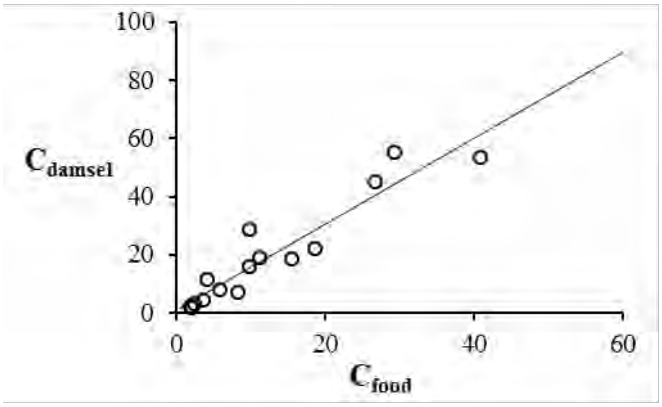


Median ratio: 2.21

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
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: 2.88

R^2: 0.89
F: 104.4
df: 13
P: <0.001

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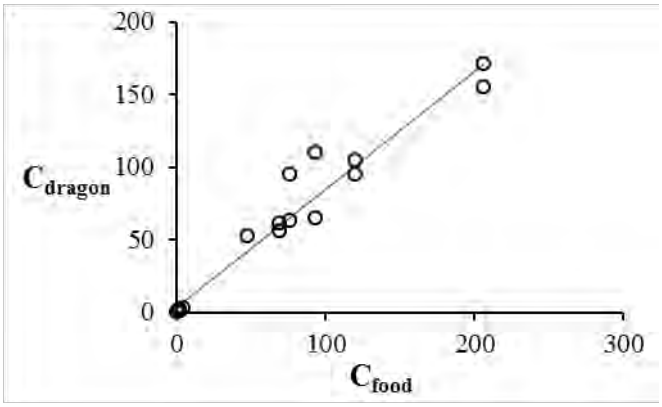
Dragonflies (*Zygoptera*)

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



Median ratio: 1.97

R^2: 0.95
F: 343.5
df: 17
P: <0.001

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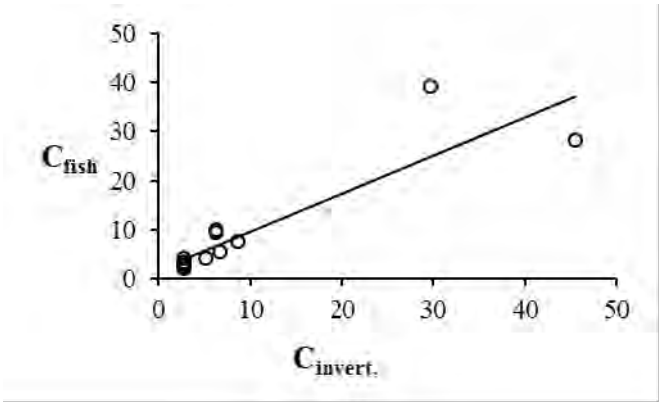
Vertebrates:

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
GEI 2013	Sand Creek at Colfax	2.81	1.95	0.70
GEI 2013	Sand Creek at Colfax	2.81	2.37	0.84
GEI 2013	Sand Creek at Colfax	2.81	2.73	0.97
GEI 2013	Sand Creek at Colfax	2.81	3.21	1.14
GEI 2013	Sand Creek at Colfax	2.81	3.96	1.41
Lemly 1985	Badin Lake	5.18	4.19	0.81
Mueller et al. 1991	Lake Meredith near Ordway, CO	6.40	9.20	1.44
Mueller et al. 1991	Lake Meredith near Ordway, CO	6.40	9.70	1.52
Lemly 1985	High Rock Lake	6.75	5.26	0.78
Mueller et al. 1991	Pueblo Reservoir near Pueblo, CO	8.70	7.40	0.85
Butler et al. 1991	Sweitzer Lake	29.80	39.00	1.31
Lemly 1985	Belews Lake	45.53	28.11	0.62



Median ratio: 0.91

R^2: 0.79
F: 38.1
df: 10
P: < 0.001

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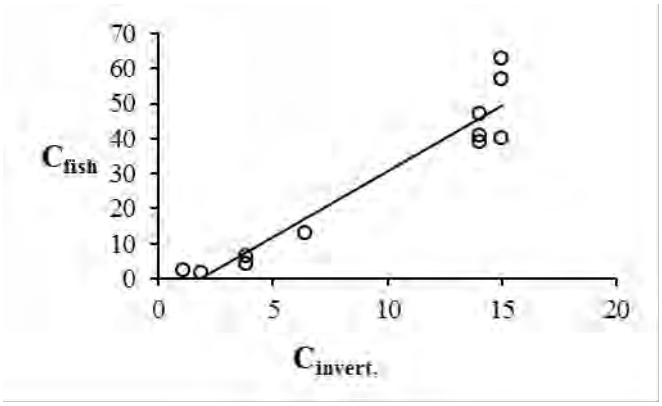
Black crappie (*Pomoxis nigromaculatus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	Totten Reservoir	1.07	2.50	2.35
Butler et al. 1995	Summit Reservoir	1.85	1.70	0.92
Peterson et al. 1991	Ocean Lake, west side	3.83	4.20	1.10
Peterson et al. 1991	Ocean Lake, west side	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



Median ratio: 2.67

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

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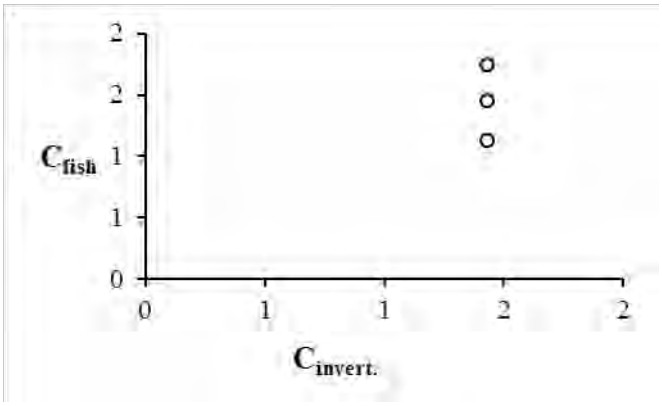
Blacknose dace (*Rhinichthys atratulus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Mason et al. 2000	BK	1.43	1.13	0.79
Mason et al. 2000	BK	1.43	1.45	1.01
Mason et al. 2000	BK	1.43	1.74	1.21



Median ratio: 1.01

R^2: 0.0
F: 0.0
df: 1
P: 1.0
Not used because P > 0.05.

Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Saiki et al. 1993	ET6	0.85	1.40	1.66
Saiki et al. 1993	ET6	0.85	2.20	2.60
Saiki et al. 1993	ET7	0.86	1.20	1.40
Saiki et al. 1993	ET7	0.86	1.20	1.40
Hermanutz et al. 1996	MSO I	0.87	1.55	1.78
Saiki et al. 1993	SJR1	0.95	0.87	0.92
Saiki et al. 1993	SJR1	0.95	1.40	1.48
Butler et al. 1995	TT	1.07	2.30	2.16
Hermanutz et al. 1996	MSO III	1.20	1.83	1.52
Saiki et al. 1993	SJR3	1.50	1.90	1.27
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Hermanutz et al. 1996	MSO II	1.70	1.55	0.91
Saiki et al. 1993	SJR2	3.30	2.70	0.82
Saiki et al. 1993	SJR2	3.30	3.30	1.00
Hermanutz et al. 1996	MSO III	3.95	4.21	1.06
Saiki et al. 1993	GT4	4.05	4.30	1.06
Saiki et al. 1993	GT4	4.05	4.50	1.11
Saiki et al. 1993	GT5	4.90	5.00	1.02
Saiki et al. 1993	GT5	4.90	6.40	1.31
Hermanutz et al. 1996	MSO II	5.05	3.86	0.76

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Bluegill (*Lepomis macrochirus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Hermanutz et al. 1996	MSO II	5.05	4.88	0.97
Hermanutz et al. 1996	MSO III	5.55	13.77	2.48
Crutchfield 2000	transect 3	8.60	7.64	0.89
Crutchfield 2000	transect 3	8.60	7.64	0.89
Crutchfield 2000	transect 3	8.60	11.90	1.38
Crutchfield 2000	transect 3	8.60	14.30	1.66
Mueller et al. 1991	R1	8.70	5.20	0.60
Crutchfield 2000	transect 3	9.25	7.64	0.83
Crutchfield 2000	transect 3	9.25	9.05	0.98
Crutchfield 2000	transect 3	9.25	16.70	1.81
Crutchfield 2000	transect 3	9.25	19.00	2.05
Hermanutz et al. 1996	MSO III	10.00	10.32	1.03
Crutchfield 2000	transect 3	11.40	30.32	2.66
Crutchfield 2000	transect 3	11.40	34.50	3.03
Crutchfield 2000	transect 3	11.95	21.28	1.78
Crutchfield 2000	transect 3	11.95	28.60	2.39
Crutchfield 2000	transect 3	15.20	38.73	2.55
Crutchfield 2000	transect 3	15.20	48.80	3.21
Crutchfield 2000	transect 4	15.70	15.16	0.97
Crutchfield 2000	transect 4	15.70	16.70	1.06
Crutchfield 2000	transect 4	15.70	20.20	1.29
Crutchfield 2000	transect 4	15.70	21.28	1.36
Crutchfield 2000	transect 4	16.45	13.10	0.80
Crutchfield 2000	transect 4	16.45	13.63	0.83
Hermanutz et al. 1996	MSO II	16.63	24.29	1.46
Crutchfield 2000	transect 3	16.95	16.70	0.99
Crutchfield 2000	transect 3	16.95	33.38	1.97
Crutchfield 2000	transect 3	16.95	38.10	2.25
Crutchfield 2000	transect 3	16.95	48.54	2.86
Hermanutz et al. 1996	MSO II	17.30	16.76	0.97
Hermanutz et al. 1996	MSO II	17.30	20.99	1.21
Crutchfield 2000	transect 3	17.90	33.38	1.86
Crutchfield 2000	transect 3	17.90	44.00	2.46
Crutchfield 2000	transect 4	18.25	16.69	0.91
Crutchfield 2000	transect 4	18.25	20.20	1.11
Crutchfield 2000	transect 3	20.35	50.07	2.46
Crutchfield 2000	transect 3	20.35	83.30	4.09
Crutchfield 2000	transect 3	20.70	27.27	1.32
Crutchfield 2000	transect 3	20.70	41.70	2.01
Crutchfield 2000	transect 4	20.90	27.27	1.30
Crutchfield 2000	transect 4	20.90	39.30	1.88

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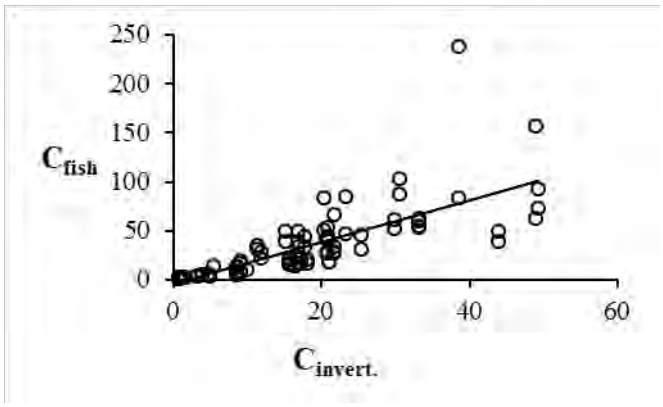
Bluegill (*Lepomis macrochirus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Crutchfield 2000	transect 4	20.90	43.96	2.10
Crutchfield 2000	transect 4	20.90	52.40	2.51
Hermanutz et al. 1996	MSO I	21.19	18.13	0.86
Hermanutz et al. 1996	MSO I	21.19	18.28	0.86
Crutchfield 2000	transect 3	21.80	26.20	1.20
Crutchfield 2000	transect 3	21.80	28.03	1.29
Crutchfield 2000	transect 3	21.80	33.38	1.53
Crutchfield 2000	transect 3	21.80	65.50	3.00
Crutchfield 2000	transect 3	23.40	46.25	1.98
Crutchfield 2000	transect 3	23.40	84.50	3.61
Crutchfield 2000	transect 4	25.40	30.32	1.19
Crutchfield 2000	transect 4	25.40	45.20	1.78
Crutchfield 2000	transect 4	30.00	51.60	1.72
Crutchfield 2000	transect 4	30.00	60.70	2.02
Crutchfield 2000	transect 4	30.70	86.51	2.82
Crutchfield 2000	transect 4	30.70	102.40	3.34
Crutchfield 2000	transect 4	33.20	53.13	1.60
Crutchfield 2000	transect 4	33.20	59.50	1.79
Crutchfield 2000	transect 4	33.25	54.66	1.64
Crutchfield 2000	transect 4	33.25	61.90	1.86
Crutchfield 2000	transect 4	38.55	83.46	2.16
Crutchfield 2000	transect 4	38.55	237.00	6.15
Crutchfield 2000	transect 4	43.90	37.97	0.86
Crutchfield 2000	transect 4	43.90	48.80	1.11
Crutchfield 2000	transect 4	48.90	62.18	1.27
Crutchfield 2000	transect 4	48.90	156.00	3.19
Crutchfield 2000	transect 4	49.30	72.75	1.48
Crutchfield 2000	transect 4	49.30	92.90	1.88



Median ratio: 1.48

R^2: 0.57
F: 115.5
df: 87
P: < 0.001

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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
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

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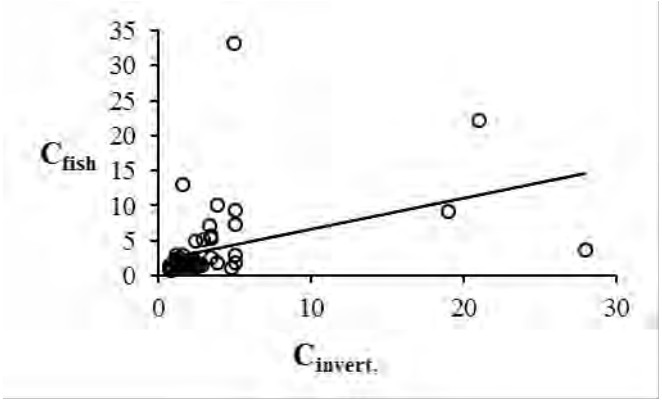
Bluehead sucker (*Catostomus discobolus*)

Study	Site	C_{invert}	C_{fish}	Ratio
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
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

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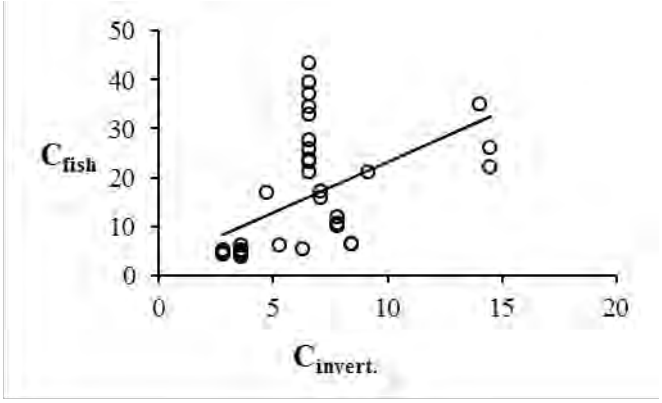
Brook stickleback (*Culaea inconstans*)				
Lambing et al. 1994	S38	4.70	17.00	3.62
Lambing et al. 1994	S37	5.30	6.10	1.15
Lambing et al. 1994	S36	6.30	5.30	0.84
GEI 2013	SW2-1	6.60	21.14	3.21
GEI 2013	SW2-1	6.60	23.21	3.52
GEI 2013	SW2-1	6.60	23.64	3.58
GEI 2013	SW2-1	6.60	25.89	3.93
GEI 2013	SW2-1	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



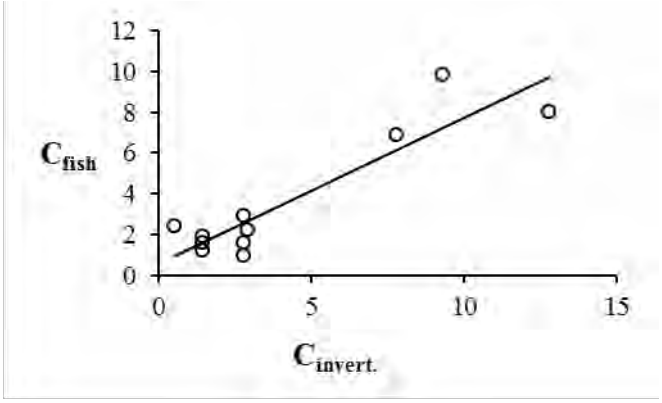
Median ratio: 1.69

R^2: 0.27
F: 13.3
df: 36
P: < 0.001

US EPA ARCHIVE DOCUMENT

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

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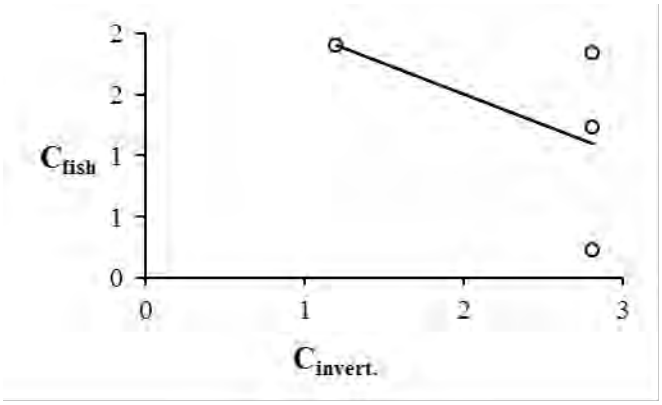

Median ratio: 0.55

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. 1993	LP2	1.00	1.60	1.60
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	LP3	1.12	2.10	1.88
Butler et al. 1993	LP3	1.12	2.80	2.51
Butler et al. 1993	B1	1.25	4.20	3.36
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	D2	1.45	3.20	2.21
Butler et al. 1993	D2	1.45	3.50	2.41
Butler et al. 1993	D2	1.45	3.50	2.41
Formation 2012	SFTC-1	1.63	6.70	4.11
Butler et al. 1993	P1	1.95	3.30	1.69
Butler et al. 1994	NFK3	2.00	5.00	2.50
Formation 2012	SFTC-1	2.42	3.68	1.52
Formation 2012	SFTC-1	2.49	2.64	1.06
Butler et al. 1993	SP2	2.75	1.20	0.44
Butler et al. 1991	12	2.80	5.40	1.93
Formation 2012	CC-75	3.11	4.05	1.30
Formation 2012	CC-75	3.11	5.35	1.72
Formation 2012	CC-350	3.16	6.28	1.99
Formation 2012	CC-350	3.16	8.53	2.70
Butler et al. 1993	LP4	3.20	1.80	0.56
Formation 2012	SFTC-1	3.21	2.25	0.70
Butler et al. 1993	SP2	3.40	3.40	1.00

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Brown trout (*Salmo trutta*)				
Butler et al. 1993	R2	3.70	5.90	1.59
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. 1991	4	3.90	3.30	0.85
Butler et al. 1991	4	3.90	3.50	0.90
Formation 2012	CC-75	3.97	3.18	0.80
Butler et al. 1993	ST2	4.10	6.00	1.46
Formation 2012	CC-75	4.16	6.60	1.59
Formation 2012	CC-75	4.16	10.32	2.48
Formation 2012	CC-350	4.20	5.78	1.38
McDonald and Strosher 1998	ER 747	4.29	4.80	1.12
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
Butler et al. 1991	10	4.80	2.00	0.42
Butler et al. 1994	SMF	4.80	8.40	1.75
Butler et al. 1994	SMF	4.80	8.54	1.78
Butler et al. 1994	SMF	4.80	9.40	1.96
Butler et al. 1994	SMF	4.80	21.44	4.47
Formation 2012	CC-3A	5.45	9.20	1.69
Formation 2012	CC-3A	5.45	10.44	1.92
Formation 2012	CC-3A	5.48	11.25	2.05
Butler et al. 1991	3	6.20	3.50	0.56
Hamilton and Buhl 2005	CC	6.70	9.70	1.45
Formation 2012	CC-150	7.03	10.14	1.44
Formation 2012	DC-600	7.83	10.54	1.35
Formation 2012	DC-600	7.83	12.83	1.64
Formation 2012	DC-600	8.53	6.20	0.73
Formation 2012	DC-600	8.53	8.54	1.00
Formation 2012	DC-600	8.65	5.85	0.68
Formation 2012	LSV-4	9.54	15.18	1.59
Formation 2012	LSV-4	9.54	16.20	1.70
Formation 2012	HS-3	11.40	18.83	1.65
Formation 2012	HS-3	11.40	20.60	1.81
Formation 2012	CC-350	11.45	7.95	0.69
Formation 2012	CC-350	11.45	11.50	1.00
Formation 2012	CC-1A	12.24	9.33	0.76
Formation 2012	CC-1A	12.24	10.51	0.86
Formation 2012	CC-1A	12.24	16.85	1.38
Formation 2012	CC-1A	12.57	9.95	0.79
Formation 2012	HS-3	13.41	17.89	1.33
Formation 2012	CC-1A	13.55	14.03	1.04
Formation 2012	CC-150	14.32	7.83	0.55

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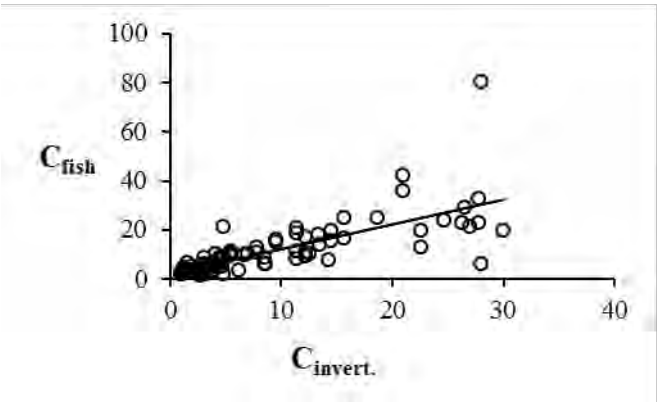
Brown trout (*Salmo trutta*)				
Formation 2012	CC-3A	14.50	15.38	1.06
Formation 2012	CC-3A	14.50	19.68	1.36
Formation 2012	HS	15.70	16.52	1.05
Formation 2012	HS	15.70	25.00	1.59
Formation 2012	HS	18.70	24.90	1.33
Butler et al. 1994	HCC1	21.00	35.68	1.70
Butler et al. 1994	HCC1	21.00	42.00	2.00
Formation 2012	LSV-2C	22.62	12.78	0.56
Formation 2012	LSV-2C	22.62	19.45	0.86
Formation 2012	HS-3	24.70	23.68	0.96
Formation 2012	LSV-2C	26.31	22.67	0.86
Formation 2012	HS-3	26.55	28.97	1.09
Formation 2012	LSV-2C	26.95	20.96	0.78
Formation 2012	HS	27.80	22.80	0.82
Formation 2012	HS	27.80	32.63	1.17
Butler et al. 1994	GUN2	28.00	5.90	0.21
Butler et al. 1994	GUN2	28.00	80.27	2.87
Formation 2012	LSV-2C	30.00	19.53	0.65



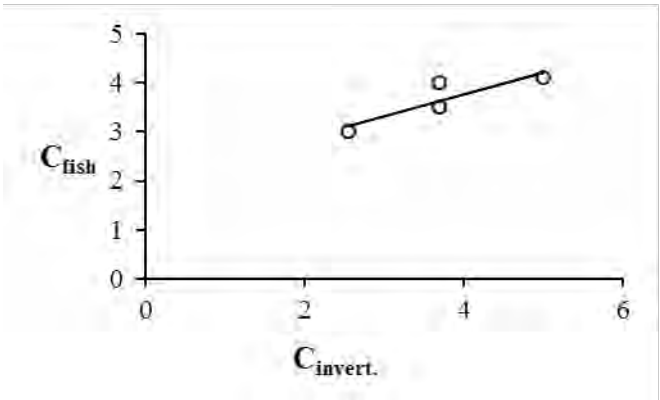
Median ratio: 1.44

R^2: 0.55
F: 102.7
df: 85
P: < 0.001

US EPA ARCHIVE DOCUMENT

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

US EPA ARCHIVE DOCUMENT

Channel catfish (*Ictalurus punctatus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	TT	1.07	1.00	0.94
Butler et al. 1995	SJ1	2.50	2.80	1.12
Butler et al. 1995	SJ1	2.50	4.10	1.64
Butler et al. 1997	MN4	2.65	4.20	1.58
Roddy et al. 1991	18	3.10	1.40	0.45
Roddy et al. 1991	18	3.10	1.50	0.48
Roddy et al. 1991	18	3.10	1.60	0.52
Roddy et al. 1991	18	3.10	1.70	0.55
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	1.80	0.58
Roddy et al. 1991	18	3.10	1.90	0.61
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	2.20	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
Butler et al. 1993	LP4	3.20	2.68	0.84
Butler et al. 1993	LP4	3.20	3.30	1.03
Butler et al. 1993	R2	3.70	3.00	0.81
Butler et al. 1993	R2	3.70	3.31	0.90
Butler et al. 1993	R2	3.90	2.17	0.56
Butler et al. 1993	R2	3.90	7.87	2.02
Butler et al. 1997	MN5	8.60	5.00	0.58
Mueller et al. 1991	R1	8.70	2.20	0.25
Butler et al. 1991	7	29.80	18.08	0.61
Butler et al. 1991	7	29.80	18.66	0.63
Butler et al. 1991	7	29.80	28.03	0.94
Butler et al. 1991	7	29.80	31.85	1.07
Butler et al. 1991	7	29.80	32.40	1.09
Butler et al. 1991	7	29.80	36.95	1.24
Butler et al. 1991	7	29.80	39.50	1.33

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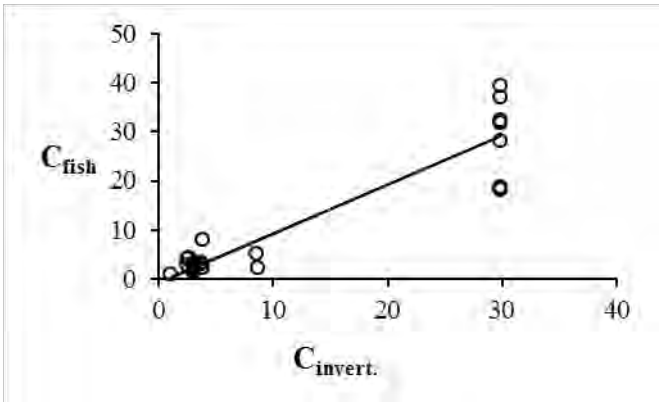
Channel catfish (*Ictalurus punctatus*)



Median ratio: 0.73

R^2: 0.89
F: 250.6
df: 32
P: < 0.001

US EPA ARCHIVE DOCUMENT

Common carp (*Cyprinus carpio*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Rinella and Schuler 1992	Malheur Lake	1.20	2.00	1.67
Butler et al. 1993	D2	1.45	3.70	2.55
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	ME4	1.55	3.90	2.52
Low and Mullins 1990	7	1.60	0.30	0.19
Rinella and Schuler 1992	Malheur Lake	2.05	2.20	1.07
Butler et al. 1995	SJ1	2.50	3.40	1.36
Butler et al. 1995	SJ1	2.50	5.30	2.12
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	MN1	2.70	5.40	2.00
Butler et al. 1995	MN1	2.70	5.80	2.15
Butler et al. 1995	MN1	2.70	9.80	3.63
Garcia-Hernandez et al. 2000		3.00	3.30	1.10
Butler et al. 1994	NFK2	3.10	4.90	1.58
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
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	SW4-1	3.33	7.29	2.19
GEI 2013	SW4-1	3.33	9.61	2.89
Butler et al. 1993	R2	3.70	3.30	0.89
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
Butler et al. 1993	R2	3.90	4.80	1.23
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
Butler et al. 1991	9	4.10	3.90	0.95

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Common carp (*Cyprinus carpio*)				
Butler et al. 1993	R2	4.30	5.00	1.16
GEI 2013	SW9	4.45	3.64	0.82
GEI 2013	SW9	4.45	3.70	0.83
GEI 2013	SW9	4.45	3.77	0.85
GEI 2013	SW9	4.45	3.80	0.85
GEI 2013	SW9	4.45	3.90	0.88
GEI 2013	SW9	4.45	4.14	0.93
GEI 2013	SW9	4.45	4.26	0.96
GEI 2013	SW9	4.45	4.41	0.99
GEI 2013	SW9	4.45	4.50	1.01
GEI 2013	SW9	4.45	4.53	1.02
GEI 2013	SW9	4.45	4.69	1.05
GEI 2013	SW9	4.45	5.61	1.26
GEI 2013	SW9	4.45	6.13	1.38
Butler et al. 1991	10	4.80	10.30	2.15
Butler et al. 1994	BSW1	5.00	12.00	2.40
Lemly 1985	Badin lake	5.18	6.50	1.26
Low and Mullins 1990	5	5.60	1.20	0.21
Mueller et al. 1991	A6	5.60	3.40	0.61
Mueller et al. 1991	A3	6.00	6.50	1.08
Butler et al. 1991	3	6.20	2.20	0.35
Mueller et al. 1991	R2	6.40	14.00	2.19
Mueller et al. 1991	R2	6.40	14.40	2.25
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	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	31.74	4.81
GEI 2013	SW2-1	6.60	36.81	5.58
GEI 2013	SW2-1	6.60	41.57	6.30
Lemly 1985	High Rock Lake	6.75	5.03	0.75
GEI 2013	SWB	7.06	12.50	1.77
GEI 2013	SWB	7.06	15.61	2.21
Butler et al. 1993	F2	7.50	5.80	0.77
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
May et al. 2008	SSW	7.60	10.40	1.37
May et al. 2008	SSAU	8.35	7.59	0.91
GEI 2013	SW11	8.41	3.56	0.42

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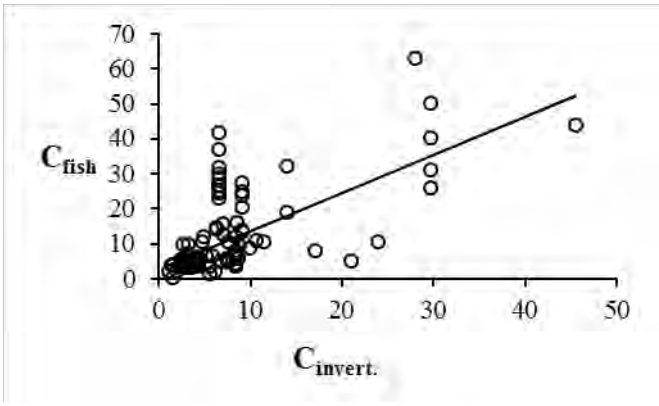
Common carp (*Cyprinus carpio*)				
GEI 2013	SW11	8.41	3.60	0.43
GEI 2013	SW11	8.41	3.79	0.45
GEI 2013	SW11	8.41	3.95	0.47
GEI 2013	SW11	8.41	4.14	0.49
GEI 2013	SW11	8.41	4.34	0.52
GEI 2013	SW11	8.41	6.43	0.77
GEI 2013	SW11	8.41	7.21	0.86
GEI 2013	SW11	8.41	7.50	0.89
GEI 2013	SW11	8.41	7.90	0.94
GEI 2013	SW11	8.41	11.84	1.41
Mueller et al. 1991	A2	8.50	7.30	0.86
Butler et al. 1997	MN5	8.60	16.00	1.86
Mueller et al. 1991	R1	8.70	5.60	0.64
May et al. 2008	NSK	8.81	9.33	1.06
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	23.65	2.59
GEI 2013	SW2-1	9.14	24.84	2.72
GEI 2013	SW2-1	9.14	27.27	2.99
May et al. 2008	SSO	10.00	8.48	0.85
May et al. 2008	NSCL	10.70	10.80	1.01
May et al. 2008	SSAL	11.50	10.50	0.91
Lambing et al. 1994	S34	14.00	19.00	1.36
Lambing et al. 1994	S34	14.00	32.00	2.29
May et al. 2008	KR	17.20	7.78	0.45
Butler et al. 1994	RB1	21.00	5.10	0.24
May et al. 2008	NSP	24.00	10.30	0.43
Butler et al. 1994	GUN2	28.00	63.00	2.25
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
Lemly 1985	Belews Lake	45.53	43.66	0.96

US EPA ARCHIVE DOCUMENT

Common carp (*Cyprinus carpio*)



Median ratio: 1.29

R^2: 0.44
F: 89.7
df: 16
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
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

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Creek chub (*Semotilus atromaculatus*)				
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
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

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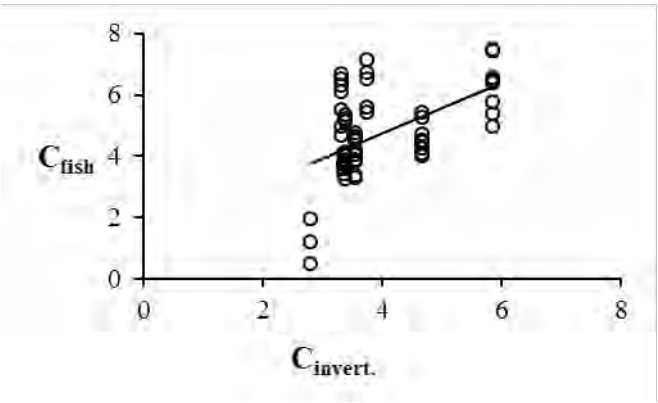
Creek chub (*Semotilus atromaculatus*)

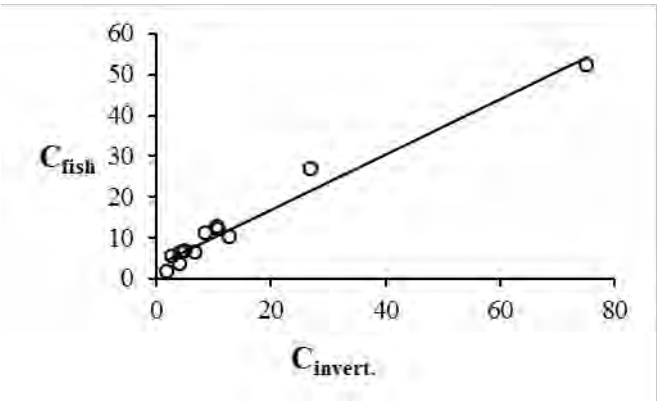


Median ratio: 1.12

R^2: 0.30
F: 25.11
df: 58
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



Median ratio: 1.07

R^2: 0.97
F: 325.2
df: 10
P: < 0.001

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	C_{invert}	C_{fish}	**Ratio**
Butler et al. 1997	MNP1	0.70	1.70	2.43
Butler et al. 1997	MNP1	0.70	1.80	2.57
Butler et al. 1995	AK	0.78	2.60	3.35
Butler et al. 1995	AK	0.78	2.80	3.61
Butler et al. 1995	AK	0.78	2.90	3.74
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	HD1	0.83	3.90	4.73
Butler et al. 1995	DD	0.86	3.40	3.95
Butler et al. 1995	DD	0.86	3.60	4.19
Butler et al. 1995	DD	0.86	3.90	4.53
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. 1993	D1	1.20	3.70	3.08
Butler et al. 1993	D1	1.20	3.80	3.17
Butler et al. 1995	ME2	1.25	4.80	3.84
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	SD	1.40	4.90	3.50
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. 1997	TRH	1.60	2.20	1.38
Butler et al. 1997	TRH	1.60	3.00	1.88
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. 1995	YJ2	1.65	4.00	2.42
Butler et al. 1995	YJ2	1.65	11.00	6.67
Birkner 1978	1	1.75	2.10	1.20
Birkner 1978	4	1.80	2.10	1.17
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
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
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
Butler et al. 1993	U1	2.45	6.40	2.61
Butler et al. 1995	ME3	2.55	4.30	1.69
Butler et al. 1995	ME3	2.55	4.40	1.73

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	ME3	2.55	5.30	2.08
Butler et al. 1994	AD	2.70	9.60	3.56
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	4.48	1.60
GEI 2013	SWA1	2.81	4.53	1.61
GEI 2013	SWA1	2.81	4.81	1.71
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
Butler et al. 1993	WSB2	3.00	8.10	2.70
Lambing et al. 1994	S48	3.05	2.50	0.82
Butler et al. 1993	SP2	3.15	8.20	2.60
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	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	6.11	1.84
GEI 2013	SW4-1	3.33	6.61	1.99
GEI 2013	SW4-1	3.33	6.67	2.01
GEI 2013	SW4-1	3.33	6.87	2.07
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
Butler et al. 1993	SP2	3.40	6.00	1.76
Butler et al. 1995	ME1	3.40	5.60	1.65
Butler et al. 1997	MUD2	3.45	6.50	1.88
Butler et al. 1997	MUD2	3.45	7.70	2.23
Butler et al. 1997	MUD2	3.45	12.00	3.48
GEI 2013	LG1	3.56	3.26	0.92
GEI 2013	LG1	3.56	3.35	0.94
GEI 2013	LG1	3.56	3.72	1.05
GEI 2013	LG1	3.56	4.09	1.15
GEI 2013	LG1	3.56	4.20	1.18

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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
Butler et al. 1993	WSB2	3.60	4.20	1.17
Butler et al. 1993	WSB2	3.60	10.00	2.78
Butler et al. 1994	CF1	3.60	7.90	2.19
GEI 2013	SWA1	3.64	3.62	1.00
GEI 2013	SWA1	3.64	3.72	1.02
GEI 2013	SWA1	3.64	4.07	1.12
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	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
Butler et al. 1993	SB2	3.65	9.90	2.71
Butler et al. 1993	R2	3.70	6.60	1.78
Butler et al. 1994	PSW1	3.70	22.00	5.95
Butler et al. 1993	SB2	3.75	5.70	1.52
Butler et al. 1993	SB2	3.75	8.60	2.29
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
Butler et al. 1993	R2	3.90	6.60	1.69
Butler et al. 1994	LSW1	3.90	73.00	18.72
GEI 2013	SW88	3.96	4.73	1.20
GEI 2013	SW88	3.96	4.96	1.25
GEI 2013	SW88	3.96	5.13	1.30
GEI 2013	SW88	3.96	5.55	1.40
GEI 2013	SW88	3.96	5.56	1.41
GEI 2013	SW88	3.96	5.86	1.48
GEI 2013	SW88	3.96	6.07	1.53
GEI 2013	SW88	3.96	6.32	1.60
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	ST2	4.10	7.60	1.85

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	ST2	4.10	16.00	3.90
Butler et al. 1997	MNP2	4.40	11.00	2.50
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
Butler et al. 1993	ST2	4.50	12.80	2.84
GEI 2013	CC1	4.69	5.92	1.26
GEI 2013	CC1	4.69	6.49	1.39
GEI 2013	CC1	4.69	7.14	1.52
GEI 2013	CC1	4.69	7.59	1.62
GEI 2013	CC1	4.69	7.68	1.64
Butler et al. 1993	WSB2	4.75	17.10	3.60
Butler et al. 1991	10	4.80	8.10	1.69
Butler et al. 1994	TGC	4.90	11.00	2.24
Lemly 1985	Badin Lake	5.18	2.54	0.49
Lambing et al. 1994	S39	5.85	7.90	1.35
Lambing et al. 1994	S39	5.85	21.00	3.59
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	9.21	1.57
GEI 2013	CC1	5.86	9.70	1.66
GEI 2013	CC1	5.86	11.69	2.00
Butler et al. 1991	3	6.20	9.50	1.53
Lambing et al. 1994	S46	6.20	5.10	0.82
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	SW2-1	6.60	12.51	1.90
GEI 2013	SW2-1	6.60	12.83	1.95
GEI 2013	SW2-1	6.60	14.80	2.24

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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	20.13	3.05
GEI 2013	SW2-1	6.60	20.66	3.13
GEI 2013	SW2-1	6.60	26.75	4.06
GEI 2013	SW2-1	6.60	30.48	4.62
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
Lemly 1985	High Rock Lake	6.75	3.21	0.48
GEI 2013	SWB	7.06	7.38	1.05
GEI 2013	SWB	7.06	8.49	1.20
GEI 2013	SWB	7.06	8.61	1.22
GEI 2013	SWB	7.06	8.72	1.24
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	7.06	9.80	1.39
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	SWB	7.44	10.97	1.48
GEI 2013	SWB	7.44	11.22	1.51
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
Butler et al. 1994	CRC	7.50	20.40	2.72
GEI 2013	SW1	7.82	8.45	1.08
GEI 2013	SW1	7.82	8.88	1.14
GEI 2013	SW1	7.82	9.11	1.16
GEI 2013	SW1	7.82	9.15	1.17
GEI 2013	SW1	7.82	9.41	1.20
GEI 2013	SW1	7.82	9.82	1.26
GEI 2013	SW1	7.82	11.07	1.42
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

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Fathead minnow (*Pimephales promelas*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1994	IW	8.35	10.00	1.20
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	SW11	8.41	5.38	0.64
GEI 2013	SW11	8.41	5.70	0.68
GEI 2013	SW11	8.41	7.05	0.84
Butler et al. 1997	MN5	8.60	7.30	0.85
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
Butler et al. 1997	WCP	9.70	10.00	1.03
Butler et al. 1997	WCP	9.70	15.00	1.55
Birkner 1978	22	11.30	11.00	0.97
Lambing et al. 1994	S34	14.00	25.00	1.79
Lambing et al. 1994	S11	14.50	11.00	0.76
Lambing et al. 1994	S11	14.50	33.00	2.28
Birkner 1978	23	15.50	34.50	2.23
Butler et al. 1994	GUN2	28.00	7.50	0.27
Butler et al. 1994	MKP	32.00	51.00	1.59
Birkner 1978	27	34.60	79.00	2.28
Lemly 1985	Belews Lake	45.53	23.03	0.51
Butler et al. 1994	OMD	73.00	13.00	0.18

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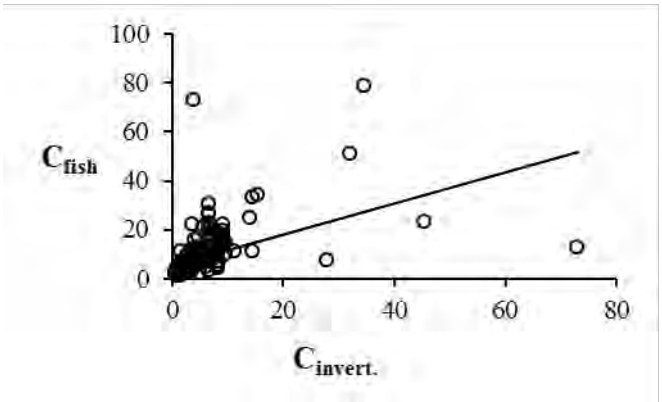
Fathead minnow (*Pimephales promelas*)

Study	Site	C_{invert}	C_{fish}	Ratio



Median ratio: 1.57

R^2: 0.22
F: 64.4
df: 232
P: < 0.001

Flannelmouth sucker (*Catostomus latipinnis*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1995	AK	0.78	0.82	1.06
Butler et al. 1995	AK	0.78	0.90	1.16
Butler et al. 1995	AK	0.78	1.10	1.42
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. 1993	LP3	1.12	0.92	0.83
Butler et al. 1993	LP3	1.12	1.40	1.26
Butler et al. 1995	ME2	1.25	1.40	1.12
Butler et al. 1995	ME2	1.25	1.60	1.28
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. 1993	P1	1.50	2.40	1.60
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

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Flannelmouth sucker (*Catostomus latipinnis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1994	COL1	1.50	1.90	1.27
Butler et al. 1995	ME4	1.55	1.30	0.84
Butler et al. 1995	ME4	1.55	1.50	0.97
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. 1994	RB3	1.60	29.00	18.13
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. 1995	YJ2	1.65	1.60	0.97
Butler et al. 1995	YJ2	1.65	2.40	1.45
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
Butler et al. 1993	P1	1.95	1.50	0.77
Butler et al. 1997	MUD	2.30	2.70	1.17
Butler et al. 1997	MUD	2.30	4.10	1.78
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	1.50	0.60
Butler et al. 1995	SJ1	2.50	2.20	0.88
Butler et al. 1995	SJ1	2.50	3.19	1.27
Butler et al. 1995	SJ1	2.50	4.20	1.68
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. 1997	MN4	2.65	5.10	1.92
Butler et al. 1997	MN4	2.65	9.60	3.62
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	MN1	2.70	6.50	2.41
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. 1993	LP4	3.20	2.40	0.75
Butler et al. 1993	LP4	3.20	2.60	0.81
Butler et al. 1994	PSW1	3.70	9.40	2.54
Butler et al. 1994	LSW1	3.90	6.70	1.72
Butler et al. 1991	4	3.90	1.70	0.44

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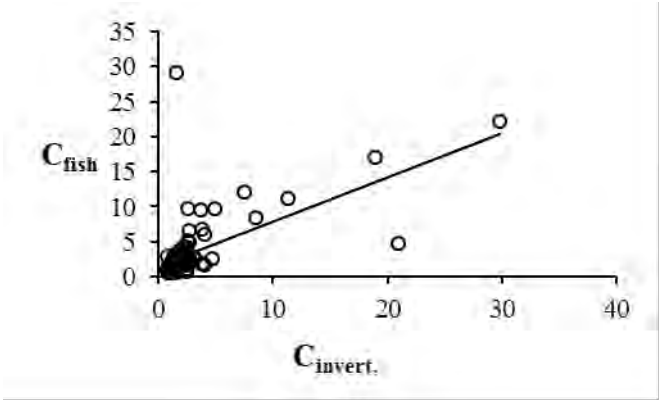
Flannelmouth sucker (*Catostomus latipinnis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1991	9	4.10	1.50	0.37
Butler et al. 1991	9	4.10	6.00	1.46
Butler et al. 1991	10	4.80	2.50	0.52
Butler et al. 1994	BSW1	5.00	9.60	1.92
Butler et al. 1994	CRC	7.50	12.00	1.60
Butler et al. 1997	MN5	8.60	8.40	0.98
Butler et al. 1997	NW2	11.40	11.00	0.96
Butler et al. 1994	LZA1	19.00	17.00	0.89
Butler et al. 1994	RB1	21.00	4.60	0.22
Butler et al. 1991	7	29.80	22.00	0.74



Median ratio: 1.06

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

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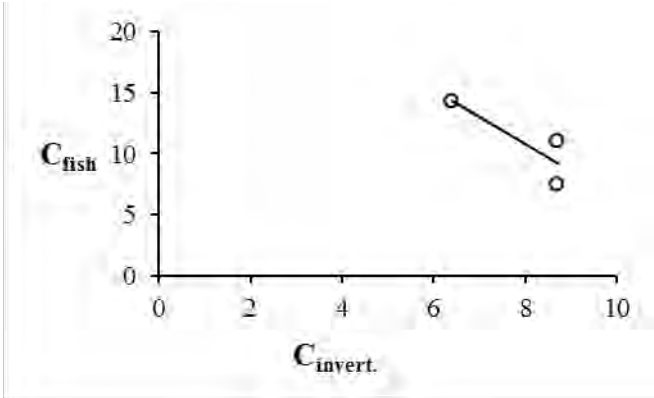
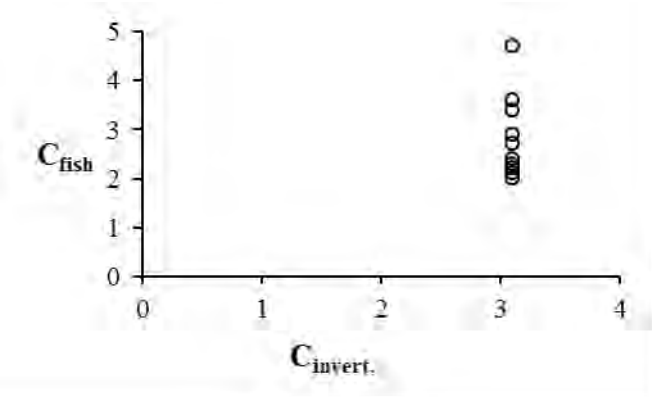


Median ratio: 1.26

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
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

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Green sunfish (*Lepomis cyanellus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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	MP	1.60	1.90	1.19
Butler et al. 1997	TRH	1.60	3.30	2.06
Butler et al. 1997	TR25	1.80	4.40	2.44
Butler et al. 1995	ME3	2.55	5.00	1.96
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
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
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
Butler et al. 1997	MUD2	3.45	7.00	2.03
Butler et al. 1997	MUD2	3.45	7.60	2.20
GEI 2013	SW88	3.96	7.14	1.81
GEI 2013	SW88	3.96	7.41	1.87
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
Butler et al. 1991	10	4.80	7.90	1.65
Lemly 1985	Badin Lake	5.18	3.43	0.66
Butler et al. 1991	3	6.20	6.40	1.03
Lemly 1985	High Rock Lake	6.75	3.30	0.49
GEI 2013	SWB	7.44	11.94	1.61
Butler et al. 1997	CH1	7.50	9.50	1.27
GEI 2013	SW11	8.41	4.54	0.54
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
Butler et al. 1994	LZA1	19.00	37.00	1.95
Butler et al. 1991	7	29.80	15.20	0.51

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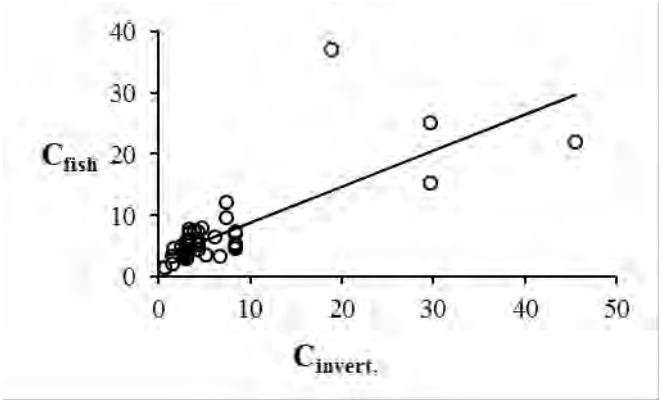
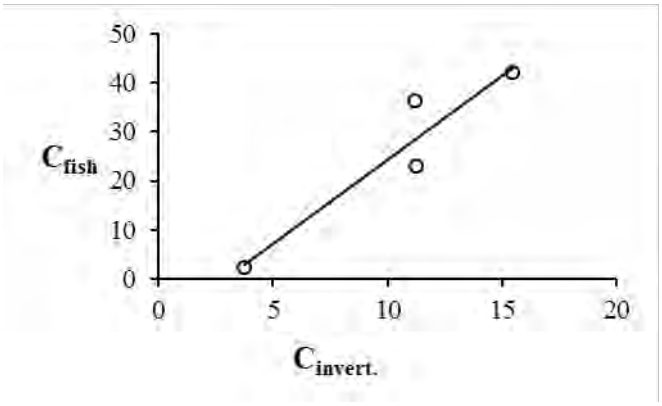
Green sunfish (*Lepomis cyanellus*)				
Butler et al. 1991	7	29.80	25.10	0.84
Lemly 1985	Belews Lake	45.53	21.96	0.48



Median ratio: 1.27

R^2: 0.59
F: 57.9
df: 41
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



Median ratio: 2.37

R^2: 0.90
F: 17.3
df: 2
P: 0.055
Not used because P > 0.05

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Largemouth bass (*Micropterus salmoides*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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	ET7	0.86	0.86	1.00
Saiki et al. 1993	ET7	0.86	1.00	1.16
Saiki et al. 1993	SJR1	0.95	0.80	0.85
Saiki et al. 1993	SJR1	0.95	1.80	1.90
Rinella and Schuler 1992	Malheur Lake	1.20	0.92	0.77
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR3	1.50	1.80	1.20
Butler et al. 1995	MP	1.60	1.40	0.88
GEI 2013	SWA1	2.81	3.17	1.13
Garcia-Hernandez et al. 2000	Cienga Wetland	3.00	5.10	1.70
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR2	3.30	2.40	0.73
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	LG1	3.39	4.29	1.27
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
Saiki et al. 1993	GT4	4.05	4.00	0.99
Saiki et al. 1993	GT4	4.05	4.70	1.16
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
Saiki et al. 1993	GT5	4.90	6.80	1.39
Saiki et al. 1993	GT5	4.90	6.90	1.41
GEI 2013	SW11	8.41	5.02	0.60
GEI 2013	SW11	8.41	5.19	0.62
GEI 2013	SW11	8.41	5.77	0.69
GEI 2013	SW11	8.41	6.26	0.74

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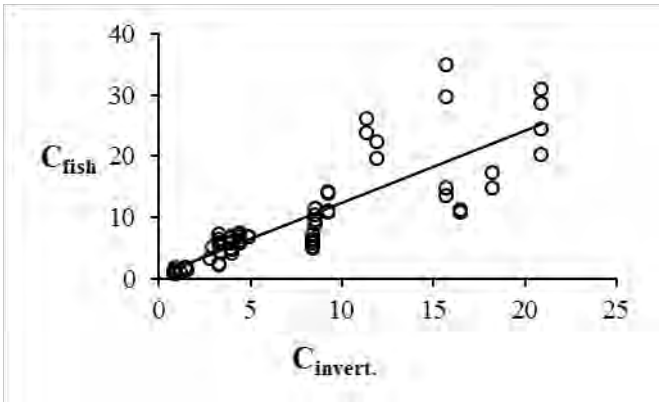
Largemouth bass (*Micropterus salmoides*)

Study	Site	C_{invert}	C_{fish}	Ratio
GEI 2013	SW11	8.41	6.48	0.77
GEI 2013	SW11	8.41	7.22	0.86
Crutchfield 2000	transect 3	8.60	8.92	1.04
Crutchfield 2000	transect 3	8.60	9.50	1.10
Crutchfield 2000	transect 3	8.60	10.32	1.20
Crutchfield 2000	transect 3	8.60	11.40	1.33
Crutchfield 2000	transect 3	9.25	10.70	1.16
Crutchfield 2000	transect 3	9.25	10.96	1.18
Crutchfield 2000	transect 3	9.25	14.00	1.51
Crutchfield 2000	transect 3	9.25	14.20	1.54
Crutchfield 2000	transect 3	11.40	23.70	2.08
Crutchfield 2000	transect 3	11.40	26.12	2.29
Crutchfield 2000	transect 3	11.95	19.62	1.64
Crutchfield 2000	transect 3	11.95	22.30	1.87
Crutchfield 2000	transect 4	15.70	13.50	0.86
Crutchfield 2000	transect 4	15.70	14.78	0.94
Crutchfield 2000	transect 4	15.70	29.69	1.89
Crutchfield 2000	transect 4	15.70	34.90	2.22
Crutchfield 2000	transect 4	16.45	10.70	0.65
Crutchfield 2000	transect 4	16.45	11.20	0.68
Crutchfield 2000	transect 4	18.25	14.78	0.81
Crutchfield 2000	transect 4	18.25	17.20	0.94
Crutchfield 2000	transect 4	20.90	20.20	0.97
Crutchfield 2000	transect 4	20.90	24.34	1.16
Crutchfield 2000	transect 4	20.90	28.60	1.37
Crutchfield 2000	transect 4	20.90	30.83	1.48



Median ratio: 1.27

R^2: 0.72
F: 164.3
df: 65
P: < 0.001

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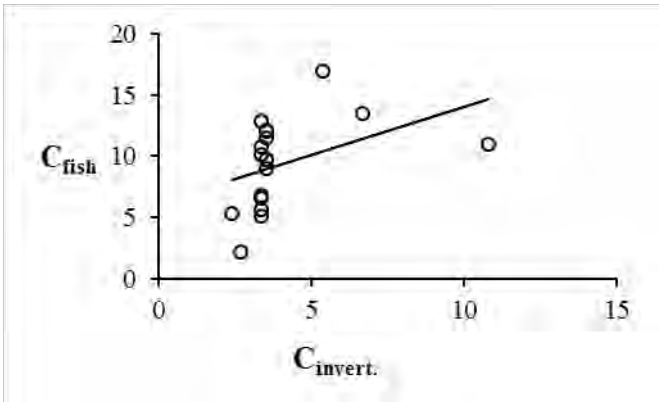
Longnose dace (*Rhinichthys cataractae*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Lambing et al. 1994	S33	2.40	5.30	2.21
Mueller et al. 1991	A1	2.70	2.10	0.78
GEI 2013	LG1	3.37	5.05	1.50
GEI 2013	LG1	3.37	5.57	1.65
GEI 2013	LG1	3.37	6.57	1.95
GEI 2013	LG1	3.37	6.75	2.00
GEI 2013	LG1	3.37	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
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: 2.52

R^2: 0.17
F: 3.16
df: 15
P: 0.07
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

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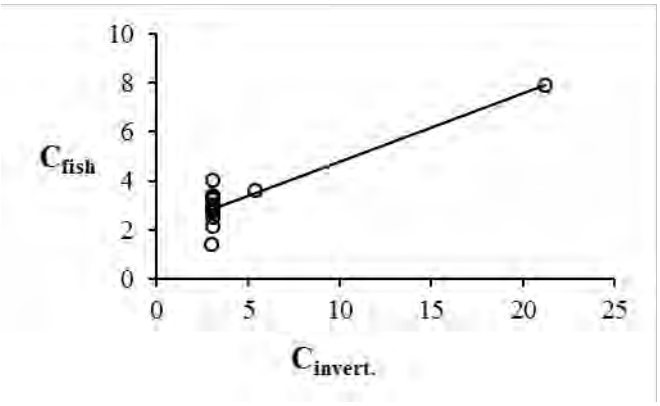
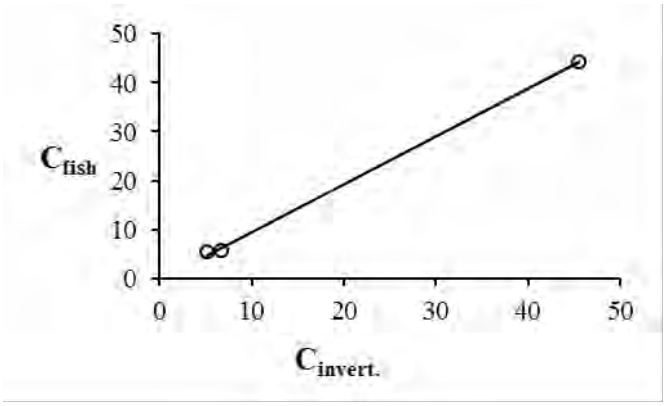
Longnose sucker (*Catostomus catostomus*)				
Butler et al. 1994	NFK2	3.10	3.00	0.97
Butler et al. 1994	NFK2	3.10	3.20	1.03
Butler et al. 1994	NFK2	3.10	3.30	1.06
Butler et al. 1994	NFK2	3.10	3.40	1.10
Butler et al. 1994	NFK2	3.10	4.00	1.29
Mueller et al. 1991	T1	5.40	3.60	0.67
Minnow 2007	FL17	21.22	7.90	0.37



Median ratio: 0.90

R^2: 0.83
F: 54.7
df: 11
P: <0.001

Mosquitofish (*Gambusia sp.*)				
Study	**Site**	C_{invert}	C_{fish}	**Ratio**
Lemly 1985	Badin Lake	5.18	5.44	1.05
Lemly 1985	High Rock Lake	6.75	5.75	0.85
Lemly 1985	Belews Lake	45.53	44.15	0.97



Median ratio: 0.97

R^2: 1.00
F: 1326
df: 1
P: 0.019

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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
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

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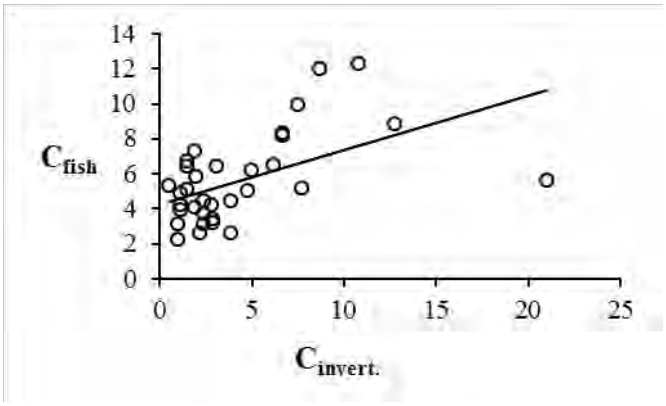
Mottled sculpin (*Cottus bairdii*)

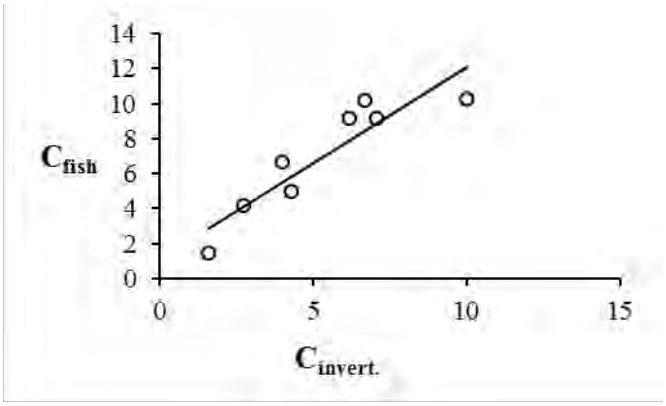


Median ratio: 1.38

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
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: 30.3
df: 6
P: <0.001

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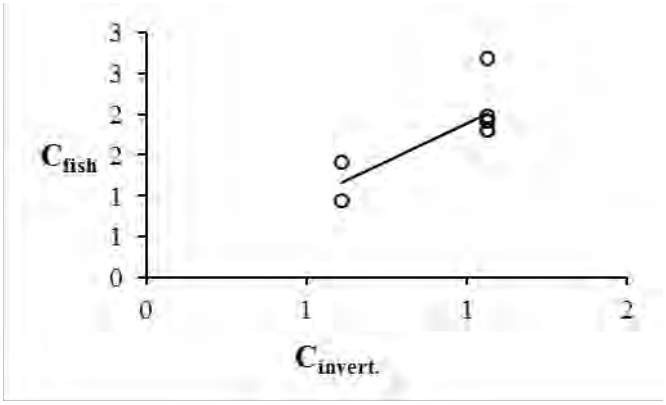
Northern pike (*Esox lucius*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	PU	0.61	0.93	1.52
Butler et al. 1995	PU	0.61	1.40	2.30
Butler et al. 1995	TT	1.07	1.80	1.69
Butler et al. 1995	TT	1.07	1.80	1.69
Butler et al. 1995	TT	1.07	1.90	1.78
Butler et al. 1995	TT	1.07	1.91	1.79
Butler et al. 1995	TT	1.07	1.97	1.85
Butler et al. 1995	TT	1.07	2.68	2.51



Median ratio: 1.79

R^2: 0.62
F: 9.63
df: 6
P: 0.013

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

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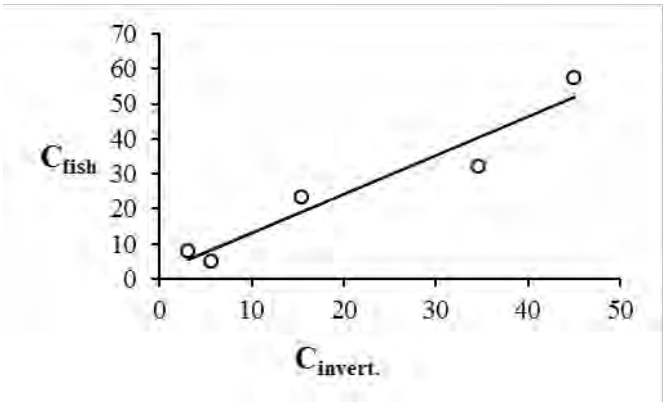

Median ratio: 1.27

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. 1993	LP2	1.00	1.27	1.27
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. 1995	MP	1.60	2.10	1.31
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	3.06	1.91
Butler et al. 1994	NFK3	2.00	4.70	2.35
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	CHK	2.40	2.20	0.92
Butler et al. 1997	CHK	2.40	2.29	0.96
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	2.60	0.96
Butler et al. 1997	MN3	2.70	3.69	1.37
Butler et al. 1997	MN3	2.70	4.90	1.81
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. 1994	NFK2	3.10	3.60	1.16
Butler et al. 1994	NFK2	3.10	35.68	11.51
Butler et al. 1993	F2	3.90	7.60	1.95
Butler et al. 1991	4	3.90	3.50	0.90
Casey 2005	Deerlick Cr.	4.45	2.09	0.47
Casey 2005	Deerlick Cr.	4.45	3.34	0.75

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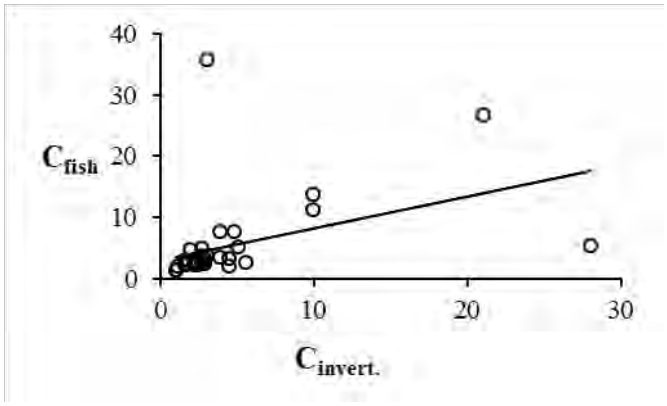
Rainbow trout (*Oncorhynchus mykiss*)				
Butler et al. 1993	F2	4.80	7.60	1.58
Butler et al. 1997	WBR	5.05	5.10	1.01
Low and Mullins 1990	5	5.60	2.60	0.46
Casey 2005	Luscar Cr.	9.95	11.16	1.12
Casey 2005	Luscar Cr.	9.95	13.71	1.38
Butler et al. 1994	HCC1	21.00	26.76	1.27
Butler et al. 1994	GUN2	28.00	5.40	0.19



Median ratio: 1.19

R^2: 0.16
F: 6.22
df: 32
P: 0.005

Red shiner (*Cyprinella lutrensis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	ME4	1.55	5.10	3.29
Butler et al. 1995	YJ2	1.65	4.50	2.73
Butler et al. 1995	SJ1	2.50	3.50	1.40
Butler et al. 1995	ME3	2.55	4.20	1.65
Butler et al. 1995	ME3	2.55	4.60	1.80
Butler et al. 1997	MN4	2.65	4.20	1.58
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
Lemly 1985	Badin Lake	5.18	3.56	0.69
Mueller et al. 1991	A3	6.00	8.10	1.35
Butler et al. 1991	3	6.20	7.70	1.24
Lemly 1985	High Rock Lake	6.75	3.70	0.55
May et al. 2008	SSW	7.60	10.00	1.32
Butler et al. 1994	IW	8.35	83.00	9.94
May et al. 2008	SSAU	8.35	11.20	1.34
Mueller et al. 1991	A2	8.50	7.90	0.93
Butler et al. 1997	MN5	8.60	4.40	0.51

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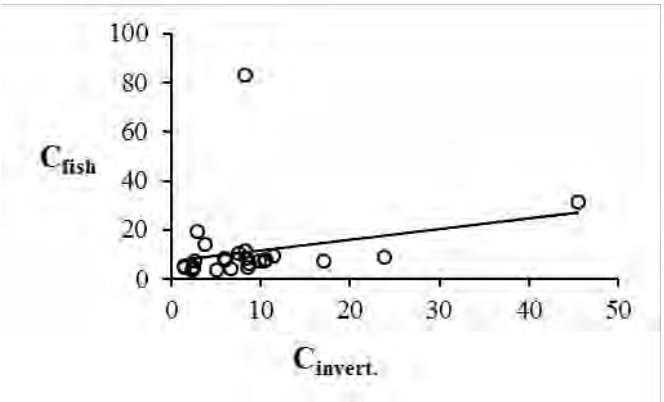
Red shiner (*Cyprinella lutrensis*)				
Study	**Site**	C_{invert}	C_{fish}	**Ratio**
May et al. 2008	NSK	8.81	5.81	0.66
May et al. 2008	SSO	10.00	7.16	0.72
May et al. 2008	NSCU	10.50	7.24	0.69
May et al. 2008	NSCL	10.70	7.36	0.69
May et al. 2008	SSAL	11.50	9.00	0.78
May et al. 2008	KR	17.20	7.03	0.41
May et al. 2008	NSP	24.00	8.62	0.36
Lemly 1985	Belews Lake	45.53	30.92	0.68



Median ratio: 1.28

R^2: 0.06
F: 1.59
df: 24
P: 0.224
Not used because P > 0.05

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

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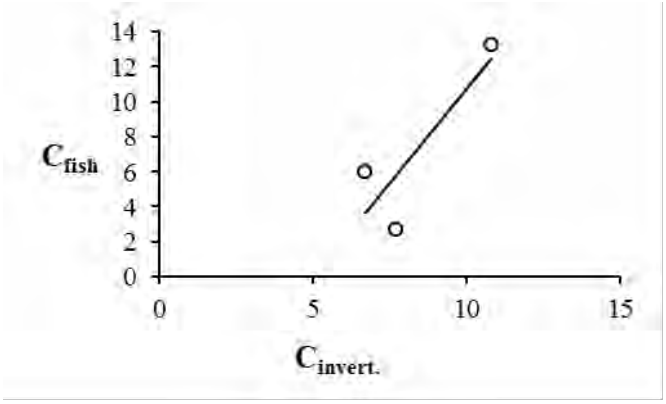

Median ratio: 0.90

R^2: 0.73
F: 2.68
df: 1
P: 0.397
Not used because P > 0.05

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

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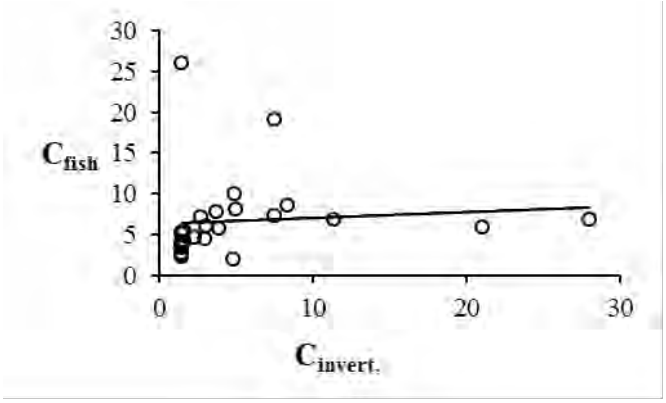
Roundtail chub (*Gila robusta*)



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

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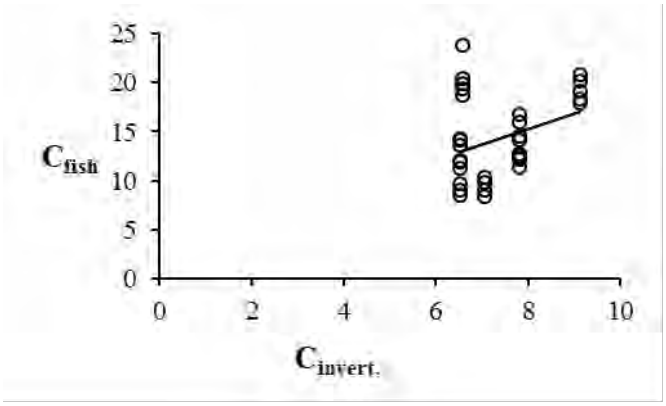
Sand shiner (*Notropis stramineus*)				
GEI 2013	SW1	7.82	14.43	1.85
GEI 2013	SW1	7.82	15.87	2.03
GEI 2013	SW1	7.82	16.63	2.13
GEI 2013	SW2-1	9.14	17.84	1.95
GEI 2013	SW2-1	9.14	18.21	1.99
GEI 2013	SW2-1	9.14	18.98	2.08
GEI 2013	SW2-1	9.14	20.12	2.20
GEI 2013	SW2-1	9.14	20.73	2.27



Median ratio: 1.83

R^2: 0.12
F: 4.12
df: 31
P: 0.026

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

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Sculpin (*Cottoidea*)				
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
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

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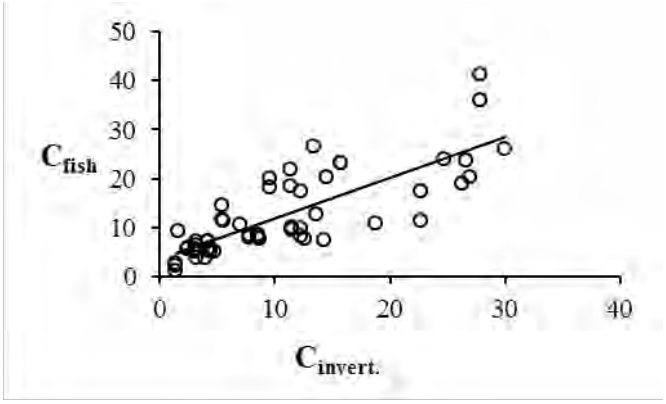
Sculpin (*Cottoidea*)

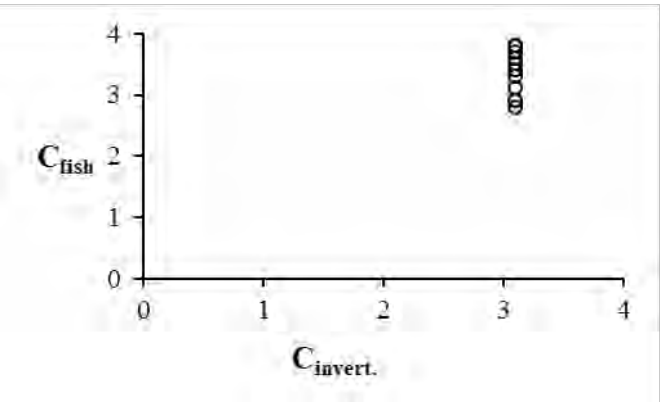


Median ratio: 1.29

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

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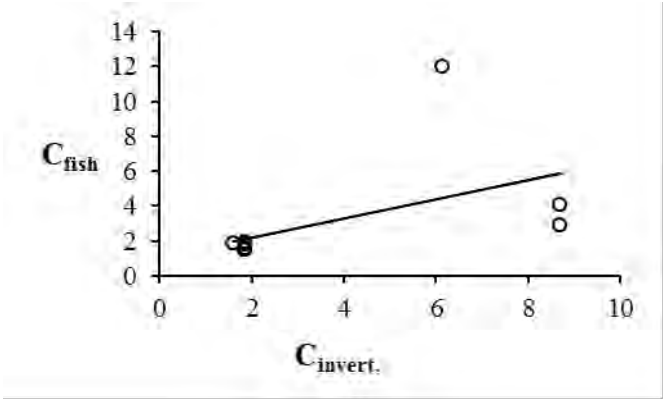
Smallmouth bass (*Micropterus dolomieu*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	MP	1.60	1.90	1.19
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	SU	1.85	1.50	0.81
Butler et al. 1995	SU	1.85	1.53	0.83
Butler et al. 1995	SU	1.85	1.55	0.84
Butler et al. 1995	SU	1.85	1.78	0.96
Butler et al. 1995	SU	1.85	1.91	1.03
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



Median ratio: 0.83

R^2: 0.26
F: 2.81
df: 8
P: 0.119
Not used because P > 0.05

Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Hamilton and Buhl 2004	USC	0.50	6.90	13.80
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	AK	0.78	4.30	5.55
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	DD	0.86	4.40	5.12
Butler et al. 1995	DD	0.86	5.60	6.51
Butler et al. 1995	DD	0.86	6.00	6.98
Butler et al. 1993	LP3	1.12	6.00	5.38
Butler et al. 1993	D1	1.20	3.40	2.83
Butler et al. 1993	D1	1.20	3.50	2.92

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Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1993	D1	1.20	3.70	3.08
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. 1995	ME2	1.25	6.10	4.88
Butler et al. 1993	B2	1.35	5.80	4.30
Butler et al. 1993	D2	1.45	4.90	3.38
Butler et al. 1993	D2	1.45	6.50	4.48
Butler et al. 1993	D2	1.45	6.80	4.69
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. 1995	YJ2	1.65	6.30	3.82
Butler et al. 1995	YJ2	1.65	6.50	3.94
Butler et al. 1995	YJ2	1.65	7.10	4.30
Butler et al. 1997	MNQ	1.80	5.90	3.28
Butler et al. 1993	P1	1.95	5.50	2.82
Butler et al. 1994	NFK3	2.00	7.10	3.55
Butler et al. 1993	SB1	2.15	7.80	3.63
Butler et al. 1993	SB1	2.15	9.50	4.42
Butler et al. 1993	SB1	2.15	10.00	4.65
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. 1993	ST1	2.25	6.80	3.02
Butler et al. 1997	MUD	2.30	6.10	2.65
Butler et al. 1997	MUD	2.30	7.20	3.13
Butler et al. 1997	CHK	2.40	3.80	1.58
Butler et al. 1997	CHK	2.40	5.20	2.17
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. 1995	SJ1	2.50	2.90	1.16

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Speckled dace (*Rhinichthys osculus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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	ME3	2.55	2.80	1.10
Butler et al. 1995	ME3	2.55	5.50	2.16
Butler et al. 1995	ME3	2.55	7.00	2.75
Butler et al. 1997	MN4	2.65	7.90	2.98
Butler et al. 1995	MN1	2.70	5.50	2.04
Butler et al. 1997	MN3	2.70	4.30	1.59
Butler et al. 1997	MN3	2.70	6.00	2.22
Butler et al. 1993	SP2	2.75	12.00	4.36
Butler et al. 1997	MN1	2.90	3.70	1.28
Butler et al. 1993	SP1	2.95	7.00	2.37
Butler et al. 1993	SP1	2.95	7.30	2.47
Butler et al. 1993	SP1	2.95	8.90	3.02
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	LW	3.00	62.00	20.67
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
Butler et al. 1994	NFK2	3.10	6.70	2.16
Butler et al. 1994	NFK2	3.10	6.90	2.23
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. 1993	LP4	3.20	8.70	2.72
Butler et al. 1993	ST2	3.35	9.30	2.78
Butler et al. 1995	ME1	3.40	6.40	1.88
Butler et al. 1993	WSB2	3.60	11.70	3.25
Butler et al. 1993	SB2	3.60	12.10	3.36
Butler et al. 1994	CF1	3.60	6.10	1.69
Butler et al. 1994	PSW1	3.70	13.00	3.51
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	R2	3.90	6.00	1.54
Butler et al. 1993	F2	3.90	8.90	2.28
Butler et al. 1994	LSW1	3.90	83.00	21.28
Butler et al. 1993	R1	4.00	8.50	2.13

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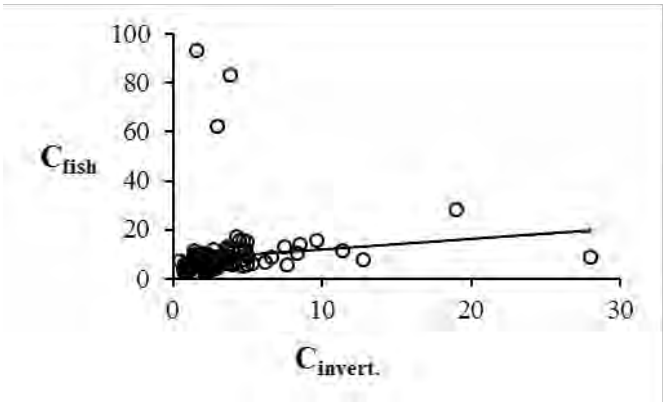
Speckled dace (*Rhinichthys osculus*)

Study	Site	C_{invert}	C_{fish}	Ratio
Butler et al. 1991	9	4.10	5.70	1.39
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	R2	4.30	17.10	3.98
Butler et al. 1993	ST2	4.50	15.70	3.49
Butler et al. 1993	WSB2	4.75	15.60	3.28
Butler et al. 1991	10	4.80	4.80	1.00
Butler et al. 1994	SMF	4.80	7.80	1.63
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. 1997	WBR	5.05	5.50	1.09
Butler et al. 1997	WBR	5.05	9.70	1.92
Butler et al. 1995	NW	5.10	8.70	1.71
Hamilton and Buhl 2005	LiB	5.40	5.80	1.07
Butler et al. 1991	3	6.20	6.50	1.05
Hamilton and Buhl 2004	ACM	6.70	8.50	1.27
Butler et al. 1994	CRC	7.50	13.00	1.73
Hamilton and Buhl 2004	LBR	7.70	5.60	0.73
Butler et al. 1994	IW	8.35	10.00	1.20
Butler et al. 1997	MN5	8.60	14.00	1.63
Hamilton and Buhl 2005	SLC	9.70	15.20	1.57
Butler et al. 1997	NW2	11.40	11.00	0.96
Hamilton and Buhl 2004	DVC	12.80	7.50	0.59
Butler et al. 1994	LZA1	19.00	28.00	1.47
Butler et al. 1994	GUN2	28.00	8.90	0.32



Median ratio: 2.79

R^2: 0.01
F: 1.71
df: 118
P: 0.185
Not used because P > 0.05

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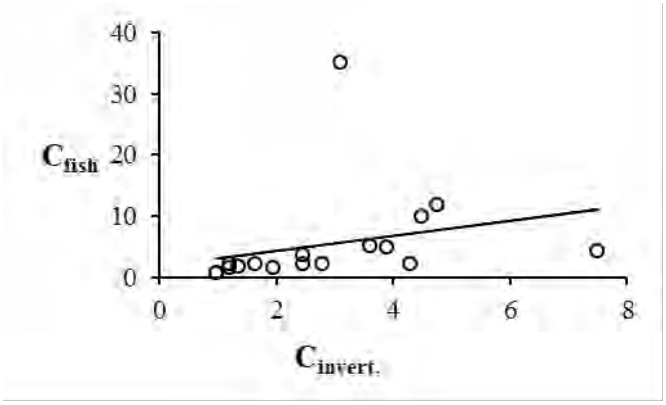
Sucker (*Catostomidae*)

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
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

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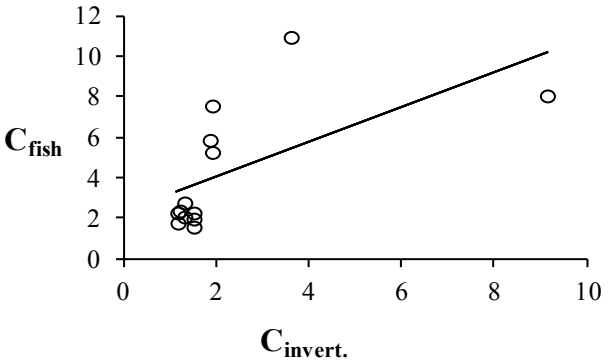
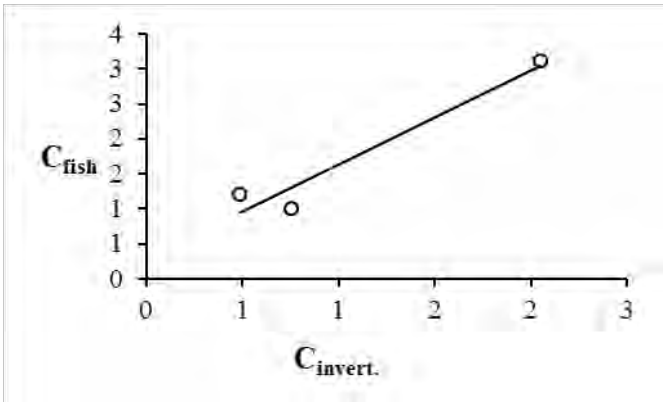
Sunfish (*Centrarchidae*)				
Welsh and Maughan 1994	Pretty Water	1.50	2.30	1.53
Welsh and Maughan 1994	Cibola Lake	1.85	5.90	3.19
Welsh and Maughan 1994	Cibola Lake	1.90	5.30	2.79
Welsh and Maughan 1994	Cibola Lake	1.90	7.60	4.00
Welsh and Maughan 1994	Oxbow Lake	3.60	11.00	3.06
GEI 2013	SW2-1	9.14	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

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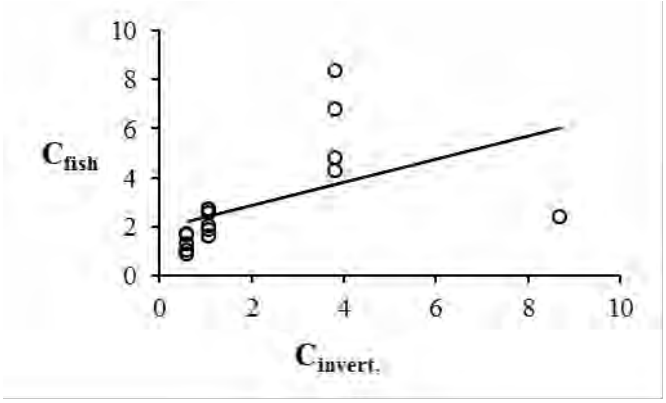
Walleye (*Sander vitreus*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
Butler et al. 1995	PU	0.61	0.89	1.46
Butler et al. 1995	PU	0.61	1.00	1.64
Butler et al. 1995	PU	0.61	1.27	2.09
Butler et al. 1995	PU	0.61	1.66	2.72
Butler et al. 1995	PU	0.61	1.72	2.82
Butler et al. 1995	TT	1.07	1.60	1.50
Butler et al. 1995	TT	1.07	1.86	1.75
Butler et al. 1995	TT	1.07	2.00	1.88
Butler et al. 1995	TT	1.07	2.55	2.39
Butler et al. 1995	TT	1.07	2.68	2.51
Butler et al. 1995	TT	1.07	2.68	2.51
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
Mueller et al. 1991	R1	8.70	2.40	0.28



Median ratio: 1.82

R^2: 0.24
F: 4.46
df: 14
P: 0.032

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Western mosquitofish (*Gambusia affinis*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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	ET7	0.86	0.90	1.05
Saiki et al. 1993	ET7	0.86	1.00	1.16
Saiki et al. 1993	SJR1	0.95	0.95	1.01
Saiki et al. 1993	SJR1	0.95	1.30	1.38
Saiki and Lowe 1987	Volta Pond 26	1.42	1.24	0.87
Saiki and Lowe 1987	Volta Pond 26	1.42	1.28	0.90
Saiki et al. 1993	SJR3	1.50	1.70	1.13
Saiki et al. 1993	SJR3	1.50	2.00	1.33
Saiki and Lowe 1987	Volta Wasteway	2.23	1.35	0.61
Saiki and Lowe 1987	Volta Wasteway	2.23	1.36	0.61
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
Saiki et al. 1993	SJR2	3.30	2.20	0.67
Saiki et al. 1993	SJR2	3.30	4.50	1.36
GEI 2013	SWA1	3.64	2.91	0.80
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	GT5	4.90	11.00	2.24
Saiki et al. 1993	GT5	4.90	16.00	3.27
Saiki and Lowe 1987	Kesterson Pond 11	60.65	104.00	1.71
Saiki and Lowe 1987	Kesterson Pond 11	60.65	130.00	2.14
Saiki and Lowe 1987	Kesterson Pond 8	102.50	164.00	1.60
Saiki and Lowe 1987	Kesterson Pond 8	102.50	223.00	2.18
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	San Luis Drain	190.00	149.00	0.78
Saiki and Lowe 1987	San Luis Drain	190.00	332.00	1.75

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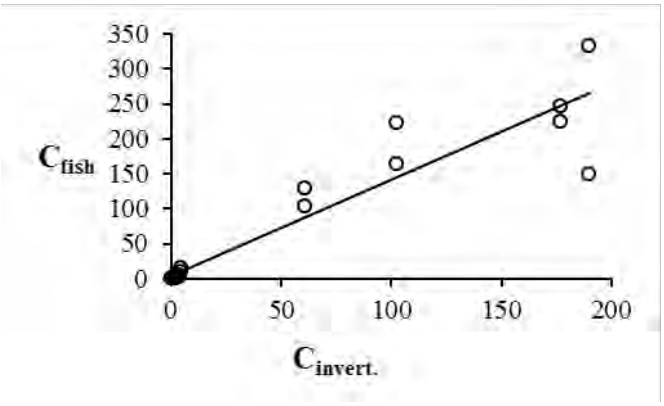
Western mosquitofish (*Gambusia affinis*)

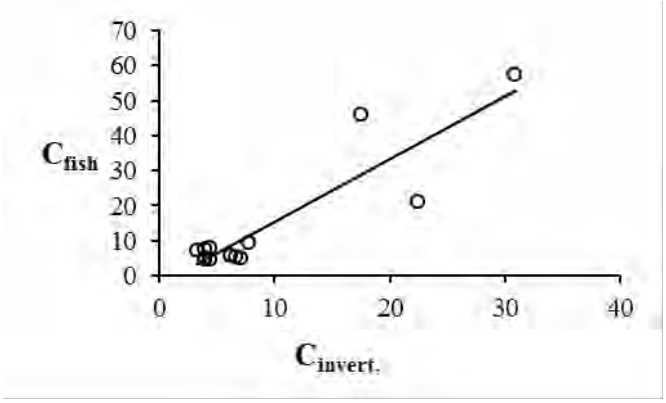


Median ratio: 1.25

R^2: 0.90
F: 256.4
df: 30
P: <0.001

Western cutthroat trout (*Oncorhynchus clarkii lewisi*)

Study	Site	C_{invert}	C_{fish}	Ratio
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
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



Median ratio: 1.20

R^2: 0.81
F: 47.6
df: 11
P: <0.001

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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
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
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
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

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White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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
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

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White sucker (*Catostomus commersonii*)				
Study	**Site**	**C_{invert}**	**C_{fish}**	**Ratio**
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
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

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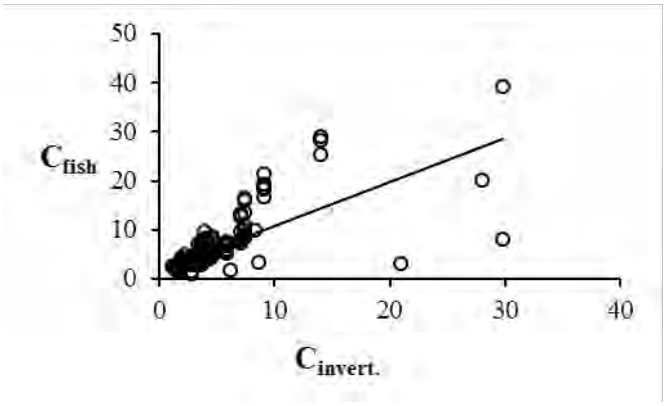
White sucker (*Catostomus commersonii*)

Study	Site	C_{invert}	C_{fish}	Ratio
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



Median ratio: 1.18

R^2: 0.49
F: 129.9
df: 134
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

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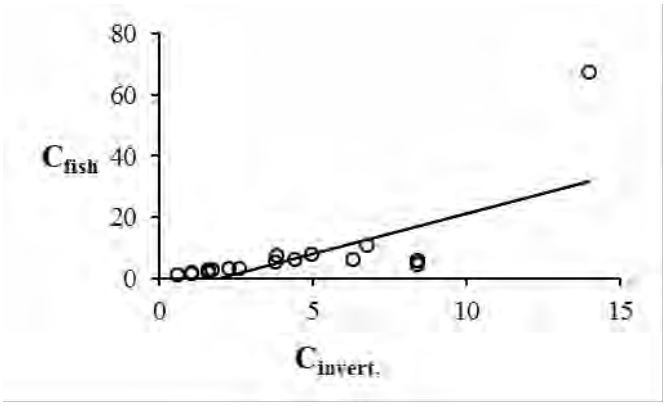
Yellow perch (*Perca flavescens*)

Study	Site	C_{invert}	C_{fish}	Ratio
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



Median ratio: 1.42

R^2: 0.46
F: 16.24
df: 19
P: <0.001

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Table B-6. Final EPA-derived Trophic Transfer Function (*TTF*)Values

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	2.18			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
Cutthroat tTrout		1.81	1.27	2.30
Dolly varden		1.26	1.27	1.61
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		1.02	1.27	1.30
Roundtail chub	2.07			2.07
Smallmouth bass	1.42			1.42
White sucker	1.41			1.41
Genus				
Catostomus				1.41
Esox				2.39
Lepomis				1.79
Micropterus				1.42
Oncorhynchus				2.37
Family				
Catostomidae				1.41
Centrarchidae				1.45
Cyprinidae				2.00
Salmonidae				1.96
Order				
Perciformes				1.45

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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				1.71

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APPENDIX C: Summaries of Chronic Studies

Considered For Criteria Derivation

White sturgeon C-2
Sacramento splittail C-6
Fathead minnow C-9
Flannelmouth & razorback suckers C-15
Northern pike C-17
Chinook salmon C-20
Rainbow trout & brook trout C-25
Cutthroat trout C-44
Dolly varden C-57
Brown trout C-60
Desert pupfish C-70
Eastern and western mosquitofish C-87
Striped bass C-89
Bluegill sunfish C-91
Largemouth bass C-133

See Appendix D for descriptions of other, less conclusive studies with:
Rainbow trout
Fathead minnow
Sacramento splittail
White sucker

See Appendix D 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

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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.

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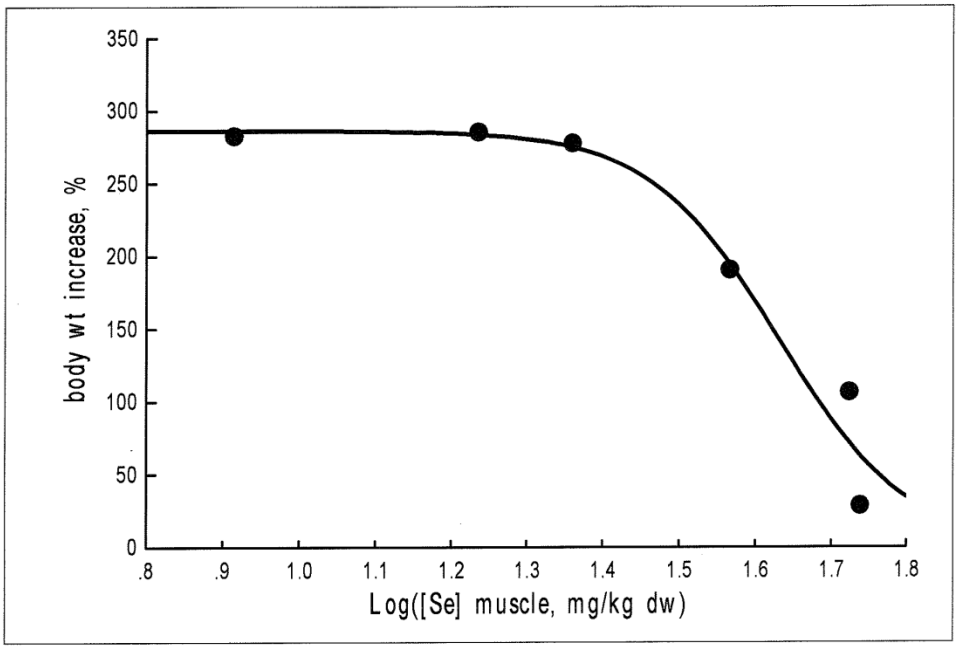
White sturgeon (Tashjian et al 2006)



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

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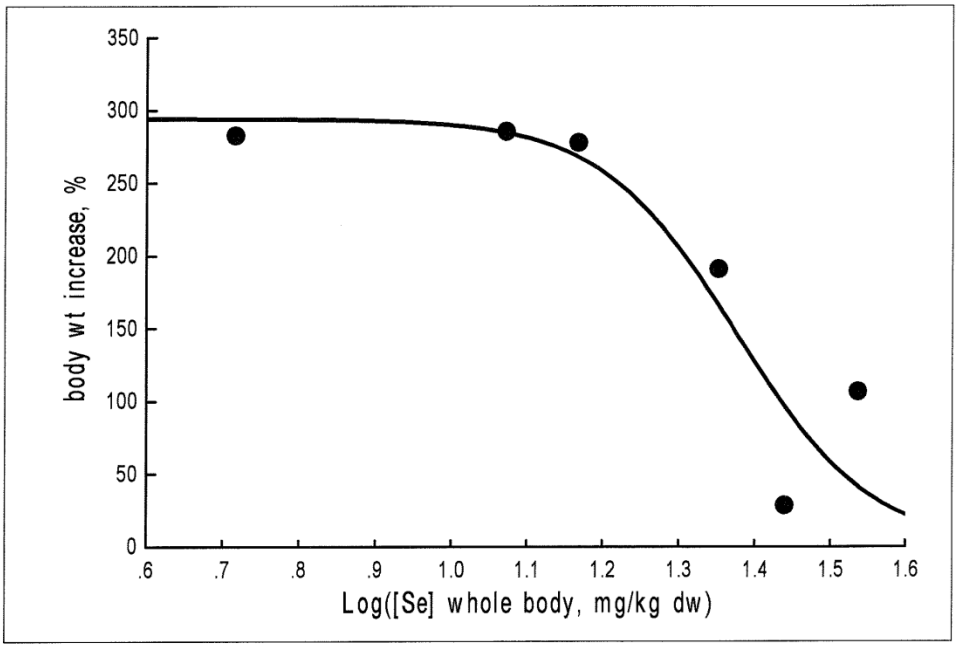
White sturgeon (Tashjian et al 2006)



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

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.

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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 degerneration	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)

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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.

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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.

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	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.

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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

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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.

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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.

US EPA ARCHIVE DOCUMENT

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 :g/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 :g 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 :g Se/L. Corresponding occurrence in control fish incubated in the egg cups was only 1 and 6 percent, respectively. Selenium residues in the ovaries of females from the control and treated streams were 0.77 and 5.89 mg Se/kg ww. Using 75.3 percent moisture content in the eggs/ovaries (average value for fathead minnow ovaries and eggs from GEI Consultants 2008 and Rickwood et al. 2008), these concentrations equate to 3.12 and 23.85 mg Se/kg dw.

Chronic Value: The NOAEC for egg/ovary is <23.85 mg Se/kg dw.

US EPA ARCHIVE DOCUMENT

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 :g/L selenate. Well water was used as the dilution water. Across the range of aqueous exposure concentrations, each test chamber was fed the same daily ration of living rotifers containing selenium at <0.702, 1.35, 2.02, 4.63, and 8.24 mg/kg dw, respectively. Rotifers accumulated selenium from algae (*Chlorella vulgaris*) exposed to 0, 25, 50, 100, and 200 :g/L selenate.

Study Design: Replicated (n=4) exposure beakers using a randomized, balanced 5x2 factorial design (1st factor - selenium; 2nd factor - species). Survival was monitored daily and growth measured at the end of the 28-day exposure. Selenium was measured in the larvae at the end of the 28-day exposure.

Effects Data : No survival effects were observed and there were no decreases in fish weight or length. Fish mass was found to increase as a function of selenium concentration.

Chronic Value: The chronic values for the flannelmouth sucker and razorback sucker were >10.2 and >12.9 mg Se/kg dw, respectively, based on the concentrations of selenium measured in whole-body tissue of larval fish at the highest water and dietary selenium concentrations.

US EPA ARCHIVE DOCUMENT

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 (1st factor - test water type; 2nd 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.

US EPA ARCHIVE DOCUMENT

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. Difference 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).

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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 10µ g/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 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.

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Mean selenium in northern pike egg and effect values for reference and exposure sites			
Sites	**[Se] in eggs, 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	13.20	0
exposure sites	34.00	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

Chronic Value: EC_{24} = 34.00 mg Se/kg dw in eggs. Note: an EC_{10} cannot be estimated with the data.

US EPA ARCHIVE DOCUMENT

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.

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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

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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.

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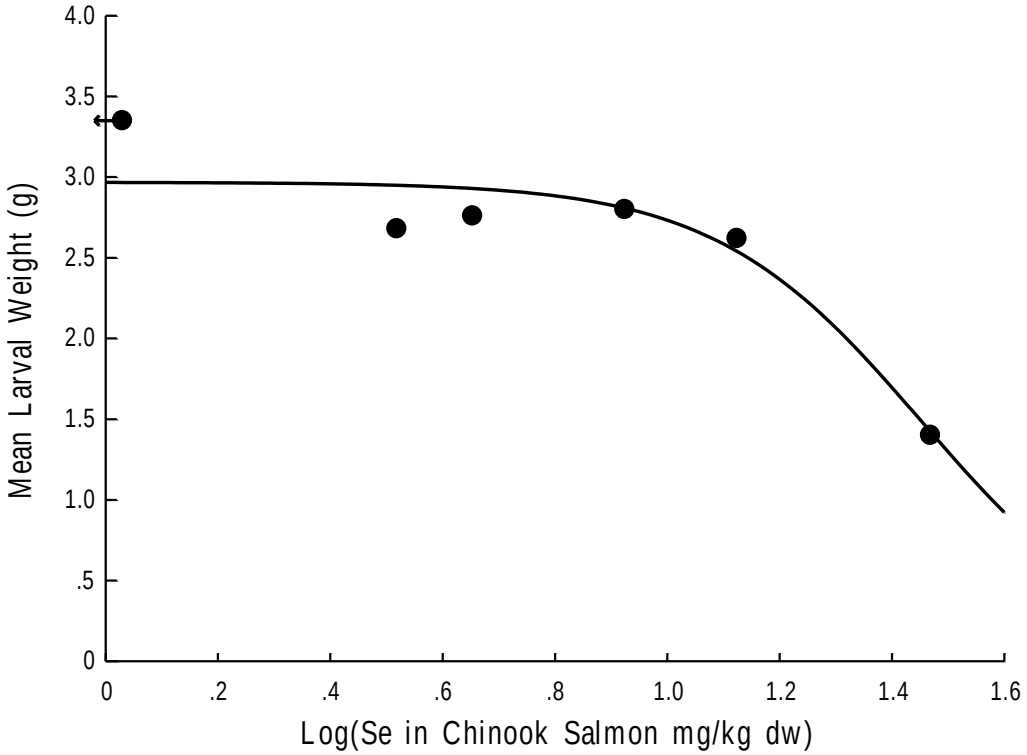
Hamilton et al (1990) Chinook Salmon fed SLD Diet
Logistic Equation, Three Parameter Model, Se concentrations $\log_{10}$ transformed



	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		

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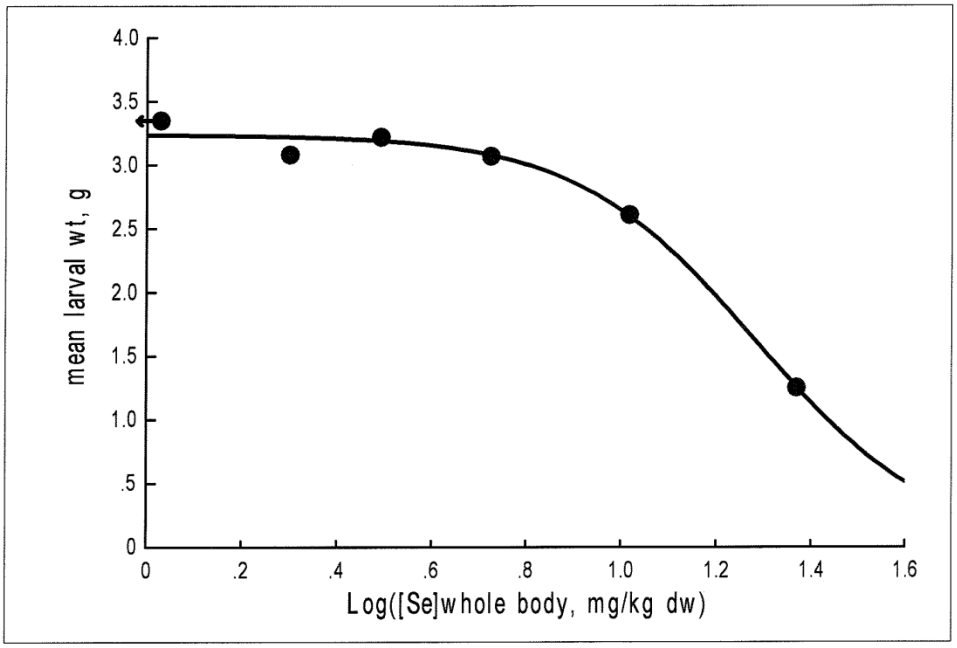
Chinook salmon SeMet diet (Hamilton et al. 1990)



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

US EPA ARCHIVE DOCUMENT

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

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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

US EPA ARCHIVE DOCUMENT

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.

US EPA ARCHIVE DOCUMENT

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.

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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

US EPA ARCHIVE DOCUMENT

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 32µg/L. Selenium was not measured at the reference streams; selenium concentrations at reference locations in the area ranged from <0.5 to 2.2µg/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 8EC in 2000 and at 5EC in 2001. The authors noted that 5EC 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

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tissue concentrations for the combined contaminated and reference stream data set with each year (*within streams approach*). This approach, which results in EC estimates (e.g., EC_{10} and EC_{20}) if the data meet the model assumptions, is explained in the *Calculations of Chronic Values* section of the text.

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 logistic curve from which EC_{10} and EC_{20} values were calculated (see table below and Figures 1 and 2). 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 3).

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Rainbow Trout EC Estimates using TRAP Logistic Equation, log([Se]$_{egg}$)

	EC_{20}		EC_{10}		
Response	**Se, mg/kg ww**	**Se, mg/kg dw[a]**	**Se, mg/kg ww**	**Se, mg/kg dw[a]**	**Comment**
100% - %craniofacial	11.4	29.4	10.3	26.5	
100% - %skeletal	11.0	28.4	8.2	21.1	
100% - %edema	9.9	25.5	9.5	24.5	Large SE for steepness

[a] ww to dw was converted using 61.2% moisture for rainbow trout eggs (Seilor and Skorupa, 2001)

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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

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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
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

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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
2001	Gregg	31	7.35	0.51	1.7	0.17	0

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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 (continued)

Year	Location	Female #	Se in egg, mg/kg	%craniofaci	%skeletal	%finfold	%edema
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

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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		

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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)

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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		

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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		

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Figure 1. Rainbow trout percent normal (100 - % craniofacial deformities) as a function of the logarithm of selenium concentration in eggs (Exposure Variable). Untransformed values reported in mg Se/kg tissue wet weight. The curve represents projections from the fitted logistic equation.

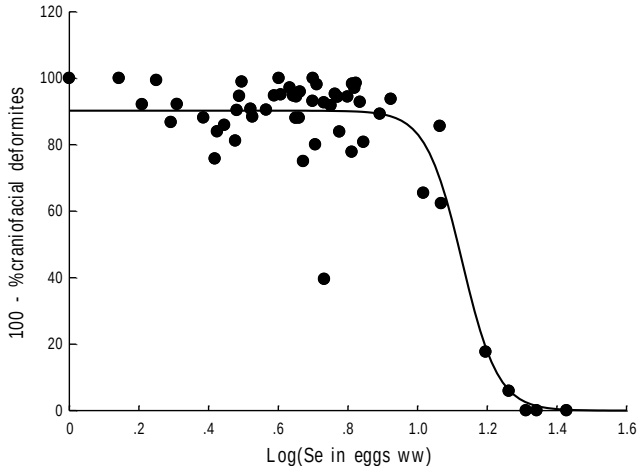

Figure 2. Rainbow trout percent normal (100 - % skeletal deformities) as a function of the logarithm of selenium concentration in eggs (Exposure Variable). Untransformed values were reported in mg Se/kg tissue wet weight. The curve represents projections from the fitted logistic equation.

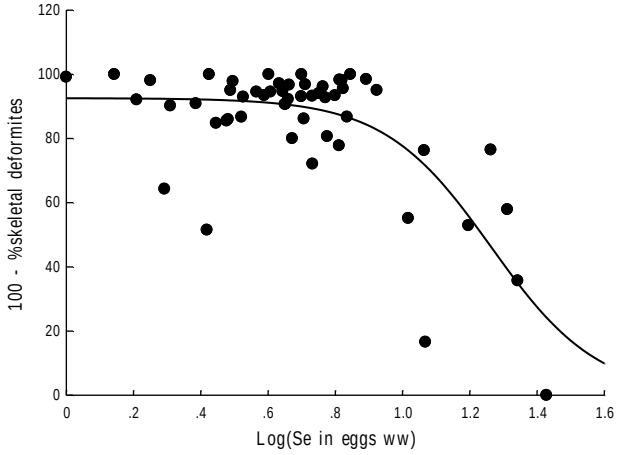

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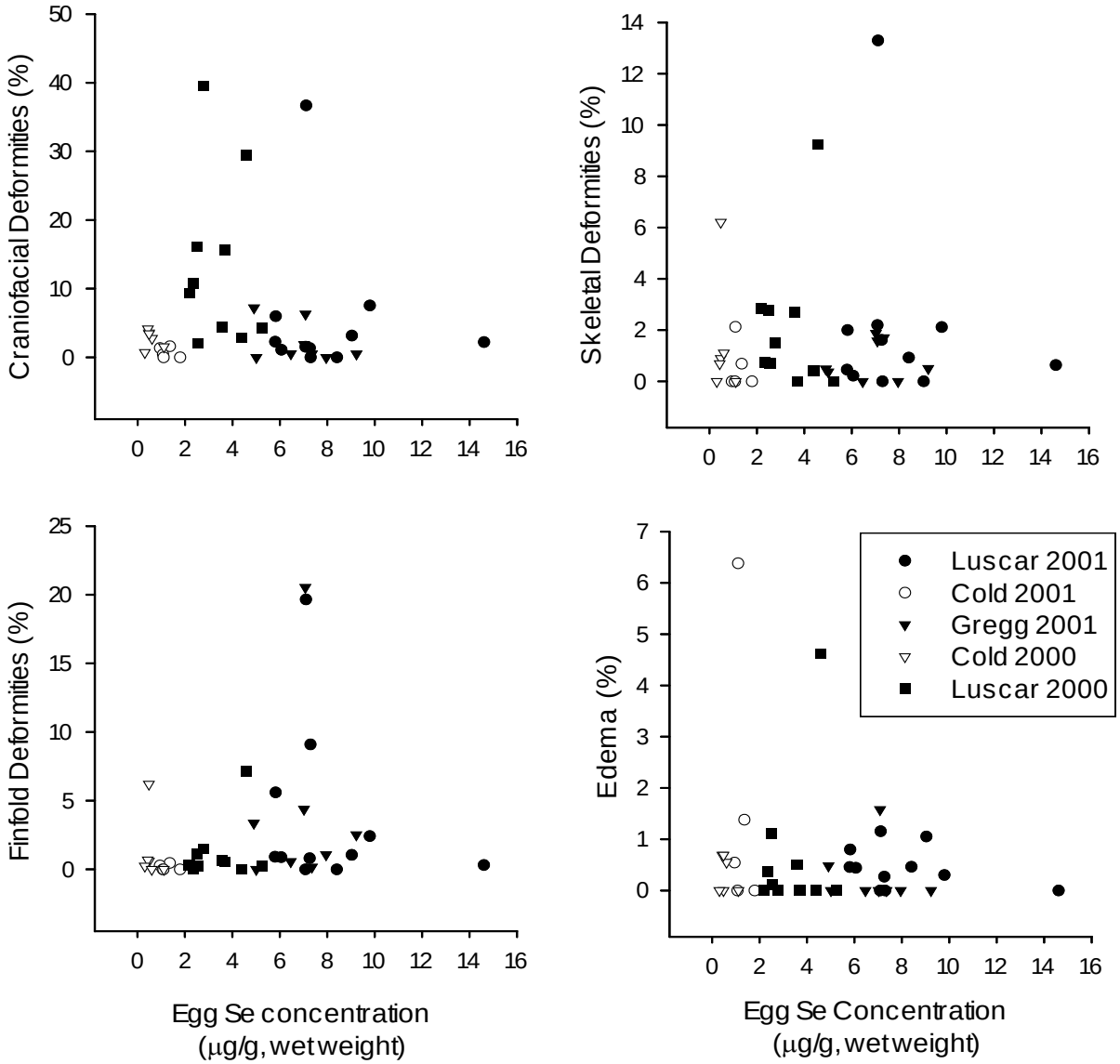
Figure 3. Plot of percent abnormal for craniofacial, skeletal and finfold deformities and edema against selenium concentration in brook trout eggs ww, 2000 and 2001 data.



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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 20.5 mg Se/kg eggs dw; egg. **Chronic value is >20.5 mg Se/kg eggs dw.**

Rainbow trout: *Within streams approach*
EC_{10} value (skeletal deformities) at 8.2 mg Se/kg egg ww or 21.1 mg Se/kg egg dw. **Chronic value is 21.1 mg Se/kg eggs dw.**

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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 clarki lewisi*). Arch. Environ. Contam. Toxicol. 39:46-52.

Test Organism: Cutthroat trout (*Oncorhynchus clarki 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

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Hardy, R.W. 2005. Effects of dietary selenium on cutthroat trout (*Oncorhynchus clarki*) growth and reproductive performance. Report for Montgomery Watson Harza. December 14, 2005.

Test Organism: Cutthroat trout (*Oncorhynchus clarki*, 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 ug/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 (14.5EC) 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.

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Rudolph, B-L, I. Andreller, CJ. Kennedy. 2008. Reproductive success, early life stage development, and survival of Westslope cutthroat trout (*Oncorhynchus clarki lewisi*) exposed to elevated selenium in an area of active coal mining. Environ. Sci. Technol. 42: 3109-3114.

Test Organism: Westslope cutthroat trout (*Oncorhynchus clarki 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 = 4922) 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 (Table 1). The percent of alevins (post hatch to swim-up stage) that died was related to the selenium concentration in the eggs; the EC_{10} estimated by TRAP is 24.1 mg Se/kg dw Figure 1). Note: The data used in the TRAP analysis excluded the variable from OL1. These are data from the reference lake in which only 57% of the larvae survived. Alevin survival was meaningfully higher in the other 15 clutches of eggs from the reference site (87.3 to 99.8%). Analysis of combined egg and alevin survival resulted in a similar EC_{10} estimate. The selenium in muscle data was not amenable for analysis with alevin survival using TRAP. EC_{10} and EC_{20} estimates for muscle were derived using a least squares regression of the egg and muscle data reported by Rudolph et al.

$$[Se_{muscle}] = 4.0853 + 0.391[Se_{egg}] \qquad (R^2 = 0.9094)$$

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

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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: 24.11 mg Se/kg dw in eggs; 13.51 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	Fertilization, %	Dead eggs, %	Dead alevins, %
Clode Pond (exposed site)					
CP1	38.8	88.3	0	100	NA
CP2	11.8	16.1	99.7	1.8	0.9
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	99	7.4	92.6
CP7	39	65.3	97.2	8.9	91.1
CP8	7	11.8	73.7	36.1	0.8
CP9	35.4	46.8	91.3	36.6	63.2
CP10	35.5	75.4	88.2	17.6	82.4
CP11	11.3	16.9	79.2	22.1	1.3
CP12	13.4	20.6	98.6	3	5.1
avg	30.3	61.6	61	44	42
SD	15.1	42.4	45	42	44
O'Rourke Lake (reference site)					
OL1	8.28	12.9	100	28.6	42.9
OL2	7.7	13.9	93.1	53.1	6.9
OL3	8.16	12.5	99.4	3.9	2.4
OL4	8.03	15	98.2	14.5	12.7
OL5	8.12	14.9	89.3	19.3	5.3
OL6	6.61	15.2	76	32	4
OL7	8.52	12.9	99.4	2.1	0.2
OL8	7.22	12.3	30.5	100	NA

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Fish ID	Muscle [Se] mg/kg dw	Egg [Se] mg/kg dw	Fertilization, %	Dead eggs, %	Dead alevins, %
OL9	7.25	16.7	96.4	12.8	4.5
OL10	7.64	13.1	99.1	2.5	5.5
OL11	8.74	15.6	96.2	10.8	2.4
OL12	8.2	13.9	99.1	16.4	3
OL13	7.86	15.1	92.6	25.9	2.8
OL14	8.5	13.1	79.5	22.2	0.5
OL15	7.62	12.3	92.4	11.8	2.6
OL16	8.13	12.7	71	45.2	4.8
avg	7.9	13.9	88	25	7
SD	0.6	1.4	18	25	10

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

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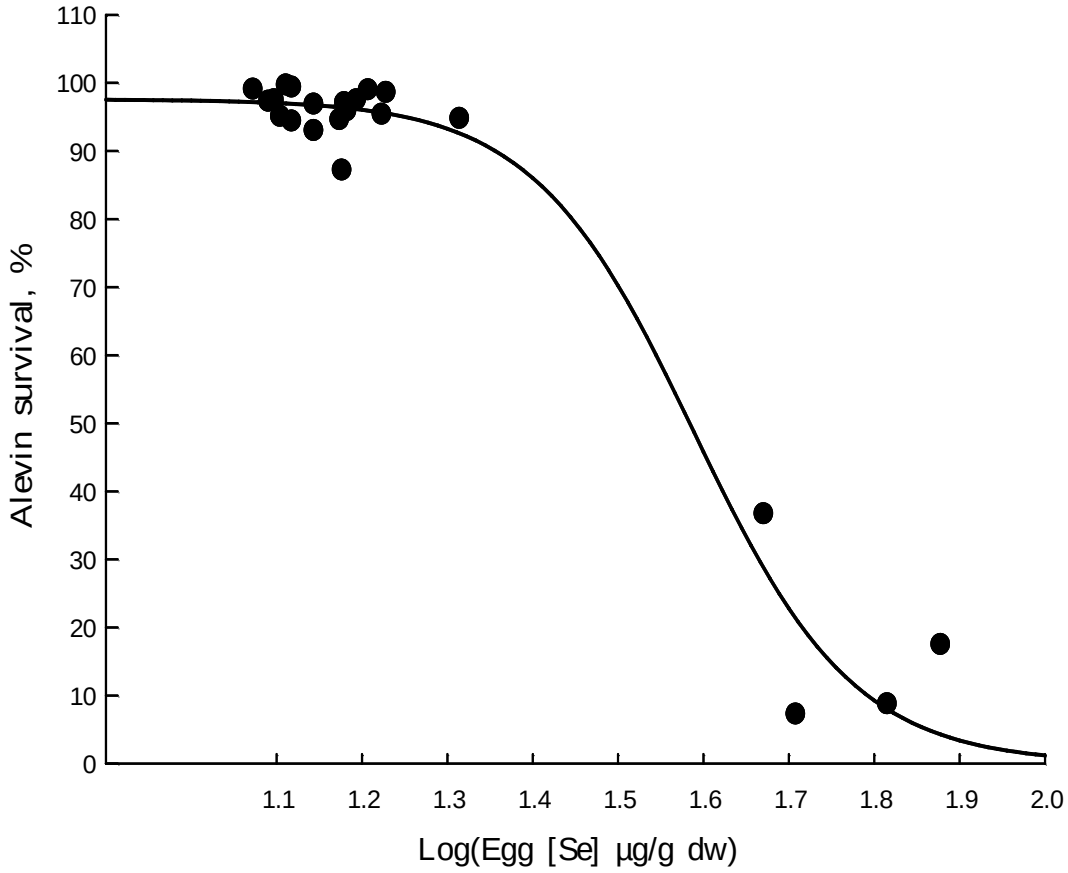
Figure 1. Survival of Westslope cutthroat trout alevin as a logistic function of the logarithm of the selenium concentration in eggs.



TRAP Output

Parameter summary

Parameter	Initial Est.	Final Est.	Std Error	95% LCL	95% UCL
Log X50	1.35	1.5885	0.0345	1.516	1.661
S	3.0	2.663	0.694	1.209	4.116
Y0	96.35	97.59	2.17	93.04	102.13

Effect concentration summary

p	Xp estimate	95% LCL	95% UCL
50	38.77	32.83	45.78
20	28.73	20.95	39.39
10	24.11	16.03	36.26
5	20.51	12.51	33.63
1	14.36	7.22	28.53

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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 clarki 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).

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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. TRAP was used to model larval survival with the entire egg Se dataset that included egg Se measurements from Laboratory A and adjusted measurements from Laboratory B (EC10 = 26.6 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 EC10 of 24.02 mg/kg egg dw is the selected effect concentration for this study.

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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

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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

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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

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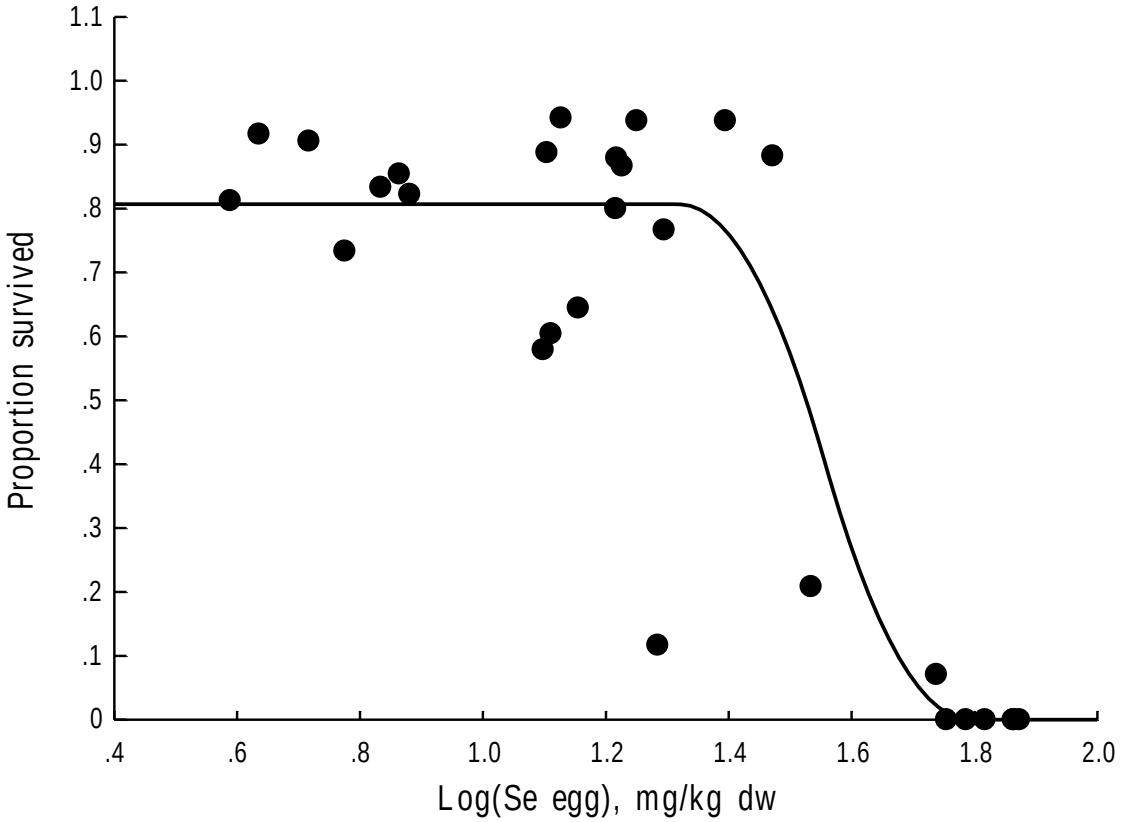

Figure 1. TRAP 1.20 Analysis type - Tolerance distribution; Model option – Triangular distribution (3 parameters). Includes Laboratories A and B datasets.

Xp Estimates

p	Xp Estimate	95% LCL	95% UCL
50	35.992	34.831	37.192
20	29.449	28.128	30.832
10	26.616	25.229	28.080
5	24.779	23.356	26.289
0	20.850	19.376	22.437

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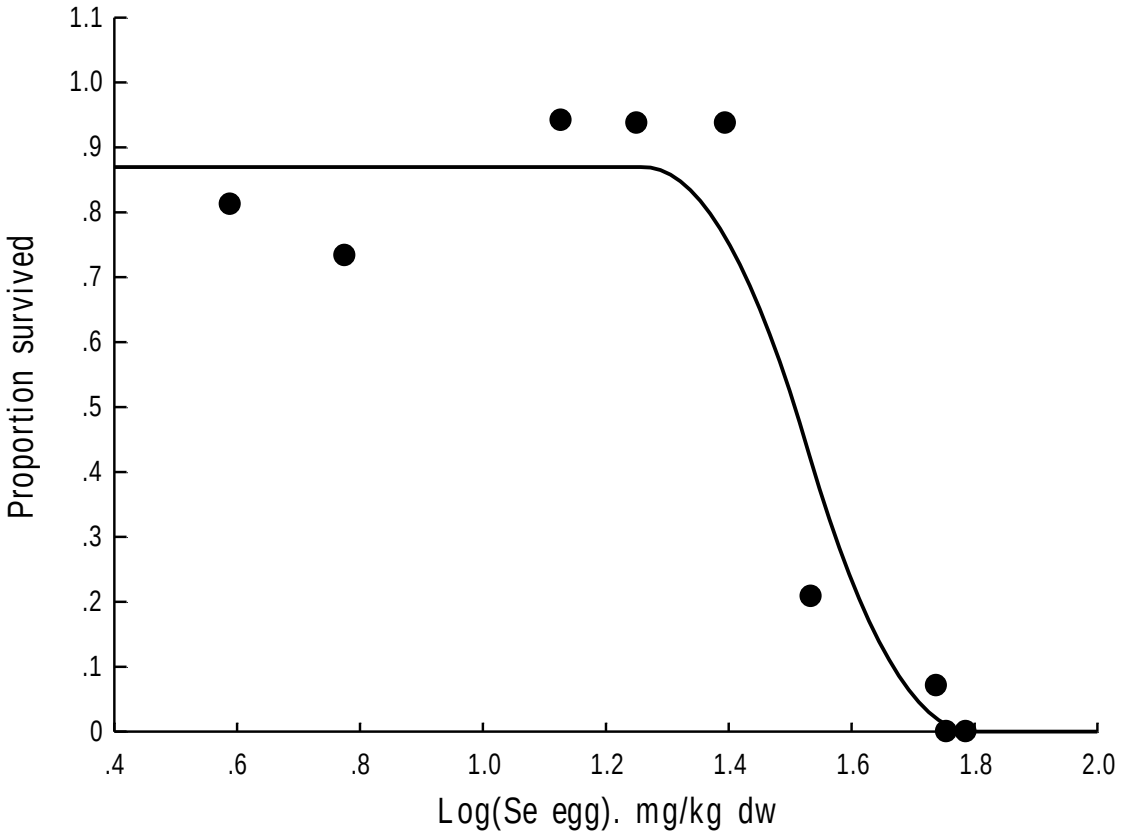

Figure 2. TRAP 1.20 Analysis type - Tolerance distribution; Model option – Triangular distribution (3 parameter). Includes Laboratory "A" dataset only. *

Xp Estimates

p	Xp Estimate	95% LCL	95% UCL
50	33.75	32.56	34.99
20	26.92	25.54	28.37
10	24.02	22.55	25.57
5	22.16	20.65	23.77
0	18.237	16.686	19.933

*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.

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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 (*Salvelnius 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 embyos 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).

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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

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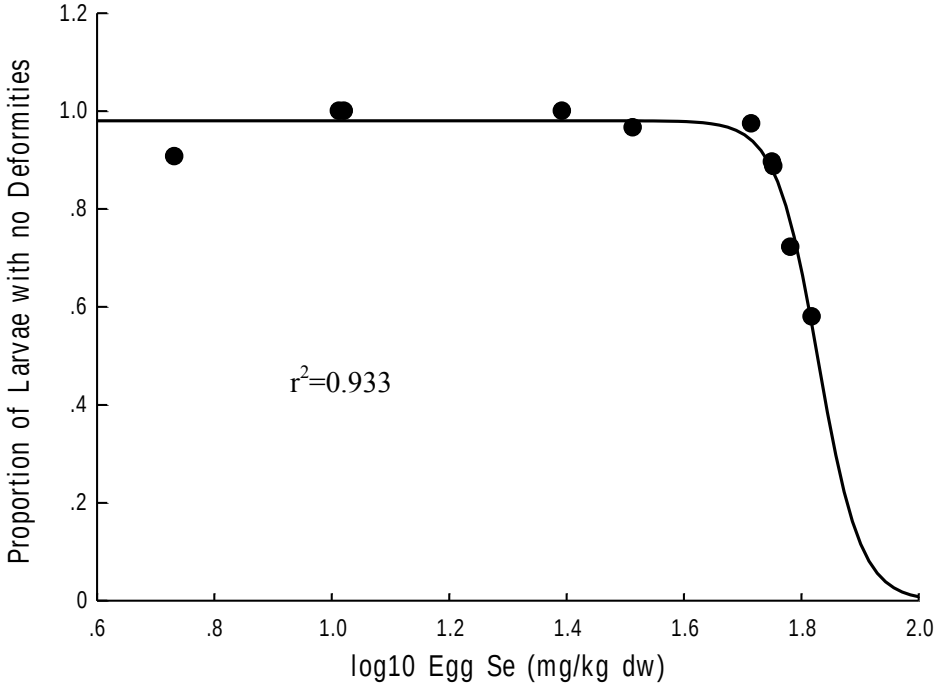
Figure 1. Proportion of Dolly Varden alevin without any type of deformity as a logistic function of the logarithm of the selenium concentration in eggs (TRAP).



	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		

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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. Se 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).

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Effects Data: Se concentrations in eggs collected from 26 ripe females at 3 field locations 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 LSV03, 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.

Because of concerns raised in a U.S. Fish and Wildlife (2012) review of the Formation Environmental (2011) report, most notably with respect to the consequences of fish lost due to an overflow event during the 15 day post swim up portion of the test, all endpoints were measured according to a both an "optimistic" and a "worst-case" scenario. The "worst-case" scenarios were introduced to examine the comment raised in the U.S. FWS (2012) review that fish lost to overflow during the post swim up test were assumed to have been dead or deformed. As an alternative to their proposal that all treatments that lost fish to overflow should be discarded from the study, we examined the effect of those individuals being either dead and/or deformed. Therefore, a total of six EC20s (3 endpoints x 2 scenarios/endpoint) were calculated here.

The U.S. FWS (2012) review also noted fish that survived but failed to reach swim up, which occurred among the offspring of the five females with the highest egg selenium concentrations (LSV2C-003, -004, -005, -010, and -021) would have likely died in the wild. We concur with this assertion, and treated all fish that failed to reach swim up as dead, with respect to survival.

Three endpoints (percentage fully free from deformities (% normal), percentage surviving from hatch through 15 day post swim up (% survival), and percentage surviving from hatch through 15 days post swim up AND fully free of deformities (% alive and normal)) were analyzed. Selenium concentrations and respective counts are shown in Table 1 (% normal), Table 2 (% survival), and Table 3 (% alive and normal). For all tables, each sample ID represents eggs hatched from a single female fish, with the exception of the 4 SPC samples, which were obtained as eyed eggs. Plots and EC_{10}s of each endpoint for both the optimistic and worst case scenarios are shown in Figure 1. All analyses were performed in TRAP (version 1.21) using tolerance distribution analysis and assuming a triangular data distribution.

For both the worst-case and optimistic scenarios, EC_{10}s were lowest for deformities, followed by survival, with the EC_{10} for the combined endpoint being the highest (Figure 1). For a given endpoint, EC_{10}s for the worst-case scenario were lower than for the optimistic scenario. The final endpoint selected was % normal, worst-case scenario (Figure 1b – top right), with an EC_{10} of 15.91 mg Se/kg dw egg.

In all of the above analyses, larvae hatched from eggs from both wild caught fish and hatchery fish were combined in this analysis. The fish from the hatchery were much larger and had many more eggs than the wild fish. In addition, the hatchery fish contained a much lower concentration of selenium in their eggs than the wild eggs. Furthermore, the fish from the Spring Creek Hatchery arrived

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as fertilized, eyed eggs, and reached swim up in 34 days, compared to 67-75 days for eggs fertilized in the field or laboratory by Formation Environmental (2011). Despite the differences between hatchery and wild-caught fish, the inclusion of larvae hatched from eggs obtained from hatcheries produced similar EC_{10} values. Figure 2 provides a comparison of the worst-case deformity endpoint, with and without the hatchery data. The EC_{10} for wild fish only is 16.89 mg/kg egg dw (Figure 2a); compared to an EC_{10} of 15.91 mg/kg egg dw for both wild caught and hatchery fish combined (Figure 2b). The EC_{10} value of 15.91 mg/kg for the wild+hatchery dataset is selected as the effect concentration because there does not seem to be an apparent reason to exclude the hatchery fish as a reference for larval survival.

Effect Concentration: 15.91 mg Se/kg dw in eggs

Table 1. Brown trout selenium concentrations and deformity data from hatch to test end. Worst case counts assumed that all fish lost to the overflow event during the post swim up portion of the study would have been deformed.

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	# Assessed for deformities plus # lost. "Worst Case"
SC-001	3.6	0.76	63	115		115
SC-002	4.1	0.94	72	113		113
SC-003	3.7	0.83	131	302	9	311
SC-004	4.3	0.92	46	140		140
SC-005	3	1.2	23	42		42
SC-006	3.1	1.2	457	535		535
SC-007	2.7	1	93	137		137
SC-008	2.5	0.96	283	359	10	369
SPC-001[c]		0.73	427	570		570
SPC-002[c]		0.73	371	545		545
SPC-005[c]		0.73	400	561		561
SPC-006[c]		0.73	427	556		556
CC-150-009	8.4	12.8	106	142		142
CC-150-011	5.6	8.4	87	266		266
CC-150-012	6.7	8.5	156	282		282
CC-150-013	5.9	8.4	137	310	26	336
CC-150-015	6	9.1	210	445		445
CC-150-016	7	7.5	13	23	43	66
CC-150-017	5.6	6.6	99	163	33	196
CC-150-018	4.7	6.9	195	486		486

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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	# Assessed for deformities plus # lost. "Worst Case"
CC-150-020	7.2	6.2	453	558		558
CC-350-006	9.2	14	120	386		386
CC-350-007	5.5	6.9	68	131	20	151
CC-350-008	8.5	9.5	269	338	28	366
LSV2C-002	8.9	12.8	483	544	16	560
LSV2C-003	13.8	40.3	2	100		100
LSV2C-004	17.9	36	16	142		142
LSV2C-005	13.6	26.8	8	149		149
LSV2C-008	9.6	17.7	147	194	45	239
LSV2C-010	22.6	38.8	5	80		80
LSV2C-012	7.2	13.2	217	554		554
LSV2C-016	9.2	13.4	440	530		530
LSV2C-017	13.2	20.5	110	150	19	169
LSV2C-019	8.6	12.5	267	390	39	429
LSV2C-020	11.3	11.2	240	296	36	332
LSV2C-021	20	28.1	8	172		172

[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.

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Table 2. Brown trout selenium concentrations and survival data from hatch to test end. Worst case counts assumed that all fish lost to the overflow event during the post swim up portion of the study would have been deformed.

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. "Optimistic Case"	Prop survival. Post swim up. "Worst Case"	Prop survival. Hatch to end. "Optimistic case"	Prop survival. Hatch to end. "Worst case"	Est. # survived. Hatch to end. "Optimistic case"	Est. # survived. Hatch to end. "Worst case"
SC-001	3.6	0.76	144	0.951	0.990	0.990	0.942	0.942	136	136
SC-002	4.1	0.94	138	0.978	0.990	0.990	0.968	0.968	134	134
SC-003	3.7	0.83	340	0.982	0.989	0.890	0.971	0.874	330	297
SC-004	4.3	0.92	189	0.868	0.971	0.971	0.842	0.842	159	159
SC-005	3	1.2	70	0.914	1.000	0.984	0.914	0.900	64	63
SC-006	3.1	1.2	564	0.988	0.990	0.990	0.978	0.978	551	551
SC-007	2.7	1	188	0.856	0.970	0.970	0.830	0.830	156	156
SC-008	2.5	0.96	396	0.985	1.000	0.900	0.985	0.886	390	351
SPC-001[c]		0.73	598	0.987	1.000	1.000	0.987	0.987	590	590
SPC-002[c]		0.73	585	0.966	1.000	1.000	0.966	0.966	565	565
SPC-005[c]		0.73	589	0.986	1.000	1.000	0.986	0.986	581	581
SPC-006[c]		0.73	593	0.971	1.000	1.000	0.971	0.971	576	576
CC-150-009	8.4	12.8	173	0.942	0.990	0.990	0.933	0.933	161	161
CC-150-011	5.6	8.4	288	0.993	1.000	1.000	0.993	0.993	286	286
CC-150-012	6.7	8.5	314	0.965	0.990	0.990	0.955	0.955	300	300
CC-150-013	5.9	8.4	402	0.891	0.973	0.720	0.866	0.641	348	258
CC-150-015	6	9.1	479	0.971	1.000	1.000	0.971	0.971	465	465
CC-150-016	7	7.5	89	0.966	1.000	0.500	0.966	0.483	86	43
CC-150-017	5.6	6.6	223	0.969	1.000	0.670	0.969	0.649	216	145
CC-150-018	4.7	6.9	522	0.969	1.000	1.000	0.969	0.969	506	506
CC-150-020	7.2	6.2	584	0.990	1.000	1.000	0.990	0.990	578	578
CC-350-006	9.2	14	432	0.944	0.980	0.980	0.926	0.926	400	400
CC-350-007	5.5	6.9	181	0.950	0.988	0.790	0.938	0.751	170	136
CC-350-008	8.5	9.5	407	0.951	0.986	0.710	0.938	0.675	382	275
LSV2C-002	8.9	12.8	584	0.993	1.000	0.840	0.993	0.834	580	487
LSV2C-003	13.8	40.3	404	0.079	0.281	0.281	0.022	0.022	9	9
LSV2C-004	17.9	36	309	0.414	0.477	0.477	0.197	0.197	61	61
LSV2C-005	13.6	26.8	287	0.387	0.622	0.622	0.240	0.240	69	69
LSV2C-008	9.6	17.7	263	0.989	0.982	0.540	0.971	0.534	255	140
LSV2C-010	22.6	38.8	108	0.231	0.440	0.440	0.102	0.102	11	11

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. "Optimistic Case"	Prop survival. Post swim up. "Worst Case"	Prop survival. Hatch to end. "Optimistic case"	Prop survival. Hatch to end. "Worst case"	Est. # survived. Hatch to end. "Optimistic case"	Est. # survived. Hatch to end. "Worst case"
LSV2C-012	7.2	13.2	591	0.971	1.000	1.000	0.971	0.971	574	574
LSV2C-016	9.2	13.4	570	0.965	1.000	1.000	0.965	0.965	550	550
LSV2C-017	13.2	20.5	217	0.885	0.963	0.780	0.852	0.690	185	150
LSV2C-019	8.6	12.5	471	0.953	1.000	0.610	0.953	0.582	449	274
LSV2C-020	11.3	11.2	357	0.986	1.000	0.640	0.986	0.631	352	225
LSV2C-021	20	28.1	424	0.288	0.730	0.730	0.210	0.210	89	89

[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.

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Table 3. Brown trout selenium concentrations and survival + deformity data (combined endpoint) from hatch to test end. Worst case counts assumed that all fish lost to the overflow event during the post swim up portion of the study would have been deformed.

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. "Optimistic case"	# Live fish assessed + # died during test + # lost during post swim up. "Worst case"
SC-001	3.6	0.76	63		63	115	8		123	123
SC-002	4.1	0.94	72		72	113	4		117	117
SC-003	3.7	0.83	131		131	302	7	9	309	318
SC-004	4.3	0.92	46		46	140	28		168	168
SC-005	3	1.2	23		23	42	6		48	48
SC-006	3.1	1.2	457		457	535	8		543	543
SC-007	2.7	1	93		93	137	30		167	167
SC-008	2.5	0.96	283		283	359	6	10	365	375
SPC-001[c]		0.73	427		427	570	8		578	578
SPC-002[c]		0.73	371		371	545	20		565	565
SPC-005[c]		0.73	400		400	561	8		569	569
SPC-006[c]		0.73	427		427	556	17		573	573
CC-150-009	8.4	12.8	106		106	142	11		153	153
CC-150-011	5.6	8.4	87		87	266	2		268	268
CC-150-012	6.7	8.5	156		156	282	12		294	294
CC-150-013	5.9	8.4	137		137	310	46	26	356	382
CC-150-015	6	9.1	210		210	445	14		459	459
CC-150-016	7	7.5	13		13	23	3	43	26	69
CC-150-017	5.6	6.6	99		99	163	7	33	170	203
CC-150-018	4.7	6.9	195		195	486	16		502	502
CC-150-020	7.2	6.2	453		453	558	6		564	564
CC-350-006	9.2	14	120		120	386	26		412	412
CC-350-007	5.5	6.9	68		68	131	10	20	141	161
CC-350-008	8.5	9.5	269		269	338	21	28	359	387
LSV2C-002	8.9	12.8	483		483	544	4	16	548	564
LSV2C-003	13.8	40.3	2	2	0	0	395		395	395
LSV2C-004	17.9	36	16	16	0	0	289		289	289
LSV2C-005	13.6	26.8	8	8	0	0	267		267	267

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. "Optimistic case"	# Live fish assessed + # died during test + # lost during post swim up. "Worst case"
LSV2C-008	9.6	17.7	147		147	194	4	45	198	243
LSV2C-010	22.6	38.8	5	5	0	0	97		97	97
LSV2C-012	7.2	13.2	217		217	554	17		571	571
LSV2C-016	9.2	13.4	440		440	530	20		550	550
LSV2C-017	13.2	20.5	110		110	150	28	19	178	197
LSV2C-019	8.6	12.5	267		267	390	22	39	412	451
LSV2C-020	11.3	11.2	240		240	296	5	36	301	337
LSV2C-021	20	28.1	8	8	0	0	404		404	404

[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.

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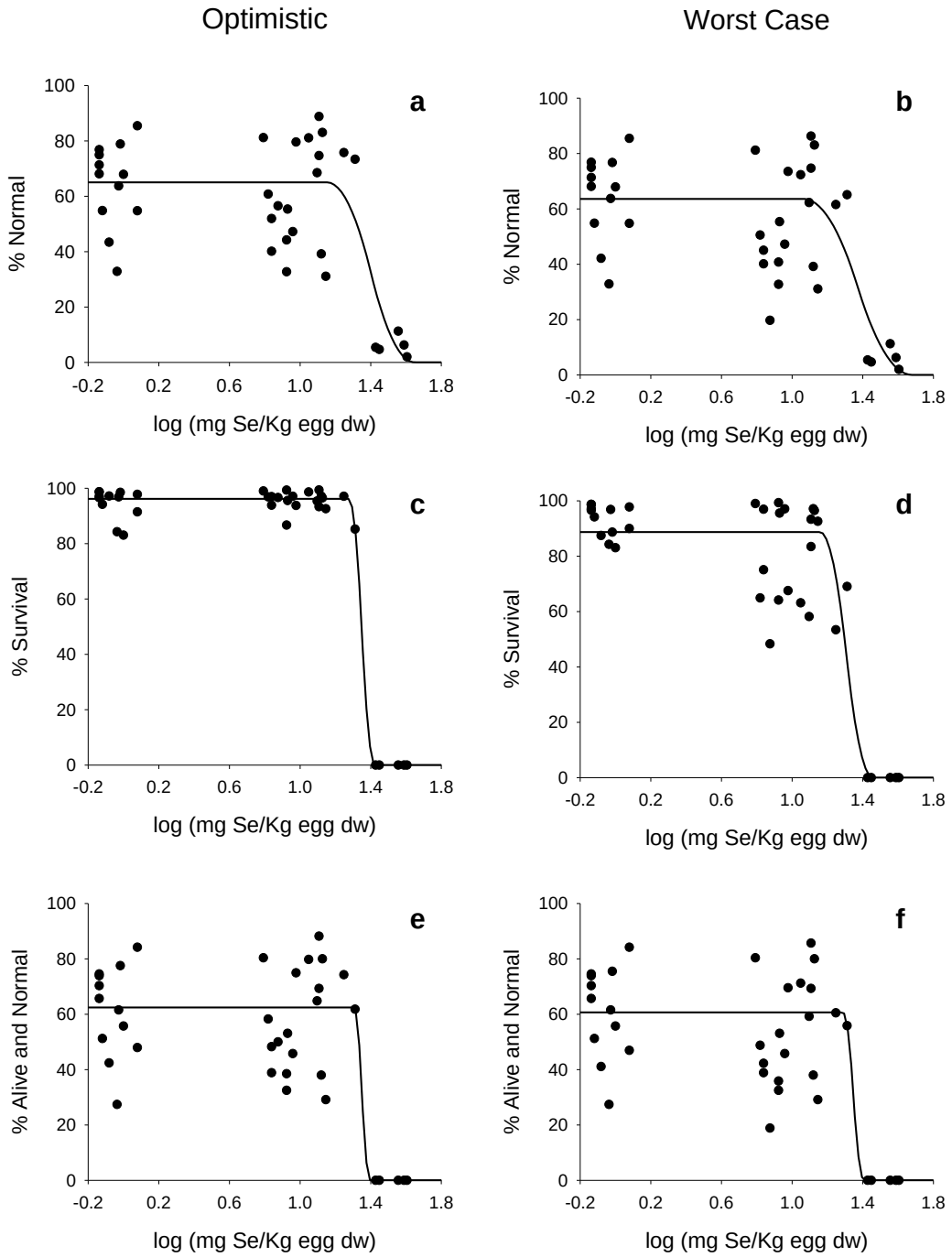

Figure 1. Concentration-response relationships of brown trout deformities (a-b), survival (c-d), and deformities+survival (e-f) in response to selenium concentrations in eggs. Each endpoint was evaluated under optimistic and worst-case scenarios with respect to larval fry lost during the 15-day post swim up test. EC_{10}s (in mg Se/kg egg dw) for each endpoint-scenario combination were as follows: Deformities (18.36 – optimistic (a); 15.91 – worst case (b)); Survival (20.40 – optimistic (c); 16.79 – worst case (d)); Combined (21.16 – optimistic (e); 20.65 – worst case (f)).

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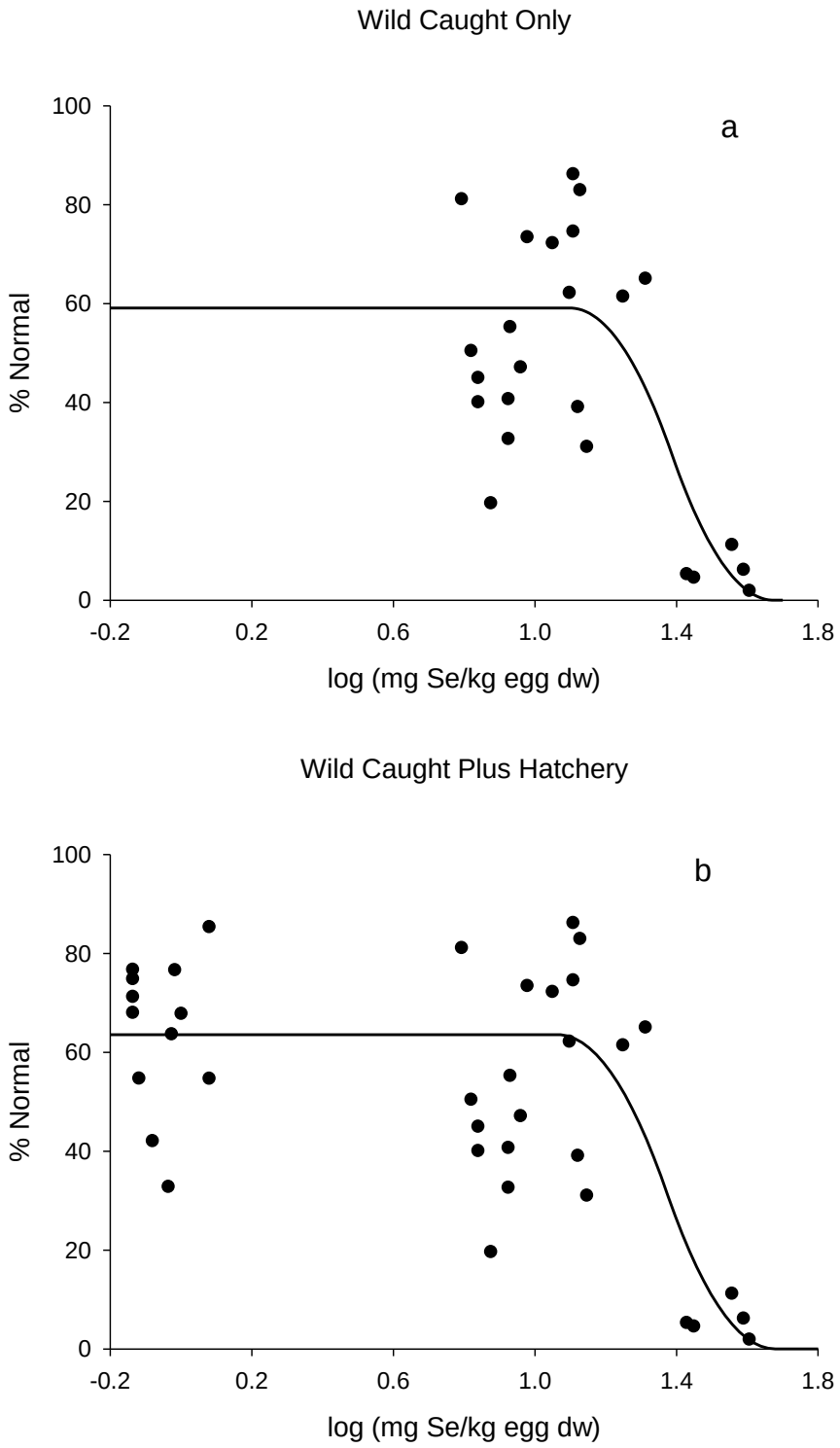

Figure 2. Proportion of brown trout larvae fully free from deformities, worst-case scenario, for larvae hatched from eggs of wild-caught fish only (a), and from eggs of wild caught+hatchery fish (b). The EC_{10} for the worst-case deformity endpoint for the wild-caught dataset was 16.89 mg Se/ kg egg dw, with a 95% confidence interval of 15.38-18.55 mg Se/ kg egg dw). The EC_{10} for the worst-case deformity endpoint for the wild caught plus hatchery dataset was 15.91 mg Se/ kg egg dw, with a 95% confidence interval of 14.77-17.13 mg Se/ kg egg dw).

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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 oligochate, *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 oligochate, *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

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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).

Endpoit[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).

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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.

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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

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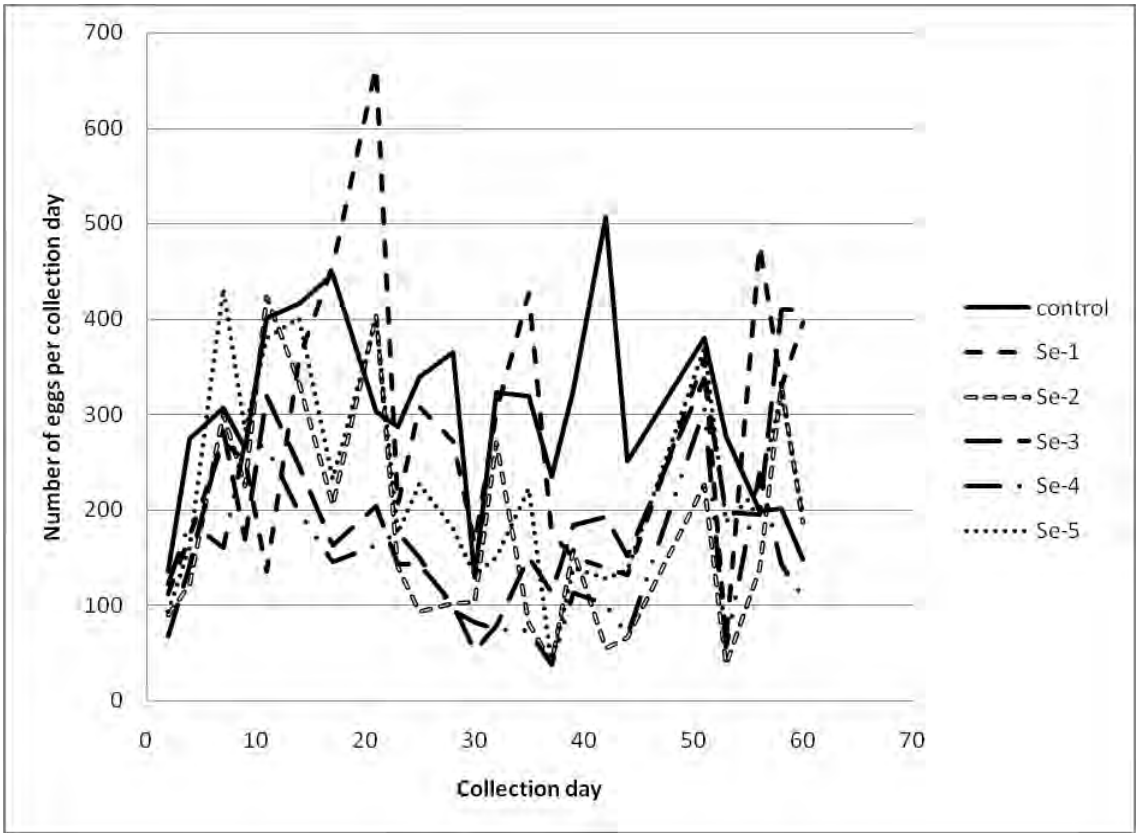

Figure 1. Pupfish egg production by sampling date

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.

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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.

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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	**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 T	110	902	1.310	0.023

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:

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$$1 - (1 - 0.05)^{\frac{1}{23}} = 0.0027$$

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.

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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

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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 Obser-vation 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 ∑ (duration · eggs/f/d) =			281.6	294.3	181.9	174.7	142.0	220.1

Larval Survival: The Besser et al. (2012b) spreadsheet also has data for larval survival after 14 and 21 days for eggs collected at three time points. The fraction surviving 21 days was used here. For each treatment, the probability of the early life stage (i=1) surviving each day equals the fraction surviving for 21 days, raised to the 1/21 power: $\sigma_1 = \sigma_L = (\text{21-d Surv})^{1/21}$, shown in Table 4.

Juvenile and Adult Survival: A second spreadsheet, Besser et al. (2012c), has data on juvenile and adult survival after 30 and 58 days. The fraction surviving

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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

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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.

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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

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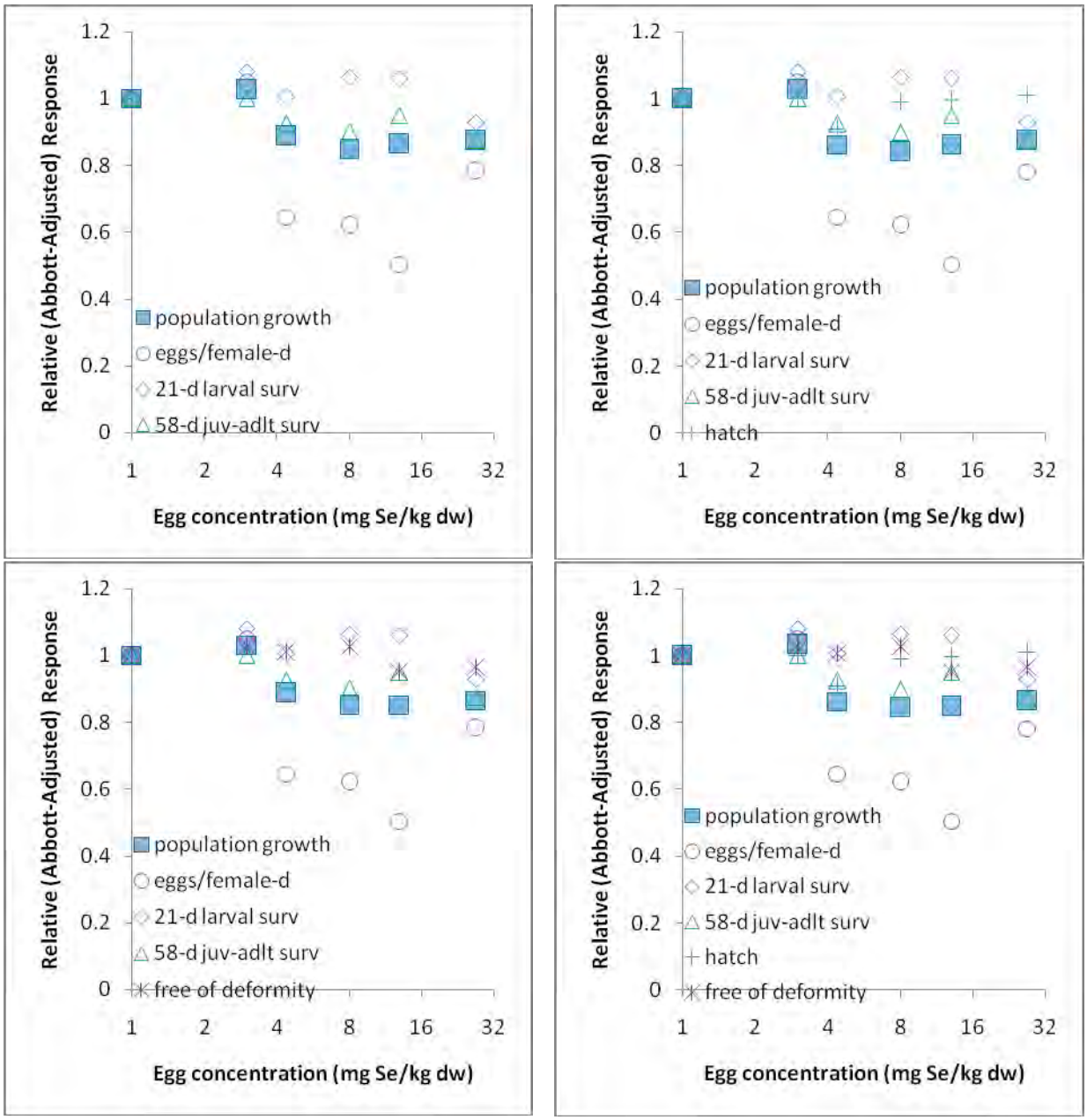

Figure 2. Abbott-adjusted pupfish response as modeled population growth rate (solid-filled symbols) and observed eggs per female per day, larval survival, and juvenile and adult survival (open symbols). Where used in the population model (to modify fecundity), hatch and deformity are shown as open symbols.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

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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.

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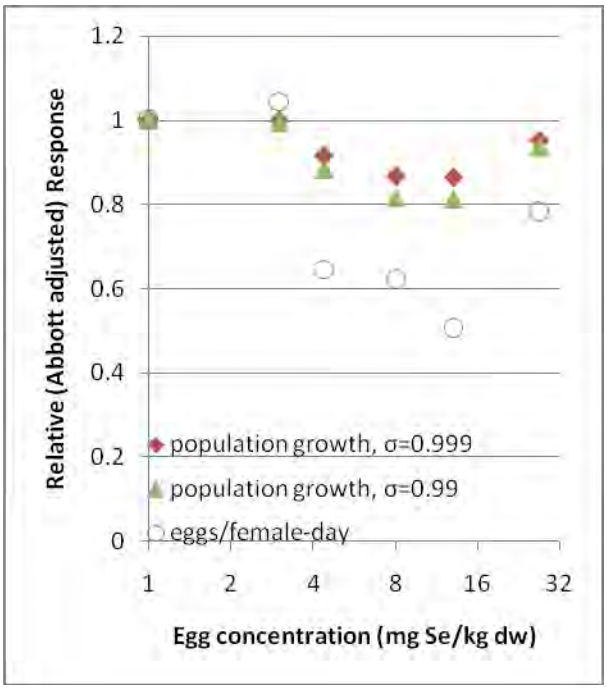

Figure 3. Predicted population growth rate calculated considering differences only in egg production and timing (having assigned uniform survival rates across treatments).

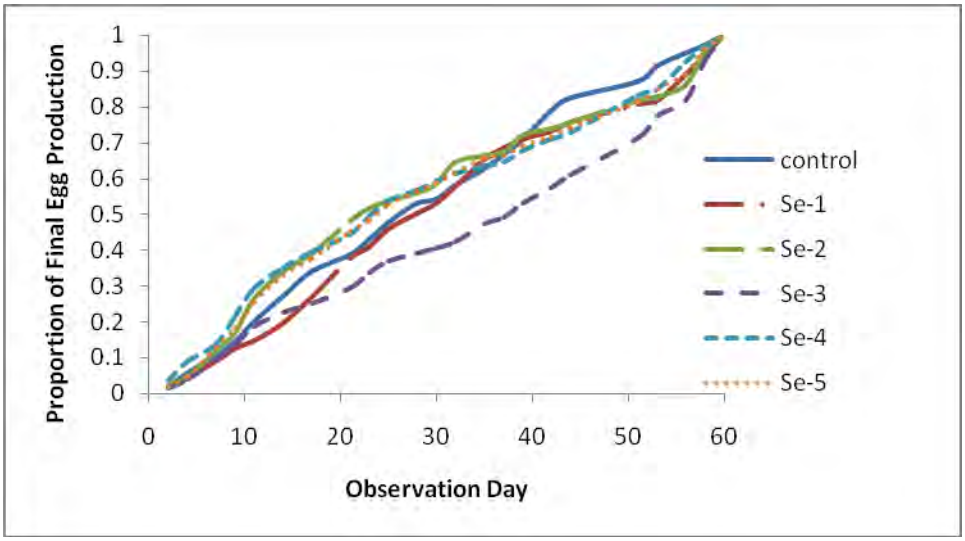

Figure 4. Cumulative pattern of egg production over time. (Control: continuous line. Se-1: dot, dot, long dash. Se-2: long dashes. Se-3: medium dashes. Se-4: short dashes. Se-5: dots.)

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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 molliesand 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).

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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

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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

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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

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meet the assumptions required for analysis.

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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 :g/L). A mean selenium for the ash pond effluent from a previous study was 53 :g/L (N=59; range 35-80 :g/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

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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.

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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

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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

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Summary of Embyo/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)

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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

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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.

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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°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.

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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: 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 21.4 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 5.5 mg/kg treatment. 3 of the 6 progenies in the 13.9 mg/kg treatment exhibited 10 to 20% larvae with similar abnormalities (in table below). The average proportion of larvae with edema were 5% in 13.9 and 95% in 21.9 mg/kg, both of these were statistically

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different from the control (0% edema).

In the 30-day larval survival bioassay, statistical difference was only in the highest test treatment for survival and growth measurements, length and weight (see table below). 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 13.9 mg/kg treatment (7.2%) was only slightly higher than the control (6.3%).

Authors present the effect level for bluegill at the 13.9 mg/kg dietary treatment (NOEC 5.5 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 13.9 and 21.4 mg/kg treated eggs.

The most sensitive endpoint, percent edema, as a function of selenium in egg dw and adult muscle dw, was fitted to a logistic curve from which EC estimates were calculated (see Figures 1 and 2). The EC_{10} and EC_{20} values are given in the following table.

Effect level	Egg, mg Se/kg dw	Maternal muscle, mg Se/kg dw
EC_{10}	20.05	11.51
EC_{20}	22.43	12.85

Chronic Value: EC_{10} value (edema) at 20.05 mg Se/kg egg dw or 11.51 mg Se/kg muscle dw
Chronic value is 20.05 :g Se/g eggs dw.

Selenium Concentrations (mg/kg dw) in Bluegills from Population A Day 113 of Bioaccumulation				
Dietary treatment	**Control**	**5.5 mg/kg dw**	**13.9 mg/kg dw**	**21.4 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)

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Selenium Concentrations (mg/kg dw) in Bluegill Parents (Population B) Used in Larval Toxicity Tests				
Dietary treatment	**Control**	**5.5 mg/kg dw**	**13.9 mg/kg dw**	**21.4 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)

5-day Larval Development Toxicity Test, average (SD)				
Dietary treatment	**Control**	**5.5 mg/kg dw**	**13.9 mg/kg dw**	**21.4 mg/kg dw**
Edema, %	0	0	5 (2)*	95.7 (2.7)*

Results from 30-day Embryo-larval Toxicity Test, average (SD)				
Dietary treatment	**Control**	**5.5 mg/kg dw**	**13.9 mg/kg dw**	**21.4 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

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Thirty day toxicity test mortalities and tissue selenium concentrations in respective females. "n" is number of eggs on Day 0, "r" is mortality on Day 30, "p" is proportions.							
Progenies	**n**	**r**	**p**	**[Se], mg/kg dw (female)**			
				Ovary	**Liver**	**Muscle**	**Eggs**
08-2C	89	17	0.191	1.95	4.04	2.25	3.54
18-4C	85	17	0.200	2.38	5.03	0.95	3.25
5.5-1S	85	64	0.753	7.72	14.89	7.07	11.49
5.5-2S	90	42	0.467	5.55	7.06	5.80	8.31
5.5-6S	85	19	0.224	4.06	10.49	1.41	6.18
13.9-1S	90	29	0.322	3.94	7.54	2.75	8.55
13.9-3S	87	34	0.391	21.82	34.74	15.44	22.06
13.9-6S	87	31	0.356	20.40	36.82	16.58	30.20
21.4-1S	88	87	0.989	29.90	38.02	NA	44.02
21.4-2S	90	89	0.989	45.82	33.96	31.10	36.31
21.4-3S	86	79	0.919	27.24	59.01	17.28	25.21
21.4-4S	88	88	1.000	23.18	62.71	27.40	52.18
21.4-5S	90	90	1.000	32.64	55.25	24.00	42.40
21.4-6S	86	86	1.000	37.63	48.14	24.66	38.47
21.4-7S	88	82	0.932	18.02	36.10	17.42	30.12

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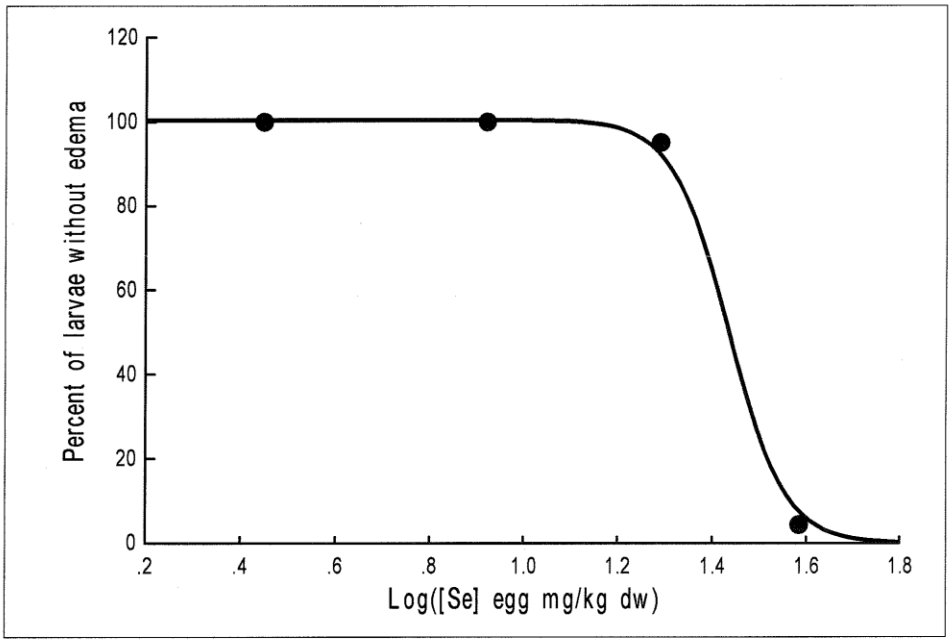
Bluegill sunfish (Doroshov et al 1992)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3830	1.4340	0.0283	1.0748	1.7933
S	1.3889	4.167	0.775	-5.681	14.014
Y0	100.00	100.47	3.09	61.19	139.75

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	27.16	11.88	62.12
20.0	22.43	8.34	60.31
10.0	20.05	6.30	63.82
5.0	18.086	4.732	69.131

06/29/2009 20:28 MED Toxic Response Analysis Model, Version 1.03

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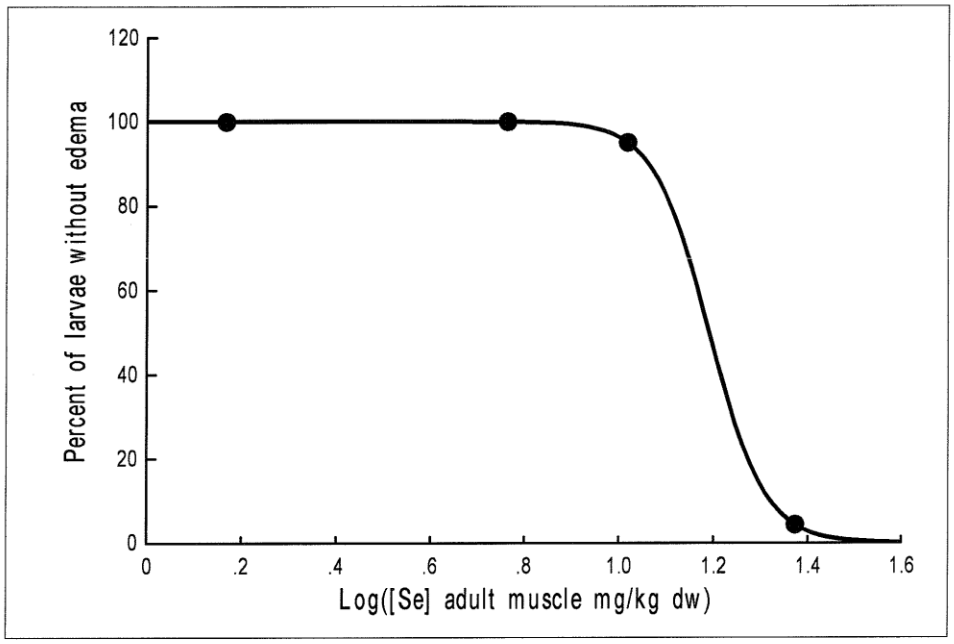
Bluegill sunfish (Doroshov et al 1992)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1522	1.1907	0.0005	1.1840	1.1974
S	1.6314	4.240	0.012	4.082	4.397
Y0	100.00	100.04	0.04	99.58	100.49

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	15.512	15.274	15.754
20.0	12.851	12.629	13.077
10.0	11.511	11.287	11.740
5.0	10.400	10.172	10.634

06/29/2009 20:33 MED Toxic Response Analysis Model, Version 1.03

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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.

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 :g/L	Recovering
3	10 :g/L	10 :g/L	Recovering
4	30 :g/L	Recovering	Recovering
5	Control	Control	Control
6	30 :g/L	Recovering	Recovering
7	Control	2.5 :g/L	Recovering
8	10 :g/L	10 :g/L	Recovering

[a] BG = Bluegill

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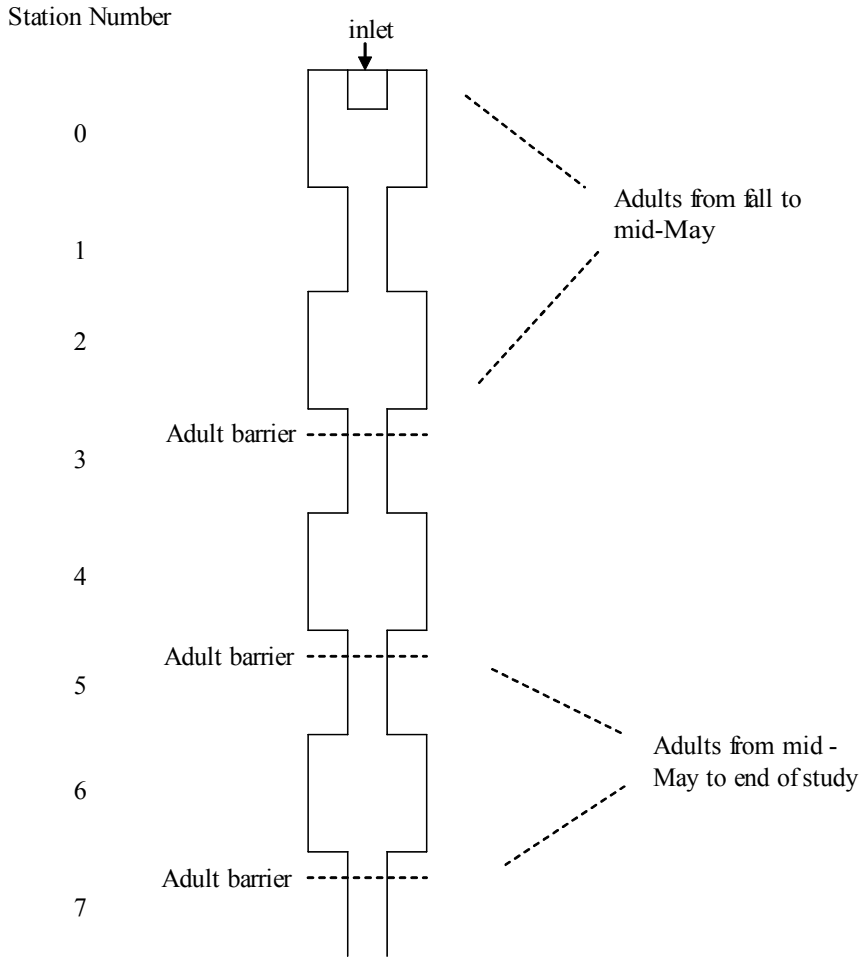
A schematic diagram of an artificial stream is provided below. For each study, a random sample of 22-50 adult bluegill were transferred from stations 0-2 (provided temperatures above 4°C during winter) to station 6 (most suitable for nests) during mid-May for spawning. Spawning activity was monitored 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.

Schematic Design of One of the Artificial Streams in the Monticello Study



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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
		Up	down	geomean							
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 :g/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 :g/L	8	4.72	6.37	5.48	22.85						
30 :g/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
		up	down	geomean							
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	0.01	0.07	0.29						
10 :g/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 :g/L	8	4.72	6.37	5.48	22.85						
30 :g/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

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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]
									up	down	avg	
control	1	6	93.0	75.2	0	0	0	97.8	1.02	0.76	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 :g/L	2	0	NA	NA	NA	NA	NA	NA		1.82	1.82	7.58
2.5 :g/L	7	4	81.4	71.6	0	0	3.6	92.2	2.02	3.36	2.61	10.86
10 :g/L	3	3	83.3	57.7	100	11.1	49.3	0		8.1	8.10	33.75
10 :g/L	8	2	91.1	57.1	100	18.2	41.1	0	6.96	12.6	9.36	39.02
rec 30 :g/L	4	0	NA	NA	NA	NA	NA	NA				
rec 30 :g/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]
											up	Down	Avg	
control	1	6	2458	0.94	3252	0.03	7	0	0	0	1.02	0.76	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 :g/L	2	1	0		2497	0.20	3	4.1	25	77.6		1.82	1.82	7.58
2.5 :g/L	7	5	1462	0	4717	0.08	8	0	0	52	2.02	3.36	2.61	10.86
10 :g/L	3	2	672	0	5376	0.50	9	81.4	5.0	55.5		8.1	8.10	33.75
10 :g/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 :g/L	4	0	NA	NA	NA	NA	NA	NA	NA	NA				
R 30 :g/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

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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 :g/L	2	3	87.3	66	0	0	0	1.84	7.67
R 2.5 :g/L	7	6	87.2	76.5	0	0	0	1.97	8.21
R 10 :g/L	3							6.25	26.04
R 10 :g/L	8	3	75.3	74.5	0	0	0	2.44	10.17
R 30 :g/L	4	5	92	78				3.82	15.92
R 30 :g/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 :g/L	2	5	5	0	0	0	1.84	7.67
R 2.5 :g/L	7	5	2	0	0	0	1.97	8.21
R 10 :g/L	3	2	4	0	0	0	6.25	26.04
R 10 :g/L	8	4	4	0	0	0	2.44	10.17
R 30 :g/L	4	9	13	0	0	0	3.82	15.92
R 30 :g/L	6							

[a] The NOAEC for the study are from recovering 30 Φg 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: The Hermanutz bluegill data from Study I&II were analyzed by combining the nest and egg cup observations for percent hemorrhage, percent lordosis, and percent edema in response to Se concentrations in parental ovaries (mg/kg dw). 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 effects and exposure data were obtained from Hermanutz et al. (1996). Adult survival was not considered because it was very low in both studies II and III. Prior to analysis, all percentages were transformed (100-% value of response) so that the response variables decreased with increasing Se. Endpoints and values used to parameterize TRAP are included in the table below. All endpoints in the table below were analyzed using a three parameter logistic equation model in TRAP with log transformed Se concentrations. Aside from %larval survival, data from nest and egg cup observations in Study II were combined with egg cup data from study I into a single analysis. For percent survival, data from egg cup observations in Study I and II were combined into a single analysis. No percent larval survival measurements were made during nest observations in Study II. Nest observation data for the relevant endpoints were not collected during Study I. Finally, neither data from the two recovering streams in Study II, nor any of the Study III data were included in these analyses. As stated on page 37 in the criteria document, the recovery streams do not reflect the type of system to which water quality criteria are most commonly applied; those receiving existing waterborne pollutant discharges.

Of the endpoints evaluated, % edema was the endpoint that was most conducive to regression analysis. Percent edema was also the most sensitive endpoint, with an EC10 of 12.68 mg/kg ovary Se dw, and a 95% confidence interval of 8.47-18.97 mg/kg ovary Se dw. TRAP results for %edema are shown below. The EC10 for % lordosis was 19.38 mg/kg ovary Se dw, but because of the large standard error surrounding the slope and inflection point parameters of the model, the corresponding confidence intervals (0.06-6103 mg/kg ovary Se dw) were extremely large. Coupled with the fact that the incidence of lordosis was low in both studies (less than 20% at the highest Se concentrations, with a maximum value of 25%), this endpoint was determined to be less appropriate than %edema. Model convergence for % hemorrhage could only be achieved in TRAP at an unrealistic y-intercept value of 150, indicating a negative 50% incidence of hemorrhage at a Se concentration of zero. Finally, model convergence for %larval survival could not be achieved in TRAP.

Chronic Value: The chronic value for bluegill was calculated as the EC_{10} value of 12.68 mg/kg Se dw (larval edema in repose to Se concentration in the parental ovaries).

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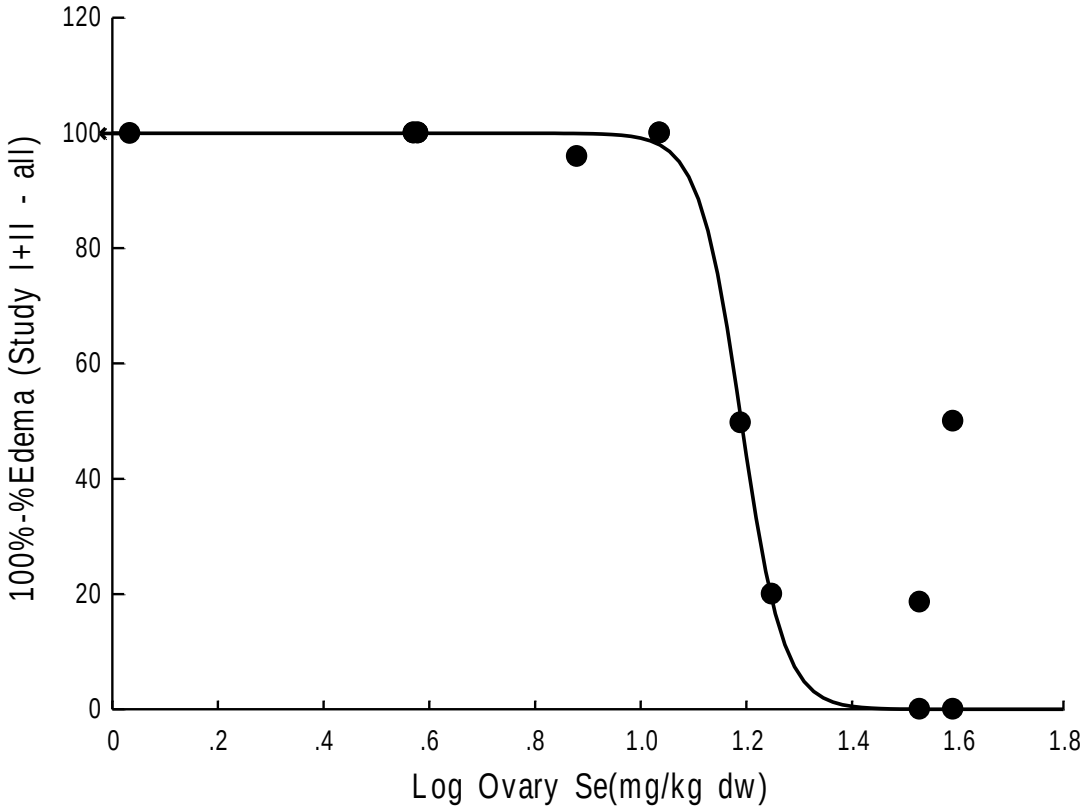
Combined Nest and Egg Cup Observations for TRAP Analysis (Study I&II)

Study	Stream	Data Source	Se Treatment	Ovary Se (mg/kg dw)	%Edema	%Lordosis	% Hemorrhage	%Survival
II	1	Egg Cup	Control	3.72	0	0	0	
II	1	Nest	Control	3.72	0	0	0	75.2
II	5	Egg Cup	Control	3.79	0	0	0	
II	5	Nest	Control	3.79	0	0	0	71.5
II	2	Egg Cup	2.5 µg/L	7.58	NA	NA	NA	
II	7	Nest	2.5 µg/L	7.58	4.1	25	77.6	
II	7	Egg Cup	2.5 µg/L	10.86	0	0	3.6	71.6
II	7	Nest	2.5 µg/L	10.86	0	0	52	
II	3	Egg Cup	10 µg/L	33.75	100	11.1	49.3	57.7
II	3	Nest	10 µg/L	33.75	81.4	5.0	55.5	
II	8	Egg Cup	10 µg/L	39.02	100	18.2	41.1	57.1
II	8	Nest	10 µg/L	39.02	50	14.7	26.7	69.7
I	5,7	Egg Cup	Control	0.79	0.1	1.8	0.1	28.8
I	3,8	Egg Cup	10 µg/L	17.71	80	11.6	28.5	9.1
I	4	Egg Cup	30 µg/L	15.46	50.3	6.3	26.8	

Incidence of larval bluegill edema as a function of the logarithm of the selenium concentration in parental ovaries.



Model Parameters (Logistic Regression Nonlinear Regression Model)

	Initial Guess	Final Estimate	S.E.	95% LCL	95% UCL
logX50	1.2	1.1905	2.52E-02	1.135	1.2459
S	6.29	6.2904	4.6679	-3.9836	16.564
Y-intercept	100	99.93	6.0437	86.628	113.23

Effect Concentration Summary

% Effect	ECx	95% LCL	95% UCL
50	15.504	13.646	17.615
20	13.657	10.232	18.229
10	12.68	8.4743	18.974
5	11.842	7.1031	19.743

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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 Φg 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 Φg 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 (Φg 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

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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 Φg/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. 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.

Effects on Adults						
Se in diet, mg/kg dw	Se in water, :g/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

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Effects on Larvae				
Se in diet, mg/kg dw	Se in water, :g/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: EC_{20} and EC_{10} estimates using logistic equation with log transformation of exposure:

effect level	egg, mg Se/kg dw	whole body, mg Se/kg dw
EC_{20}	26.30	8.954
EC_{10}	24.10	7.936

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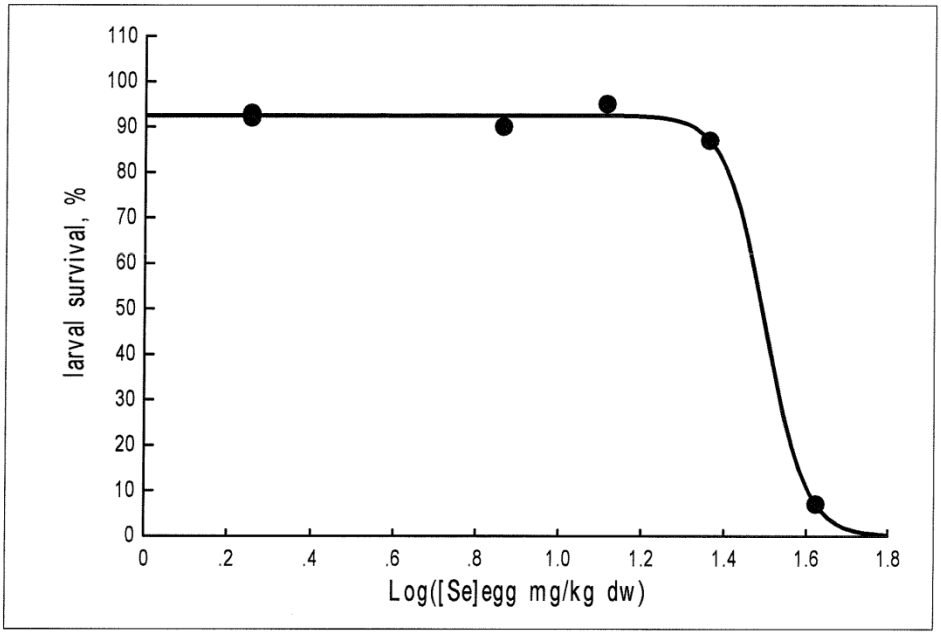
Coyle et al. 1993 bluegill larval survival - TRAP logistic



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4626	1.4990	0.0137	1.4554	1.5427
S	1.8812	5.041	0.529	3.358	6.723
Y0	92.50	92.50	1.05	89.15	95.85

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	31.55	28.54	34.89
20.0	26.93	23.75	30.54
10.0	24.55	21.19	28.45
5.0	22.54	19.02	26.72

06/29/2009 14:04 MED Toxic Response Analysis Model, Version 1.03

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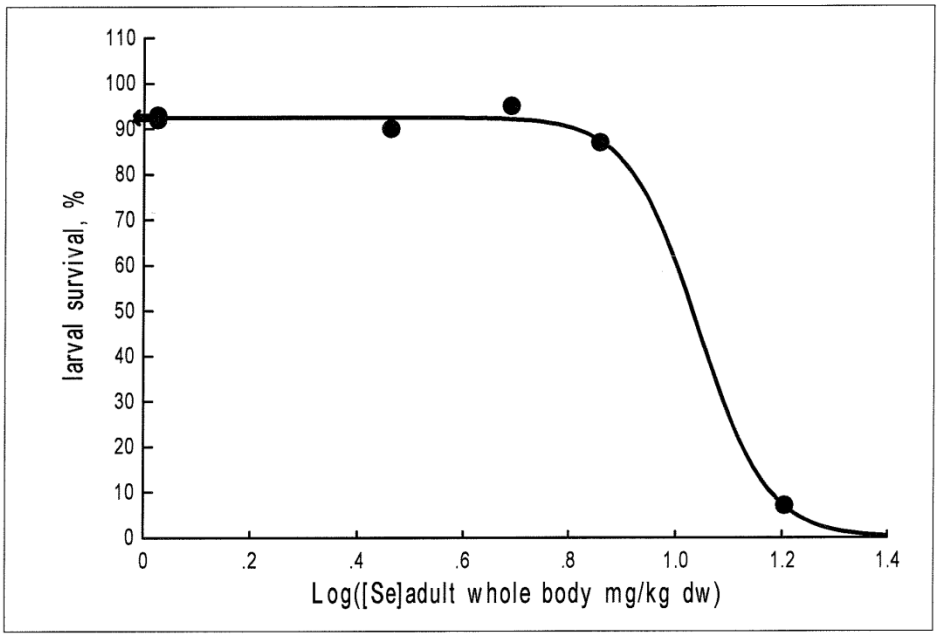
Coyle et al. 1993 bluegill larval survival - TRAP logistic



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0102	1.0408	0.0202	0.9764	1.1051
S	1.9483	3.851	0.452	2.412	5.290
Y0	92.50	92.52	1.17	88.81	96.23

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	10.985	9.472	12.739
20.0	8.929	7.398	10.777
10.0	7.910	6.342	9.865
5.0	7.074	5.484	9.125

06/29/2009 14:10 MED Toxic Response Analysis Model, Version 1.03

US EPA ARCHIVE DOCUMENT

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.

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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

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.

US EPA ARCHIVE DOCUMENT

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 4E and 20EC 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 20EC and then decreased in the cold treatment at a rate of 2EC per week for 8 weeks to reach 4EC and then maintained at that temperature for the remainder of the 180 days.

Effects Data : In the 20EC 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 4EC 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: 20EC, >6 mg Se/kg whole-body; 4EC, <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.

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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

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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	

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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 :g Se/L. For ES2, fish were exposed to a control and one nominal concentration, 5 :g 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).

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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 :g 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 were 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.

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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

ES 1	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)	

ES 3	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)	

ES 2	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

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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 EC10 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.

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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.

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 lower 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.

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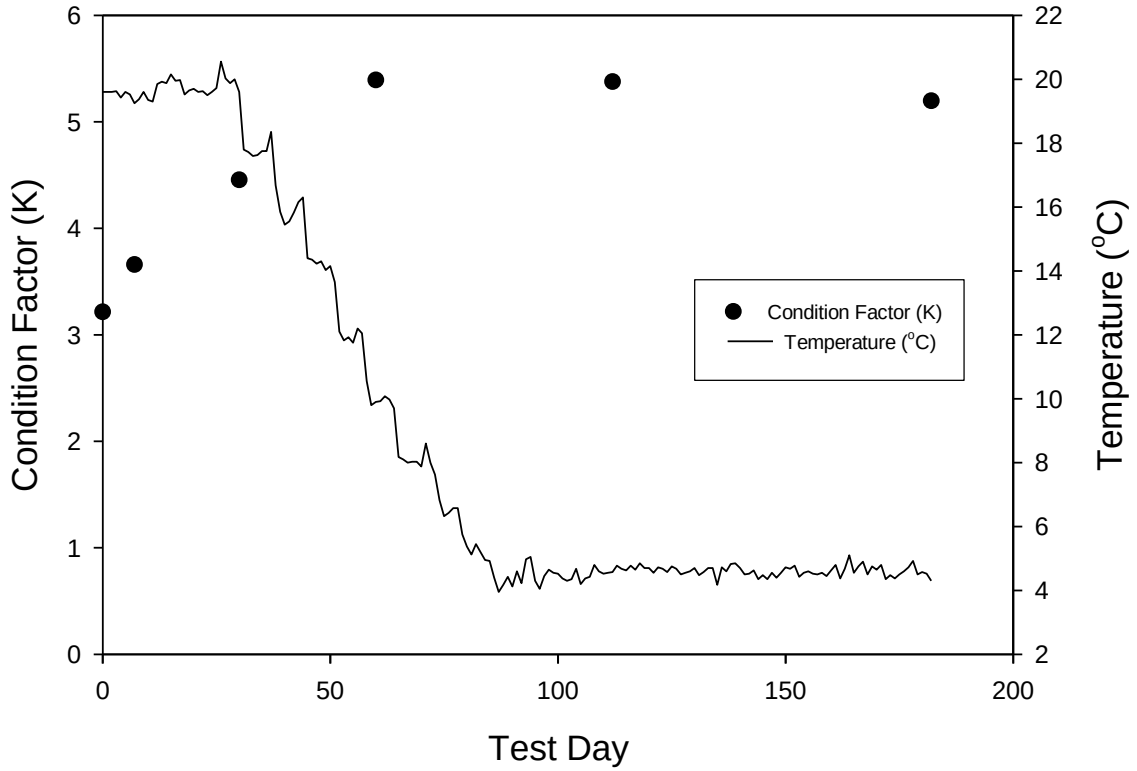

Relationship between body condition factor (K) and temperature in juvenile bluegill fed a diet of Se-enriched TetraMin in the McIntyre et al. (2008) study.

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. Such loss is not generally 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.

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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 logarithm of selenium concentration in the parental female ovary using TRAP produced an EC10 of 20.35 mg Se/kg dw (Figure 1).

Effect Concentration: 20.35 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 ovary, mg/kg dw		Larval survival, %	
		Individual	Average	Individual	Average
0.9 ± 0.1 (0.7 – 1.3)	6	5.38	3.1	75.5	95.3
	12	7.34		99.7	
	13	3.51		96.2	
	26	5.74		88.9	
	34	1.58		99.5	
	35	1.36		96.8	
	3	2.09		98.8	
	4	1.85		100	
	10 (2F)	2.11		97	
	13	1.86		97.1	
	14	1.40		98.4	
2.9 ± 0.5 (2.1 – 3.8)	9	9.59	8.8	84.9	94.8
	12	8.03		100	
	15	9.73		98.5	
	18	7.66		95.9	
7.5 ± 0.6 (6.3 – 8.4)	1	8.43	10.8	75	85.8
	2	25.15		63.9	
	5	15.31		90.6	
	7	1.20		79.1	
	8	6.78		95	
	16	8.25		96.8	
	19	10.20		100	
11.2 ± 1.4 (9.3 – 14.1)	6	35.44	25.0	91.5	88.7
	11	15.08		77.9	
	17	24.59		96.7	
26.7 ± 1.7 (23.6 – 29.5)	2	37.14	40.0	91.2	18.3
	5	44.67		0	
	11	34.26		75.9	
	16	35.58		0	
	17	33.48		9.9	
	19	48.24		0	
	36	35.81		6.3	
	37	37.88		0	
	51	32.95		0	
	52	59.89		0	
53.1 ± 4.8 (45.5 – 61.9)	22	46.22	61.0	0	0
	25	70.45		0	
	30	81.62		0	
	31	54.99		0	
	32	53.96		0	
	41	51.48		0	
	48 (2F)	84.31		0	
	50 (2F)	32.87		0	
	55	73.33		0	

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Measured Se in diet fed to parents, mg/kg dw[a]	Spawn No.	Se in parent ovary, mg/kg dw		Larval survival, %	
		Individual	Average	Individual	Average
78.4 ± 4.3 (73.2 – 87.0)	4 (2F)	66.81	77.6	66	5.5
	7	56.98		0	
	8	86.49		0	
	10	65.99		0	
	18	72.35		0	
	21	71.89		0	
	24	62.44		0	
	28	99.02		0	
	38	52.37		0	
	44	102.82		0	
	47	88.15		0	
	49	105.29		0	

[a] ± standard error; range of concentrations in parentheses.

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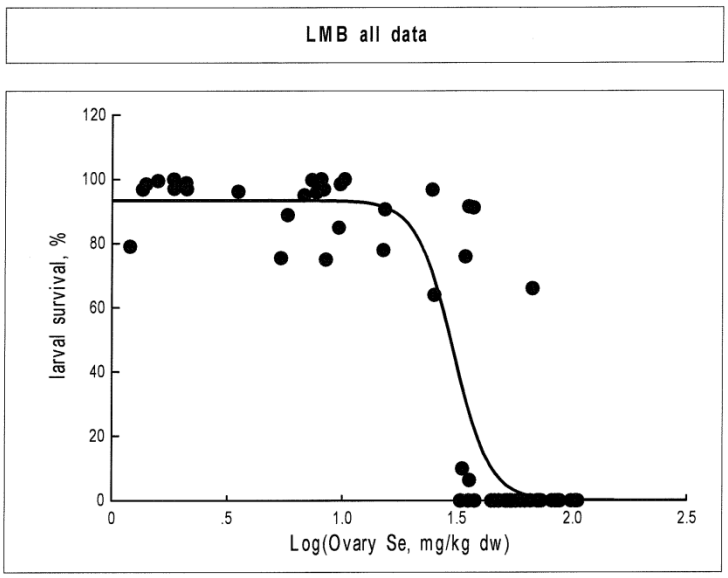
Figure 1. Percent larval survival as a function of the logarithm of the selenium concentration in the parental ovaries.



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4000	1.4828	0.0309	1.4207	1.5449
S	6.000	3.154	1.138	0.872	5.435
Y0	97.00	93.37	4.44	84.46	102.28

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	30.40	26.35	35.07
20.0	23.60	17.65	31.56
10.0	20.35	13.77	30.09
5.0	17.758	10.920	28.876

03/27/2009 11:39 MED Toxic Response Analysis Model, Version 1.03

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APPENDIX D: Other Data

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Selenite

Additional data on the lethal and sublethal effects of selenium on aquatic species are presented in Table D-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 D-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.

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 D-1.

Watenpaugh and Beitinger (1985a) found that fathead minnows did not avoid 11,200 µg/L selenate during 30-minute exposures (Table D-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.

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Table D-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*	Selenious acid	-	20 days	Inhibited growth	3,140	Albertano and Pinto 1986
Red alga, *Galdieria sulphuraria*	Selenious acid	-	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,	Sodium	-	48 hr	Incipient	62	Bringmann and

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Chilomonas paramecium	selenite			inhibition		Kuhn 1981; Bringmann et al. 1980
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*	Sodium selenite	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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
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
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,	Sodium	135	96 hr	LC50	8,200	Hodson et al.

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
Oncorhynchus mykiss	selenite		9 days	(fed)	6,920	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
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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
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
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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
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
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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
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
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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration	Effect	Concentration[a]	Reference
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 (ug/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 (Microtox>)	68,420	Yu et al. 1997

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Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (ug/L)[a]	Reference
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
			Selenium (VI)			
Bacterium, *Vibrio fisheri*	Sodium selenate	-	15 min	50% decrease in light output (Microtox>)	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

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Species	Chemical	Salinity (g/kg)	Duration	Effect	Concentration (ug/L)[a]	Reference
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
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 Φg 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.

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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.

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The experimental groups were subdivided into those receiving reference water (hatchery water; 24-Road Fish Hatchery) or site water (Table D-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 D-2. Treatment conditions during the 30-day larval study

Source of Larvae	Treatments	Se in food (mg/kg dw)	Dissolved Se in water (Φg/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

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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.

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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 D-3.

Table D-3. Treatment conditions during the 30-day larval study

Water Treatments	Se in food (mg/kg dw)	Se in water (Φg/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

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Water Treatments	Se in food (mg/kg dw)	Se in water (Φg/L)
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 year↘s 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

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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.

Other Data – Chronic Studies with Fish Species

Some chronic studies met the requirements of an acceptable chronic test but were excluded from Table 1 for a variety of reasons. Summaries of these studies are provided below.

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Vidal, D., S.M. Bay and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contamin. 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 D-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).

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Table D-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).

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 D-6). 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 D-6. 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. Contamin. 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.

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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 D-7) and two high (Fish 1 and 4 in Table D-7). Larval mortality and developmental deformities were not related to selenium concentrations in eggs (Table D-7). 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 D-7. 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).

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[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.

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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 D-5).

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Table D-5. 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.

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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

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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 D-9 for summary data; see Table D-10 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 D-1).

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 D-11; Figures D-2 and D-3). 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 D-12). 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.

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Table D-9. 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 D-10. Fathead minnow first brood embryo parameters and adult whole-body (WB) selenium concentrations (dw) for each site (± 1SE); for site acronyms see Table D-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

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T-1f-1	ETC	39.76	141	0.52	0.70
T-3b-1	ETC	47.47	208	0.88	0.92
T-5a-1	ETC	46.37	634	0.07	0.17

Table D-11. 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 D-12. 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)

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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 D-1. The fraction survival of embryos (left) and the fraction of embryos successfully fertilized (right) relative to the maternal whole body selenium concentration.

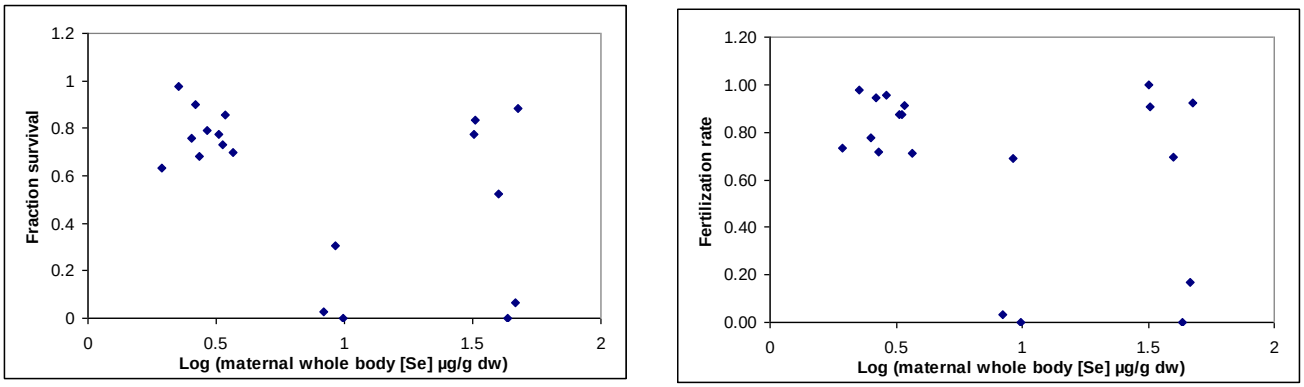

Figure D-2. Percent 2-day post-hatch larvae without edema (A), finfold deformity (B), craniofacial deformity (C), and spinal deformity (D) relative to maternal whole body selenium concentration.

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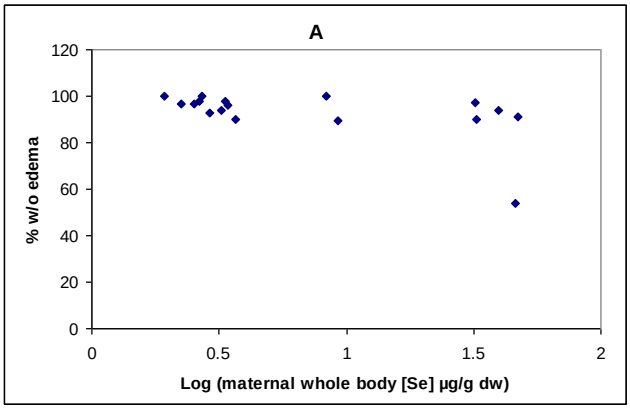

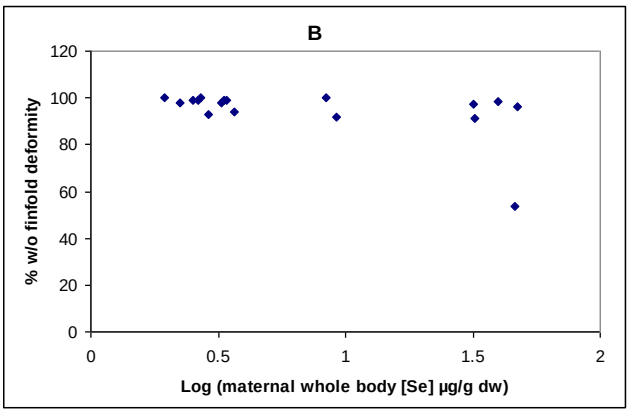

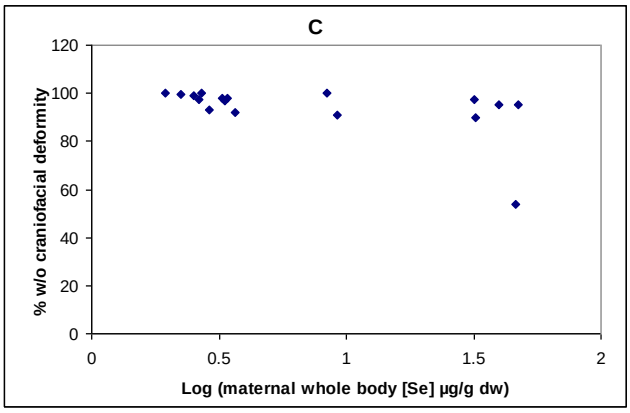

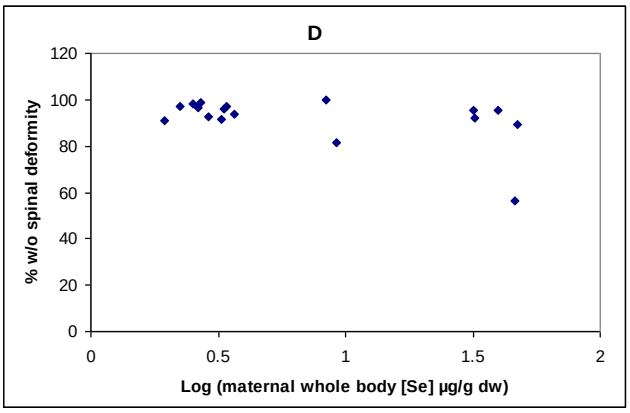

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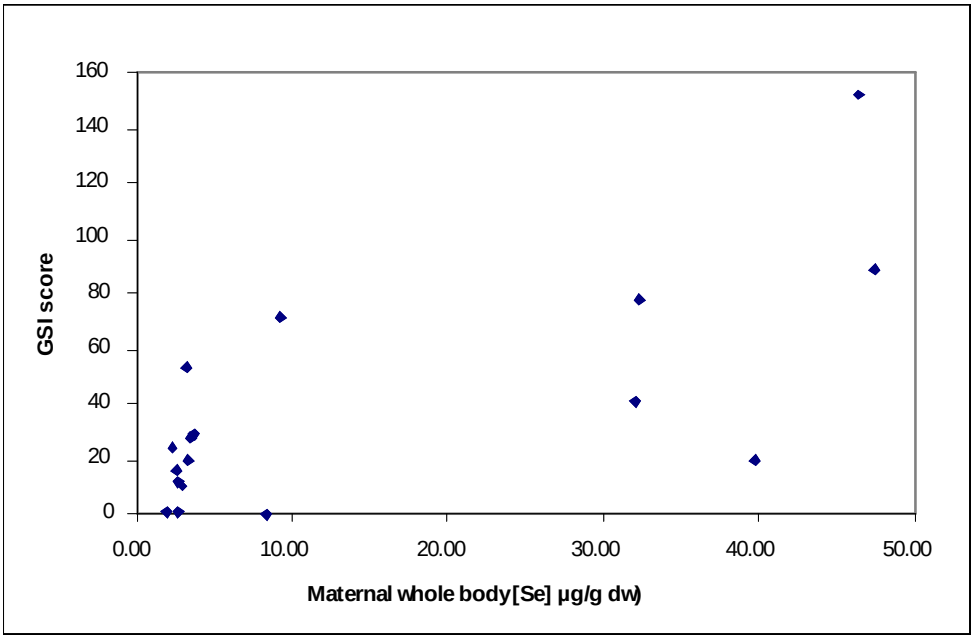
Figure D-3. Percent 2-day post-hatch larvae Graduated Severity Index (GSI) relative to maternal whole body selenium concentration



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 three tests with a rotifer, and annelid, and an insect (mayfly) were found suitable for establishing species sensitivity.

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.

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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 oligochate, *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.

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 D-10). 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 D-13. 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 D-13). 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

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periphyton Se concentration of 11 mg/kg dw translates to an adult mayfly Se concentration of 24.2 mg/kg dw.

The following invertebrate studies were inconclusive for establishing species sensitivity because of limitations in the experimental designs, as explained for each.

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 Φg Se/g dry weight resulted in a 46% reduction in growth relative to the controls. At a larval tissue concentration of 8.6 Φg 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 Φg 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 *C. decorus* 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.

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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.

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APPENDIX E: Toxicity of Selenium to Aquatic Plants

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Selenite

Data are available on the toxicity of selenite to 13 species of freshwater algae and plants (Table E-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 E-1). Wehr and Brown (1985) reported that 320 µg/L increased the growth of the alga *Chrysochromulina breviturrita*. Thus, the sensitivities of freshwater algae to selenite cover about the same range as the acute and chronic sensitivities of freshwater animals.

The 96-hr EC_{50} for the saltwater diatom, *Skeletonema costatum*, is 7,930 µg/L, based on reduction in chlorophyll a (Table E-1). Growth of *Chlorella* sp., *Platymonas subcordiformis*, and *Fucus spiralis* increased at selenite concentrations from 2.6 to 10,000 µg/L (Table E-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.

Selenate

Growth of several species of green algae was affected by concentrations ranging from 100 to 40,000 µg/L (Table E-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 E-1). Wheeler et al. (1982) reported that concentrations as low as 10 µg/L reduced growth of *Porphyridium cruentum* (Table E-1).

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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.

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Table E-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

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
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
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,	Sodium	-	14	NOEC	>2,400	Jenner and

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Species	Chemical	Hardness (mg/L as $CaCO_3$)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
Lemna minor	selenate			(mult. Rate)		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*	Sodium selenate	-	60	NOEC growth	10,433	Wong and Oliveira 1991a

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Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L)[a]	Reference
quadruplicatum						
Diatom, *Chaetoceros vixvisibilis*	Sodium selenate	-	60	NOEC growth	1,043	Wong and Oliveira 1991a
Coccolithophore, *Cricosphaera elongata*	Sodium selenate	-	14	Reduced growth	41,800	Boisson et al. 1995
Dinoflagellate, *Amphidinium carterae*	Sodium selenate	-	60	NOEC growth	10,433	Wong and 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.

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APPENDIX F: Unused Data

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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)

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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)

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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)

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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)

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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).

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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)

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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)

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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)

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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)

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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)

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APPENDIX G: Supplementary information on Selenium Bioaccumulation in Aquatic Animals

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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 the 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

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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. 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.

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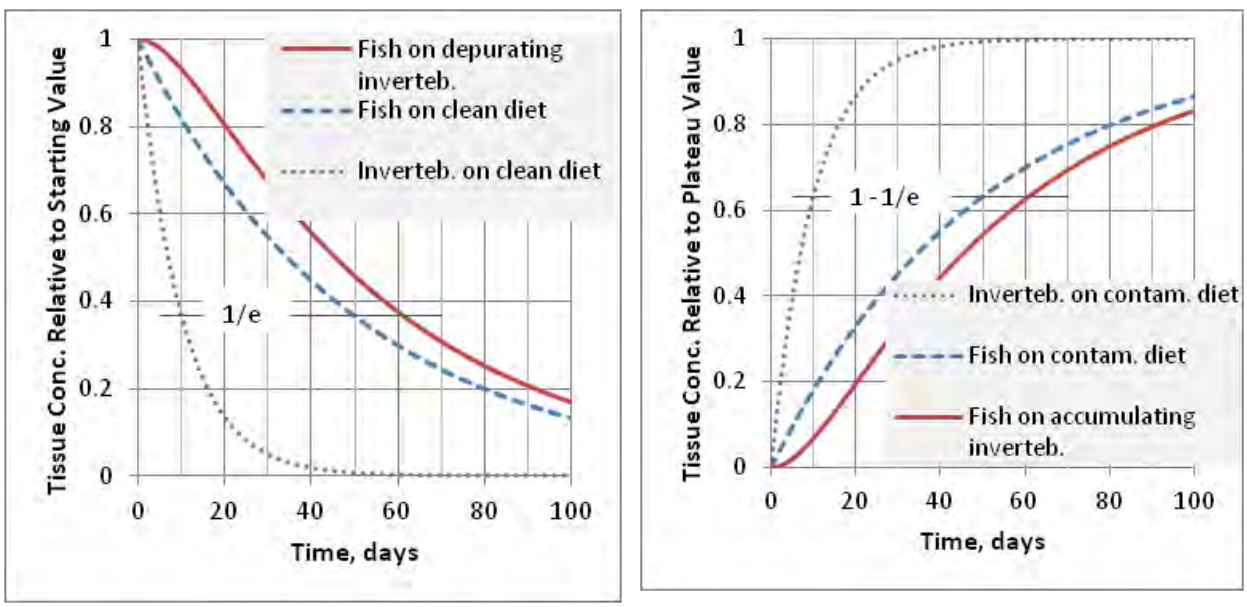
3.0 Kinetics of Accumulation and Depuration: Averaging Period

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 reach a value of 1/e, as shown in Figure G-1a. The accumulation curve is the inverted depuration curve, as shown in Figure G-1b.



Figures G-1 a & b. Depuration and accumulation behavior for invertebrate k=0.1/day and fish k=0.02/day

In the Figures G-1 a & b examples, the characteristic time for invertebrates on an invariant diet is 10 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 G-1 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. Lacking any k values for Trophic Level 1 (algae-detritus-sediment), this analysis makes the worst-case assumption that these respond instantaneously to water concentrations. Thus, at any time t, after normalization to their respective benchmarks:

Algae-detritus-sediment: $C_{TL1}[t] = C[t]water$

The above is not saying that in ordinary units of concentration, the Trophic Level 1 equals water, but only when normalized to their respective benchmarks. In ordinary (non-normalized) units of concentration, TL2 would equal TL1 x ER, the enrichment function.

For invertebrates and fish, accumulation at time t equals accumulation at time t-1 plus intake minus depuration, as follows:

Invertebrates:

$$C_{TL2}[t] = C_{TL2}[t\text{-}1] + AE_{TL2}\, IR_{TL2}\, C_{TL1}[t\text{-}1] - k_{TL2}\, C_{TL2}[t\text{-}1]$$

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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 invertebrates, values for k_{TL2} are tabulated in elsewhere in the document. It was assigned here a value of 0.1/day, considerably higher than those for Lumbriculus, Asian clam, zebra mussel, but a bit lower than that for 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. 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 G-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

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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 G, 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:

$$\textit{Fractional Effect}[t] = NORMSDIST(z[t])$$

where z[t] is given by:

$$z[t] = LN(C_{TL3}[t]/1.5)/0.3164$$

Exposure Scenarios. A range of exposure scenarios were evaluated under which the criterion was attained while maximizing exposure and effects. These begin with absolute worst case scenarios, followed by more realistic situations. The following represent worst case situations because the 30-day average water concentration remains continuously at the criterion concentration at all times.

1. Steady concentrations at the criterion: this is worst-case continuous exposure.

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.

   Because they lack real-world random variability, the above two scenarios are not realistic, but are used as absolute worst cases for purposes of comparison. The following two scenarios represent somewhat more realistic possibilities for intermittent releases.

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3. Normally distributed 1-day spikes having CV=0.12, again occurring at uniform 30-day intervals (separated by 29 days of zero concentration). Because a normal distribution has no upper bound, some percentage of 30-day average exceedances must be provided for. For this scenario, 5% of the 1-day spikes exceeded 30X the 30-day average water criterion concentration.[2]

4. Log-normally distributed 1-day spikes, with log standard deviation = 0.20, again occurring at uniform 30-day intervals. As with the previous scenario, 5% of the 1-day spikes exceeded 30X the 30-day average water criterion concentration.

Finally, the following represents a realistic and indeed typical situation for continuous exposure:

5. Log-normally distributed, smoothly variable concentrations, continuous rather than intermittent exposure, 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 (Delos 2008).

With respect to maximizing toxic effects while attaining the criterion, Scenarios #1 and #2 are absolute worst cases. Scenarios #3 and #4 are somewhat more reasonable worst cases. While relatively low variability of concentrations occurring in intermittent releases might correspond to some real-world situations, the uniformity of spacing between spikes is unrealistic. That uniformity of spacing allows maximizing exposure while attaining the criterion a high percentage of the time. Were the spacing not uniform, exposures concentrations would have to be substantially less in order to continue to attain the 30-day average water criterion concentration. In contrast to the others, Scenario #5 represents typical time variability in ambient waters.

Scenarios 3, 4, and 5 require 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. In Scenario 5, the concentrations at each half-day time step were generated by the following formula:

[2] Concentrations of selenium in contaminated groundwater tend to be rather stable. Dilution by rainfall events may punctuate these rather stable elevated concentrations with inverted spikes of low concentrations. The 2008-2012 selenium data reported by FTN Associates, Ltd., in "Evaluation of Selenium Concentrations in Fish Collected in the Receiving Stream for Mueller Copper Tube Products, Inc., Wynne, Arkansas" dated September 17, 2012, showed that the upper half of the concentration distribution tended to fit a normal distribution having the low variability represented by CV=0.12. In contrast to situation assessed by FTN Associates, this scenario envisions intermittent spike releases at uniform intervals where the spike concentrations have this low variability.

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*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 (Delos 2008). 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.

Figure G2. 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).

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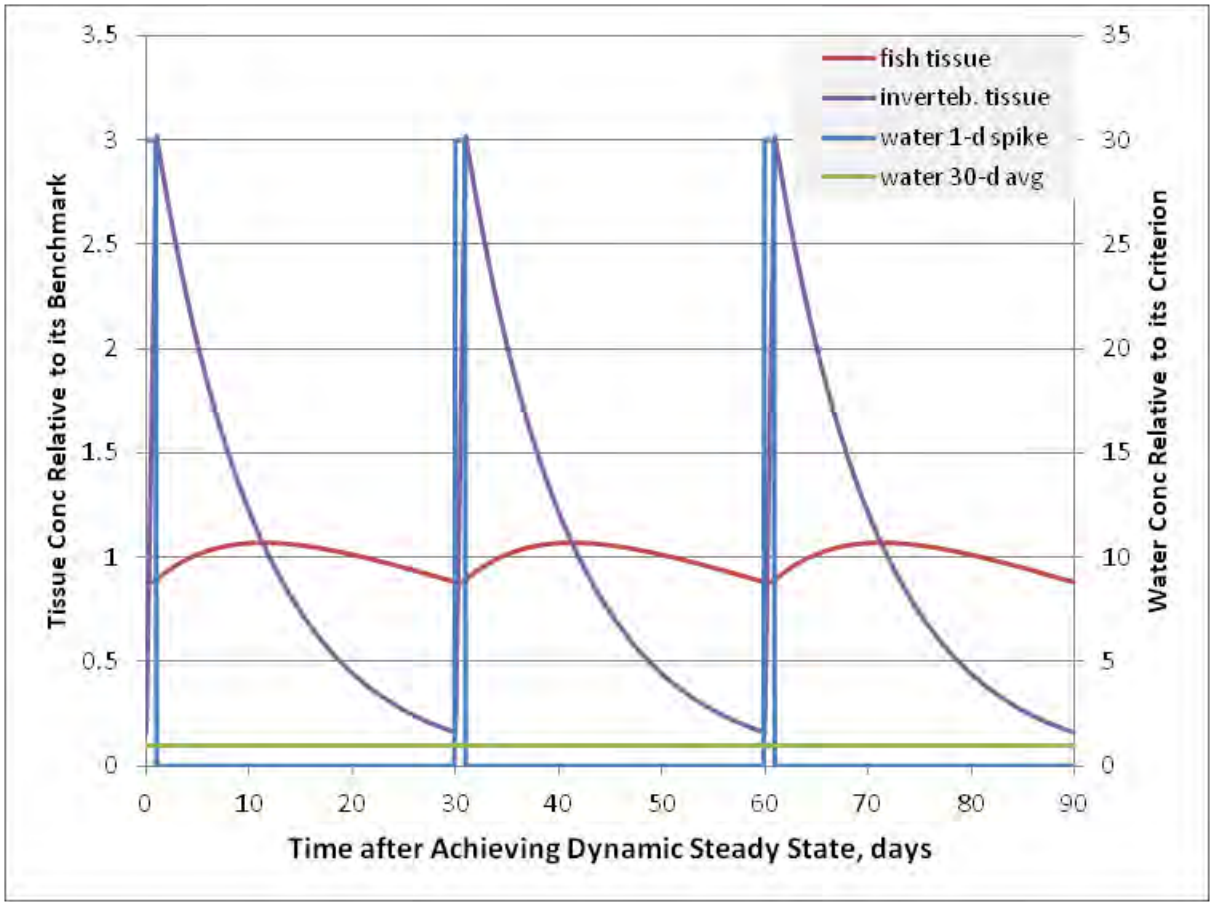

Figure G2. Uniform 1-day spikes at 30X the water criterion concentration, occurring at uniform 30-day intervals such that the 30-day average always equals the criterion.

With their more rapid kinetics, invertebrate tissue concentration swings are much more drastic than fish tissue concentration swings, but were the spike to continue as a steady exposure 30-fold above the water benchmark, both invertebrate tissue and fish tissue would 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. However, the nonlinearity of the log-probit concentration-response curve means that the reduction in toxicity while below the criterion does not quite balance the increase in toxicity while above the criterion. The average effect ends up being 10.3% instead of 10.0%.

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3.2.1.2 Normally distributed 1-day spikes having CV=0.12, uniformly separated by 29 days at zero concentration.

This scenario simulates the low variability of elevated selenium concentrations sometimes observed in releases of groundwater selenium, but discharged here in uniformly spaced spikes.

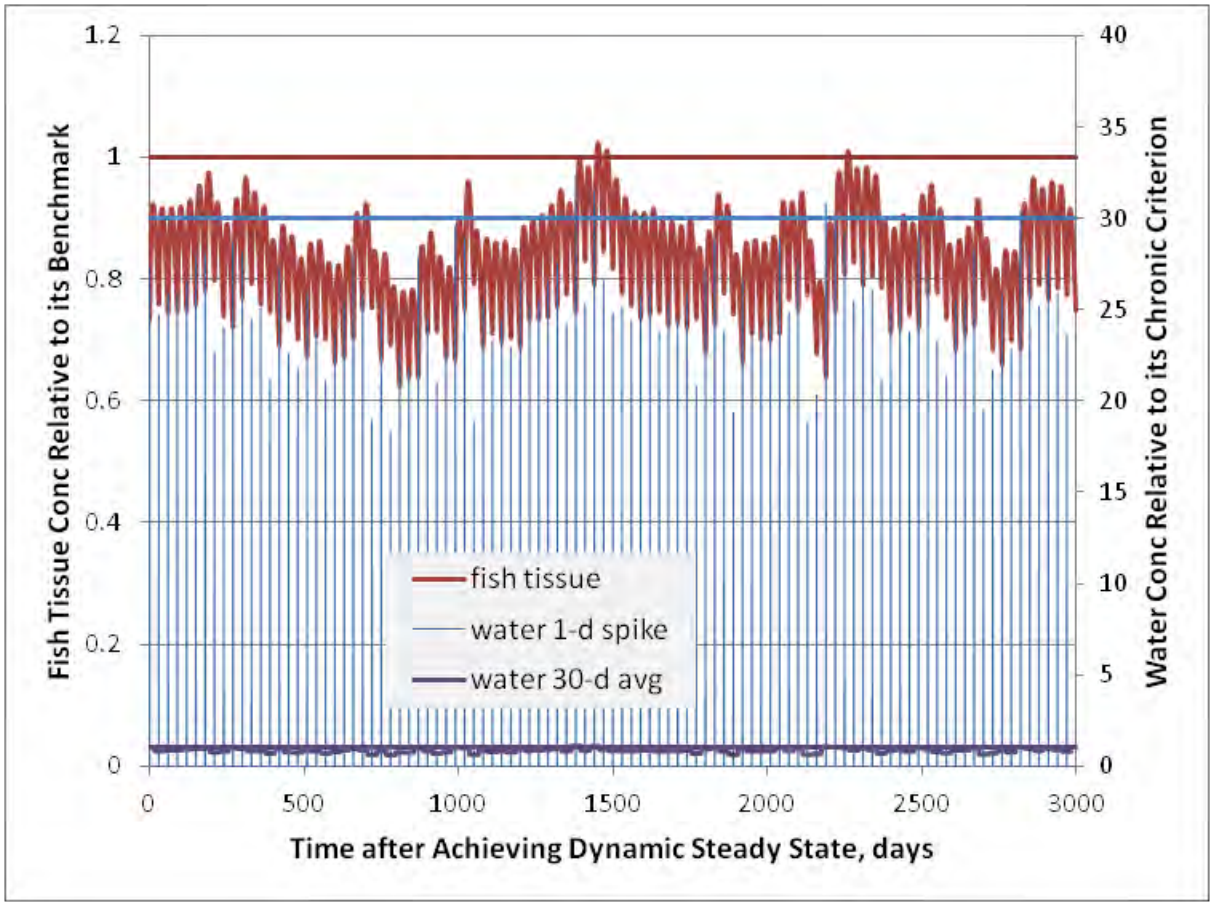

Figure G3. A typical random series for Scenario 3, normally distributed 1-day spikes having CV=0.12, uniformly separated by 29 days at zero concentration. Again read fish tissue concentrations from left scale, water concentrations from right scale. Note: (a) the time scale is 3000 days (100 sets of 30 days), compared to 90 days in Figure G-2, and (b) the left axis is scaled differently than Figure G-2 because the wide concentrations swings of invertebrate tissue are not presented here. Note also that on 29 days of each 30 day period the water concentration is zero – because of pixel limitations, the spike line thickness on the graph overstates the spike duration relative to the intervening white space. And note that Time=0 does not represent the beginning of exposure; prior to Time=0 the intermittent spikes had been at their median concentration for thousands of days.

In the Figure G-3 example run, 5 of the 100 spikes yield exceedance of the criterion, possibly representing a detection frequency resolution limit in an ambitious real-world monitoring program. On

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0.78% of the days, the fish tissue criterion exceeded its criterion, and the aggregate effect (considering all days, whether exceeding or not) was 3.73%. The effect in this somewhat more realistic scenario is less than in the previous absolute worst case because the average spike concentration must necessarily be less here in order to prevent the variable spikes from causing exceedance of the 30-day average water target.

3.2.1.3 Log-normally distributed 1-day spikes, with log standard deviation = 0.20, uniformly separated by 29 days at zero concentration.

Although still representing low variability when comparing among spike concentrations, the spikes in this scenario have greater and more typically skewed variability than the previous scenario.

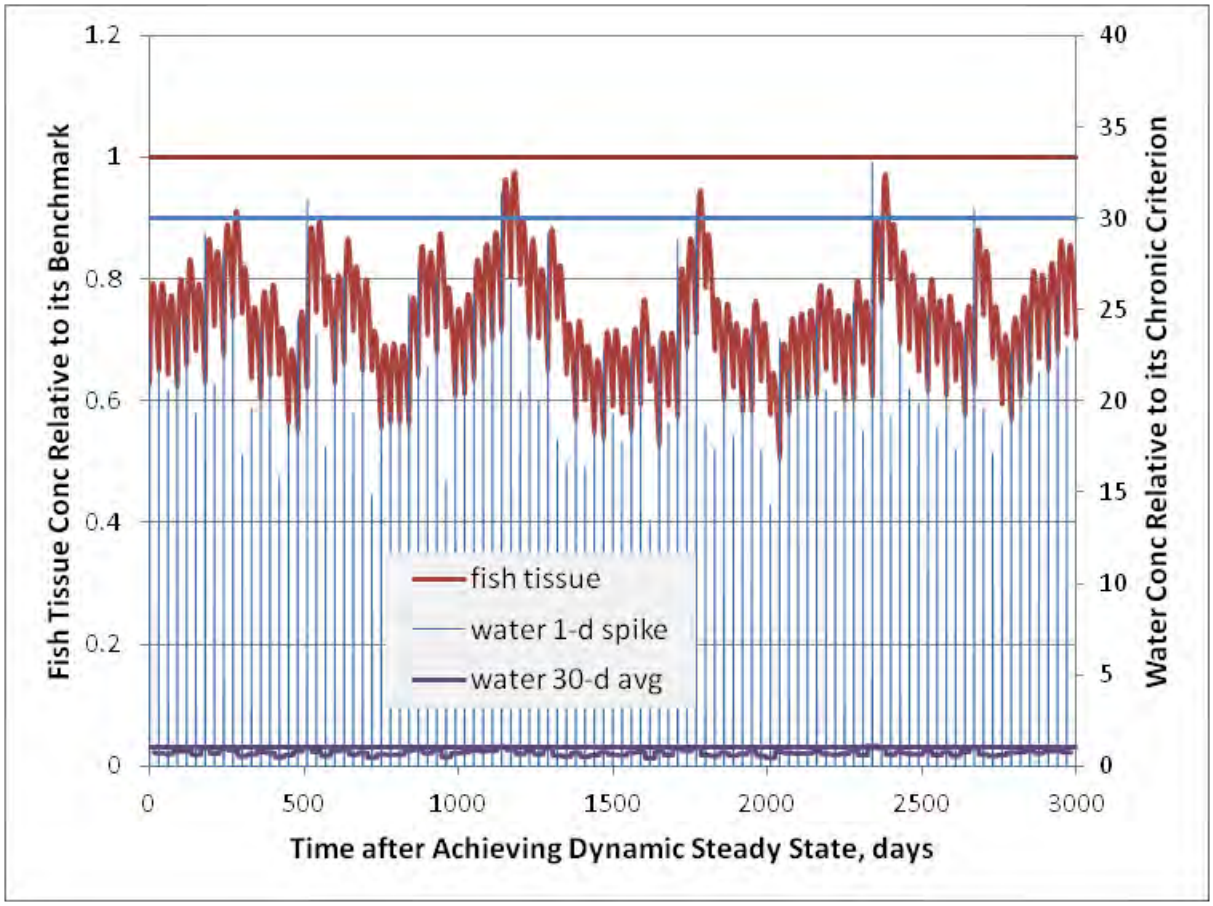

Figure G4. A typical random series for Scenario 4, log-normally distributed 1-day spikes, with log standard deviation = 0.20, uniformly separated by 29 days at zero concentration. This figure is structured like Figure G-3, and again exposure had long preceded Time=0.

In the Figure G-4 example, 5 of the 100 the spikes cause exceedance of the criterion, but did not cause any exceedance of the fish tissue criterion. The aggregate effect was 1.64%. Because the exposure is more variable than in the previous scenario, the average exposure (and aggregate effect) must necessarily be less in order to prevent exceedance of the 30-day average water target.

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3.2.1.4 Log-normally distributed, smoothly variable concentrations, continuous rather than intermittent exposure

This is the most realistic of the scenarios, corresponding to typical variability observed in streams.

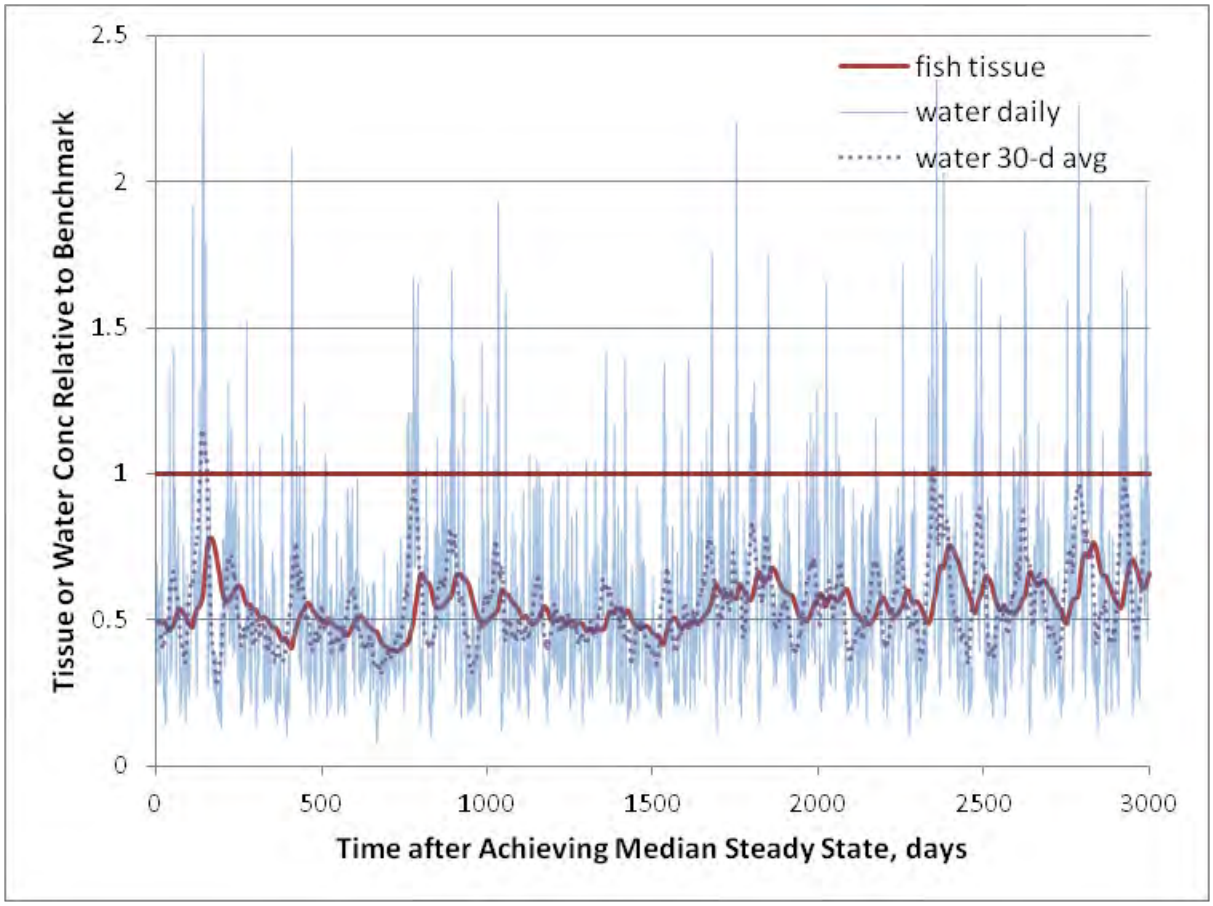

Figure G-5. A typical example of log-normally distributed, smoothly variable concentrations, continuous rather than intermittent exposure. The standard deviation of natural logs is 0.5 and the serial correlation coefficient of logs is 0.8 for daily values, both typical real-world situations. (The compression of 3000 days into the graph might make it difficult to recognize that the time series is smoothly varying – it has serial correlation.) Prior to Time=0, exposure had been at the median concentration for a long period of time.

In the Figure G5 example run, instantaneous water concentrations exceed the 30-day average criterion 7.5% of the time. The 30-day average concentrations exceed the criterion 1.15 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 0.17%.

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In contrast to the previous scenarios, the elevated concentrations here are random in their spacing and duration. This additional 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 Scenarios 3, 4, and 5 involve generation of random concentrations, the above graphs show just one run (3000 days) for each. Full results for the 20 runs for each 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	10.3	30-d avg water conc. remains steady at benchmark (Fig. 2)
3. Norm. spikes	3.58	5.00	0.59	3.60	Spike median=25.1 x benchmark, spike CV=0.12 (e.g., Fig. 3) [2]
4. Lognorm spikes	3.59	5.00	0.13	1.57	Spike median=21.6 x benchmark, spike log stdev=0.2 (e.g., Fig. 4) [3]
5. Smooth variable	0.99	7.45	0.00	0.18	Median=0.49 x benchmark, log stdev=0.5, rho(daily)=0.8 (e.g., Fig. 5) [4]

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 exactly 5% exceedances. Runs not yielding 5% exceedances were not used. Among the 20 runs with 5% exceedances, the effect CV=0.05 (coefficient of variation).
3. Results for Scenario 4 are average of 20 runs of 3000 days, each run with exactly 5% exceedances. Runs not yielding 5% exceedances were not used. Among the 20 runs with 5% exceedances, the effect CV=0.11.
4. Results for Scenario 5 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=0.28.

3.2.3 Conclusion

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.

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APPENDIX H: Binary Classification Statistics

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Sensitivity, Specificity, and Related Statistics in Applying Water-Column Criteria

The ability of any water criterion to predict exceedance of the egg-ovary FCV can be evaluated in terms of sensitivity, specificity, and related statistical measures of a binary classification test. In this case, each species-site combination in our data set of aquatic sites can be classified as (a) either above or below the egg-ovary criterion and (b) either above or below the water criterion concentration. Although such a binary classification scheme does not consider the degree to which measurements are above or below a criterion, water quality standards are usually implemented as a binary decision (a water body either attains or exceeds criteria). Thus, a statistical analysis using binary classification can provide valuable information about how well the recommended selenium water-column criteria values ensure attainment of the egg-ovary FCV in a regulatory context.

Separate binary classification tables for the 49 lotic species-site combinations and 83 lentic species-site combinations were generated using the following format:

	Tissue concentration greater than tissue criterion	**Tissue concentration less than tissue criterion**
Water concentration greater than water criterion	True Positive (*TP*)	False Positive (*FP*)
Water concentration less than water criterion	False Negative (*FN*)	True Negative (*TN*)

Below are Tables 9a and 9b reproduced from the main text showing the frequency of binary classifications for lentic and lotic waters in the confirmation dataset:

Lentic waters

	Tissue concentration greater than tissue	**Tissue concentration less than tissue criterion**
Water concentration greater than water criterion	92	13
Water concentration less than water criterion	14	21

Lotic waters

	Tissue concentration greater than tissue	**Tissue concentration less than tissue criterion**
Water concentration greater than water criterion	248	206
Water concentration less than water criterion	52	182

From the counts in the binary classification tables, the sensitivity, specificity, and related statistics (Fawcett 2006, Lowry 2011) are given as:

- Sensitivity or True Positive Rate *(TPR)* = $TP / (TP + FN)$
- Specificity or True Negative Rate *(TNR)* = $TN / (FP + TN)$
- Positive Predictive Value (*PPV)* = $TP / (TP + FP))$
- Negative Predictive Value (NPV) = $TN / (TN + FN))$

Sensitivity-specificity and PPV-NPV look at the problem from different perspectives. Sensitivity-specificity indicate probabilities given *tissue value* exceedance or attainment; PPV-NPV indicate probabilities given *water value* exceedance or attainment.

These statistics evaluate the performance of the water criterion values as predictors of attainment or exceedance of the egg-ovary FCV. In this application, sensitivity addresses how well exceedance of the water criterion detects exceedance of the tissue criterion. It is the ratio of the species-site combinations exceeding both water and tissue criteria compared to the total number actually exceeding the tissue criterion. Thus, given actual exceedance as determined by tissue measurement, sensitivity indicates the probability that the water measurement will correctly indicate such exceedance. Conversely, specificity addresses how well attainment of the water criterion indicates attainment of the tissue criterion. It is the ratio of the number of species-site combinations attaining both water and tissue criteria compared to the total number attaining the tissue criterion. Thus, given actual attainment as determined by tissue measurements, specificity indicates the probability that the water measurement will correctly indicate such attainment. For both sensitivity and specificity, higher values (i.e., closer to 1.0) are better, respectively indicating few false negatives or false positives. However, even if selenium bioaccumulation (the relationship between tissue and water concentrations) were not variable across sites, such that water concentrations have limitations for predicting selenium risks (as discussed for example by Chapman et al. 2009, 2010), statistical uncertainty in the tissue and water concentration measurements themselves would

generally prevent perfect sensitivity and specificity. Consequently, establishing a reliable criterion involves a tradeoff between sensitivity and specificity. Lowering the water criterion to increase its sensitivity generally decreases its specificity (possibly making it over-protective). Raising the water criterion to increase its specificity generally decreases its sensitivity (possibly making it under-protective).

The positive predictive value (*PPV*) addresses how well water criterion exceedance (test positive) predicts tissue criterion exceedance. That is, given a water criterion exceedance, the *PPV* indicates the probability that the tissue criterion is also exceeded. It is the ratio of the number of species-site combinations exceeding both water and tissue criteria compared to the total number actually exceeding the water criterion (i.e. the proportion of true positives out of all positive results). Conversely, the negative predictive value (*NPV*) addresses how well water criterion attainment (test negative) predicts tissue criterion attainment. That is, given water criterion attainment, the *PPV* indicates the probability that the tissue criterion is also attained. It is the ratio of the number of species-site combinations attaining both water and tissue criteria compared to the total number attaining the water criterion (i.e. the proportion of true negatives out of all negative results). Again, *PPV* and *NPV* values closer to 1.0 are better; however, these statistical measures are also subject to the same tradeoffs as described for selectivity and sensitivity. Thus, the limitations of the statistical parameters presented here should be recognized when evaluating the performance of the water criterion concentration values, and no single parameter should be relied upon at the exclusion of the others.

Sensitivity and specificity are inherent to the firmness of relationship between water and tissue concentrations and to the particular water criterion concentration chosen. PPV and NPV likewise depend on that, but they also depend on the prevalence of tissue concentrations exceeding the criterion in the study population. When the binary statistics are taken from a relatively high risk population (such as from studies dominated by sites having elevated selenium inputs), the following alternative forms of the equations are useful for calculating PPV and NPV for a lower risk population (such as represented by fish tissue concentrations observed in the EPA (2012) National Rivers and Streams Assessment, representing in all U.S. flowing waters) having known prevalence, P, of exceeding the tissue criterion:

- $PPV = Sens \cdot P / ((Sens \cdot P) + (1 - Spec)(1 - P))$
- $NPV = Spec \cdot (1 - P) / (Spec \cdot (1 - P) + (1 - Sens) \cdot P)$

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Where Sens and Spec are the calculated sensitivity and specificity (from the data on the higher risk population). Where prevalence is very low, as in the nationwide survey population, PPV is calculated to be low and NPV high.

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APPENDIX I: Site Specific Criteria

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1.0 Translating the concentration of selenium in tissue to a concentration in water

The EPA is recommending a selenium criterion to protect aquatic life that is expressed as a concentration in the eggs or ovaries of fish. Although the selenium concentration in eggs or ovaries is the most sensitive and reliable basis for a criterion, implementation can be challenging because most state and tribal Clean Water Act programs require the expression of water quality criteria as an ambient concentration in the water-column. Therefore, the EPA is also recommending two water-column criterion values, one for lotic or flowing waters, and the other for lentic or still waters.

The EPA derived the water-column criterion values by developing a translation equation based on selenium bioaccumulation modeling. The EPA worked with the United States Geologial Survey 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; Presser and Luoma 2010; Presser 2013). The selenium egg-ovary FCV is translated to water concentration values at a set of lentic and lotic aquatic systems, and the distribution of site-specific water concentrations from these sites is used to derive water-column criterion values protective of aquatic life. This appendix describes how states and tribes may use this methodology to translate the egg-ovary FCV into site-specific water-column concentrations to more precisely manage selenium in specific aquatic systems. The use of a Bioaccumulation Factor (BAF) approach is also briefly discussed. States and tribes may also derive site-specific water concentration values using other scientifically defensible methods.

The relationship between the concentration of selenium in the eggs or ovaries of fish and the concentration of selenium in the water-column can vary from one aquatic system to another. The species of fish, the species and proportion of prey, and a variety of site-specific biogeochemical factors can substantially affect selenium bioaccumulation and thus determine the allowable concentration of selenium in surface waters that is protective of aquatic life. Because most state and tribal Clean Water Act programs require the expression of water quality criteria as ambient concentrations in water, implementation of the selenium criterion expressed as a concentration in the eggs or ovaries of fish requires the ability to translate the egg-ovary FCV into site-specific water-column concentrations. The EPA considered two different modeling approaches to implement the selenium egg-ovary FCV: mechanistic model of bioaccumulation, and a site-specific, field-derived bioaccumulation factor (BAF).

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The mechanistic modeling approach uses scientific knowledge of the physical and chemical processes underlying bioaccumulation to establish a relationship between the concentrations of a chemical in the water-column and the concentration of a metal in the tissue of aquatic organisms. The EPA used this approach to develop a mathematical equation that allows states and tribes to formulate site-specific models of trophic transfer of selenium through aquatic food webs and translate the egg-ovary FCV into an equivalent site-specific water concentration.

The BAF approach is an empirical model of bioaccumulation based on the measured concentration of a chemical found in the tissue of aquatic organisms and the water from where the aquatic organisms reside (U.S. EPA 2001c). The concentration of the chemical is measured in both fish tissue and the water-column, and a BAF is calculated by taking the ratio of the two concentrations. The BAF can then be used to estimate the concentration of the chemical in one media when only the concentration of the chemical in the other media is known.

Both the mechanistic modeling approach and the BAF approach have advantages and disadvantages that states and tribes should consider 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 and relatively simple measurements of selenium from the aquatic system that are easier and less expensive to obtain. However, uncertainty can be introduced if inappropriate parameters are chosen to model selenium bioaccumulation in the aquatic ecosystem. On the other hand, the BAF approach is conceptually and computationally simpler because it is completely empirical, relying only on field measurements with no need for any knowledge of the physical, chemical, or biological characteristics of the waterbody. However, the BAF approach requires multiple measurements of selenium in fish tissue that may be unavailable or expensive to obtain.

The appropriate modeling approach to use when translating the selenium egg-ovary FCV to a site-specific water-column concentration depends on individual circumstances. Although the mechanistic modeling approach may be a cost-effective method in situations where there is little or no current information about selenium bioaccumulation, the BAF approach may be desirable in circumstances where substantial resources have already been invested in fish tissue sampling and analysis. Because the national egg-ovary selenium criterion is intended to apply to all waters of the United States, and site-specific BAF values or the data required to derive site-specific BAF values are not available for the vast majority of those waters, the EPA developed and utilized a translation methodology based on the mechanistic modeling approach.

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Below is a description of how states and tribes may use this methodology to translate the egg-ovary FCV to a site-specific water-column concentration for site-specific management of selenium.

1.1 Relating the Concentration of Selenium in Tissue and Water using the mechanistic modeling approach

To relate the concentration of selenium in the eggs or ovaries of fish to the concentration of selenium in the water-column, the EPA derived the equation:

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \times EF \times CF} \qquad \textit{(Equation 9)}$$

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 function (TTF) values of the fish species that is the target of the egg-ovary FCV 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 9 are illustrated in the conceptual model shown in Figure I-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* in Equation 9 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.

Particulate material is consumed by trophic level 2 organisms (usually aquatic invertebrates) 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 fish. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer function (*TTF)*. A *TTF* is a single value that represents the steady state proportional concentration of selenium in

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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 9 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. Because the selenium criterion 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. The parameter *CF* in Equation 9 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.

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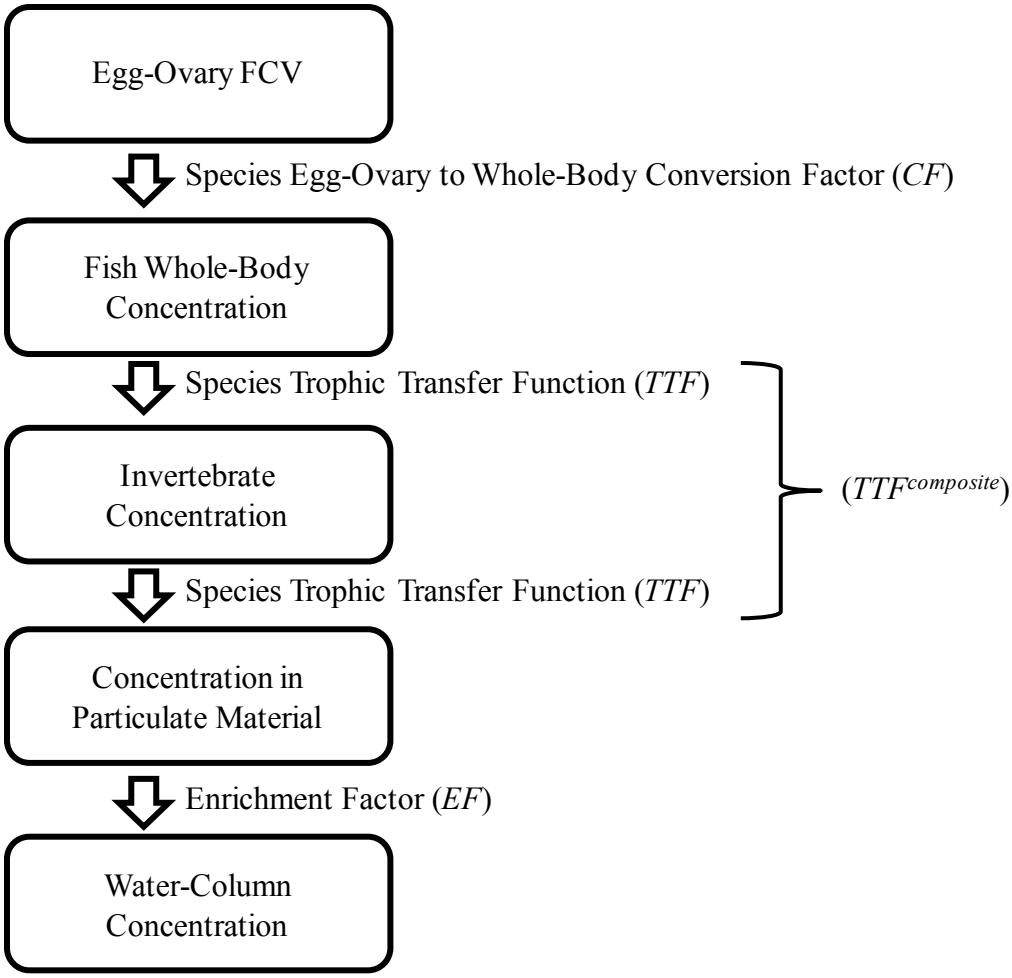

Figure I-1. Conceptual model for translating the egg-ovary FCV to a water-column concentration.

Once the parameters that quantify the transfer of selenium through each step in this pathway are identified, they can be used with Equation 9 to translate the concentration of selenium in eggs and ovaries to a concentration of selenium in the water-column.

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 as:

$$TTF^{composite} = TTF^{TL2} \times TTF^{TL3} \times \ldots \times TTF^{TLn} \qquad \textit{(Equation 10)}$$

where n is the highest trophic level.

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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:

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

where:

TTF_i^{TLx} = the trophic transfer function of the ith species at a particular trophic level

w_i = the proportion of the ith 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 I-2 illustrates four 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 9 to translate the egg-ovary FCV to a site-specific water concentration value. The general steps required to derive a site-specific translation of the egg-ovary FCV are described below.

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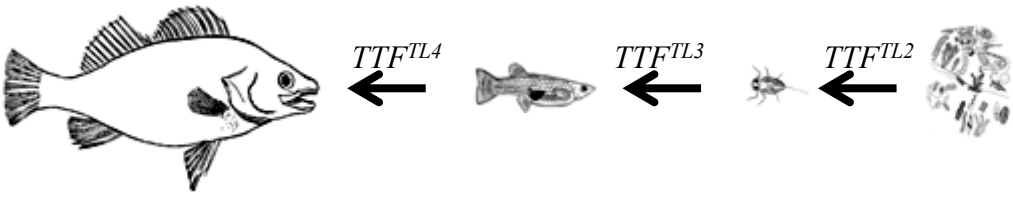
A) Three trophic levels (simple):

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

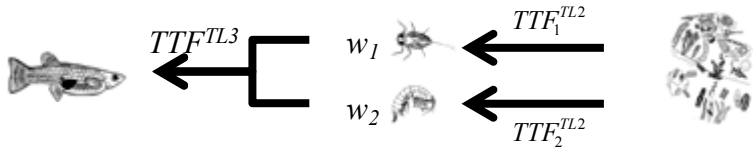
B) Four trophic levels (simple):

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



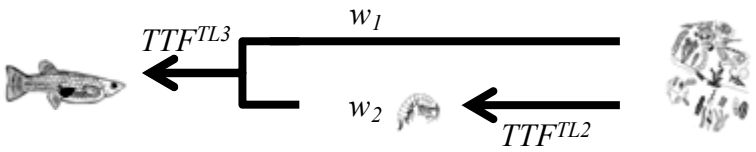
C) Three trophic levels (mix within trophic levels):

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



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):

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

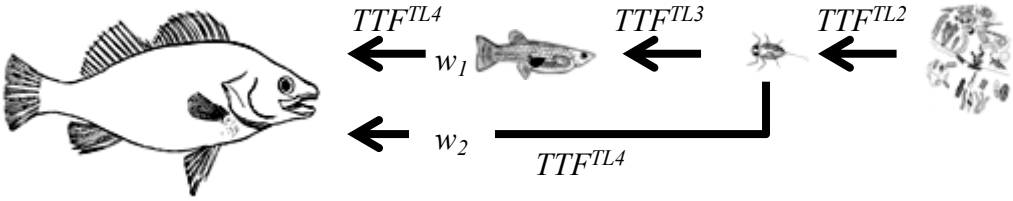


Figure I-2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios. $TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food-web model is used 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.

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1.2 Steps for deriving a site-specific water concentration value from the egg-ovary FCV

Below are the steps that can be taken to derive a site-specific water concentration value the selenium egg-ovary FCV by completing the following steps:

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, or
5) Determine the appropriate *CF* value by either,
    a. selecting the appropriate *CF* value 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 FCV into a site-specific water concentration value using Equation 9.

Below are detailed descriptions of each step followed by example calculations using a variety of hypothetical scenarios.

1.2.1 Identify the appropriate target fish species

1.2.1.1 When fish are present

The EPA's selenium criterion is expressed as a concentration in the eggs and ovaries of fish because the selenium concentration in the eggs and ovaries is the toxicological endpoint of selenium and thus the most sensitive and consistent indicator of toxicity. Nonetheless, the relationship between selenium concentration in these tissues and selenium toxicity vary across species due to differences in sensitivity and bioaccumulation potential. Therefore, states and tribes should choose the fish species that resides in the aquatic system with the greatest risk of selenium toxicity.

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The species most sensitive to selenium are those in the Salmonidae family. Thus, states and tribes should target nonanadromous species in the Salmonidae family such as trout when they are present. Members of the genus Lepomis (in the family Centrarchidae) that include bluegill are also sensitive and should be targeted when no fish in the Salmonidae family are present. Other members of the Centrarchidae family (such as bass) should be targeted if no fish of the genus Lepomis are present.

States and tribes should target nonanadromous species (species that do not migrate from salt water to spawn in fresh water), because selenium exposure and subsequent bioaccumulation occurs over a relatively long period of time through the consumption of locally contaminated aquatic organisms. If nonanadromous fish species in the Salmonidae family is absent, states and tribes should target the resident fish species likely to have the highest exposure and sensitivity to selenium. In aquatic systems with resident fish species of unknown selenium sensitivity and bioaccumulation potential, factors such as ecological significance can be factors in choosing which species to target. If the state or tribal monitoring program uses lethal tissue sampling procedures, threatened or endangered species should not be used for tissue monitoring.

Targeting fish species that consume organisms known or suspected to bioaccumulate selenium can be an alternative approach to selecting fish species when species-specific information on selenium sensitivity and bioaccumulation potential is unavailable. Prey organisms that pose a selenium toxicity risk to predators usually have physiological characteristics that predispose them to selenium bioaccumulation and/or are in close proximity to relatively high levels of selenium. For example, high levels of selenium found in San Francisco Bay white sturgeon were linked to consumption of *Potamocorbula amurensis*, a bivalve that was known to rapidly accumulate selenium and was in close proximity to selenium-contaminated sediments (Stewart et al. 2004). In contrast, striped bass from the same aquatic system had substantially lower levels of selenium due to their zooplankton-based food web with substantially lower selenium bioaccumulation characteristics (Schlekat et al. 2004; Stewart et al. 2004).

States and tribes can use data from fisheries or biological surveys or other biological assessments to determine the fish species that reside in specific surface waters. If such information is not available, general information (often online) on the fish species that are present in state or tribal surface waters can be found in:

- State Fish and Game agencies.
- U.S. Fish and Wildlife Service (http://www.fws.gov).

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- 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).

Figure I-3 shows a decision tree that states and tribes may use to help select the appropriate fish species for deriving a site-specific water concentration value from the selenium egg-ovary FCV. EPA recommends this sequence of choices on the basis of taxonomic hierarchies that begin with taxa having the highest sensitivity to selenium. The use of taxonomic hierarchies utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993).

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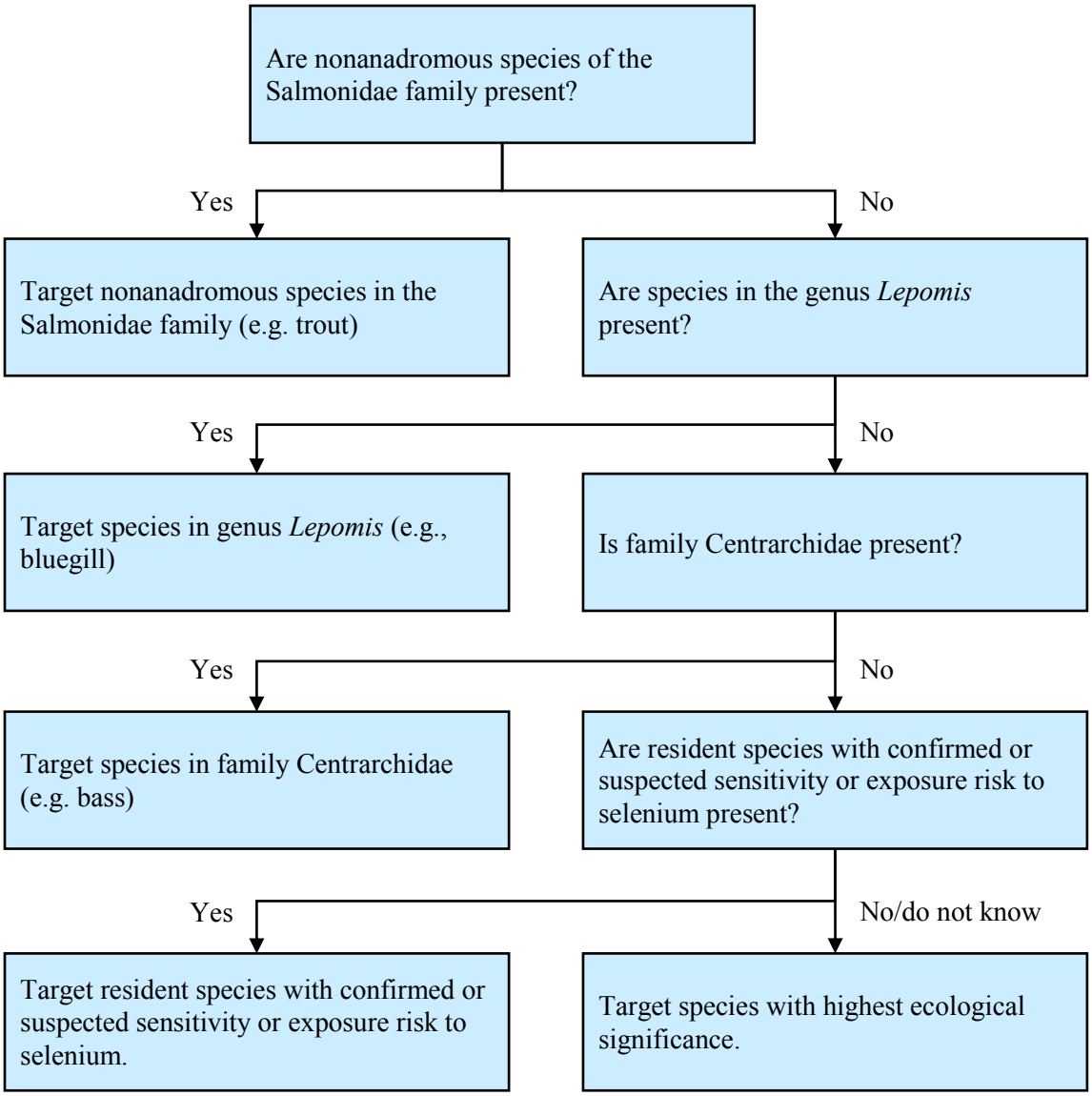

Figure I-3. Decision process for selection of the fish species to use when deriving a water concentration from the selenium egg-ovary FCV.

1.2.1.2 When fish are absent

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 naturally occurring 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.

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When fish are naturally absent from a waterbody, states and tribes should target the most sensitive fish species inhabiting downstream waters. 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 if they are transported downstream. In such cases, states and tribes may still use the decision tree in Figure J-3 to identify downstream fish species to protect. In addition, states and tribes may 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. A site-specific selenium criterion protecting a limited aquatic environment may be appropriate if selenium levels are naturally high and fish were not previously present in the aquatic system.

1.2.2 Model the food-web of the targeted fish species

After selecting the target fish species, states and tribes 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 might also be used to obtain information about its food web. The types and proportions of food organisms consumed by the targeted fish species can be directly determined through studies that examine stomach contents, or from information gathered through biological assessments. If site-specific, field-derived information is not available, the food-web characteristics of the target species can be estimated using publicly available databases such as NatureServe (http://www.natureserve.org). For example, in the HUC watershed #5040004 in Ohio, the NatureServe database record for fathead minnow 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:

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- 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). States and tribes can determine the appropriate *TTF* values to calculate $TTF^{composite}$ by either using one of the following four procedures, or by using other scientifically defensible methods.

1.2.3.1 Select the appropriate *TTF* values from a 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. TTF values for aquatic invertebrates and fish are provided in Tables J-1 and J-2 (see main text for a complete explanation of how these values were derived).

Table I-1. EPA-derived Trophic Transfer Function (TTF) values for freshwater aquati c 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*	0.390	0.720	0.220	1.28
midge	*Chironomidae*	-	-	-	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

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Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	*Zooplankton*	-	-	-	1.89

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

Table I-2. EPA-derived Trophic Transfer Function (*TTF*) values for freshwater fish

Common name	Scientific name	AE	IR	ke	TTF
Cypriniformes					
bluehead sucker	*Catostomus discobolus*	.	.	.	1.04
common carp	*Cyprinus carpio*	.	.	.	1.29
creek chub	*Semotilus atromaculatus*	.	.	.	1.12
fathead minnow	*Pimephales promelas*	.	.	.	1.57
flannelmouth sucker	*Catostomus latipinnis*	.	.	.	1.06
longnose sucker	*Catostomus catostomus*	.	.	.	0.90
sand shiner	*Notropis stramineus*	.	.	.	1.83
white sucker	*Catostomus commersonii*	.	.	.	1.18
Cyprinodontiformes					
mosquitofish	*Gambusia sp.*	.	.	.	0.97
northern plains killifish	*Fundulus kansae*	.	.	.	1.27
western mosquitofish	*Gambusia affinis*	.	.	.	1.25
Esociformes					
northern pike	*Esox lucius*	.	.	.	1.79
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	.	.	.	1.69
Perciformes					
black crappie	*Pomoxis nigromaculatus*	.	.	.	2.67
bluegill	*Lepomis macrochirus*	.	.	.	1.48
green sunfish	*Lepomis cyanellus*	.	.	.	1.27
largemouth bass	*Micropterus salmoides*	.	.	.	1.27
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	.	.	.	1.82
yellow perch	*Perca flavescens*	.	.	.	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	.	.	.	0.88
brown trout	*Salmo trutta*	.	.	.	1.44

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cutthroat trout	*Oncorhynchus clarkii*	.	.	.	1.07
mountain whitefish	*Prosopium williamsoni*	.	.	.	1.38
rainbow trout	*Oncorhynchus mykiss*	.	.	.	1.19
westslope cutthroat trout	*Oncorhynchus clarkii lewisi*	.	.	.	1.20
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	.	.	.	1.38
sculpin	*Cottus sp.*	.	.	.	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	.	.	.	0.91
channel catfish	*Ictalurus punctatus*	.	.	.	0.73

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, states and tribes should refer to other approaches to obtain an appropriate value.

1.2.3.2 Deriving *TTF* values from existing data

If one or more appropriate *TTF* values cannot be found in Tables J-1 and/or J-2, states and tribes could derive species-specific *TTF* values 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 3.

If the *TTF* value of a particular species in a food web is not available, *TTF* may be derived in several different ways. One method is to obtain the physiological coefficients of food ingestion rate (*IR*), assimilation efficiency (*AE*), or efflux rate (k_e) and apply those values to Equation 3 given as:

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

Where:

TTF	=	species-specific trophic transfer function
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 k_e may be obtained from published literature or may be derived from laboratory studies. 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

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in the food they consume using 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.

The physiological coefficients *AE*, *IR*, and k_e are species-specific values. Coefficients *AE* and k_e can only be derived from laboratory experiments, but *IR* can be derived from either laboratory or field studies. After the three physiological coefficients are obtained, a *TTF* value can be calculated using Equation 3. Another approach for deriving species-specific *TTF* values is to use paired selenium measurements of consumer organisms and their potential resources from field studies that directly measure the trophic transfer of selenium in those organisms. The *TTF* for any trophic level can be defined by the equation:

$$TTF^{TLn} = \frac{C_{tissue}^{TLn}}{C_{food}^{TLn}} \qquad \textit{(Equation I-1)}$$

Where:

TTF^{TLn} = The trophic transfer function 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.

At a given site, the empirical relationship between an organism and its food is first confirmed with linear regression analysis. If the regression is both statistically significant ($P < 0.05$) and the slope of the relationship is positive (i.e., selenium concentrations in the consumer increases with increasing selenium in food), then the data are considered acceptable and the species-specific *TTF* is determined as the median ratio of the paired consumer-food selenium concentration data. Both of the above methods were used to derive the *TTF* values provided in Tables I-1 and I-2.

1.2.3.3 Deriving *TTF* values by conducting additional studies

States and Tribes may conduct additional studies to collect the data needed to derive *TTF* values for specific needs or to revise existing *TTF* values. *TTF* values could be derived from new data using the methodology described above, or other scientifically defensible methods. If available *TTF* values do not apply to the species in a waterbody, there are no site-specific data available, and the collection of necessary data by a state is impractical; then the state could require data collection where appropriate based on Section 308 of the CWA (or comparable State authority).

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1.2.3.4 Extrapolating *TTF* values from existing values

When one or more *TTF* values are not available for a species, and the information needed to derive a species-specific *TTF* value is not available or impractical to obtain, a *TTF* value can be extrapolated from 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 this extrapolation procedure to derive the *TTF* values of some of the organisms at some representative aquatic sites that were used to derive the recommended water concentration values for lotic and lentic waters. For example, the *TTF* value for *Gila robusta*, the roundtail chub, was not listed in Table J-2. However, the roundtail chub is in the family Cyprinidae, which also includes *Pimephales promelas,* the fathead minnow. Because Cyprinidae is the lowest taxonomic classification where the fish species being considered matches a taxon in Table J-2, the *TTF* value for the fathead minnow was used for the roundtail chub. In another example, *Etheostoma exile*, the Iowa darter, is not listed in Table J-2. However, the Iowa darter is in the order Perciformes, which is common to the mangrove red snapper, striped bass, largemouth bass, and bluegill. Thus the average of the *TTF* values from those five taxa in Table J-2 was used for the Iowa darter.

1.2.4 Determine the appropriate *EF* value

The selenium enrichment function EF represents the bioavailability of selenium at the base of the aquatic food web. 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, and the biogeochemical characteristics of the waterbody. Because EF can vary greatly across waterbodies, this parameter has the greatest potential to introduce uncertainty in the translation from an egg-ovary concentration of selenium to a water concentration and should be considered carefully. States and tribes can determine an appropriate EF value either by using one of the following four procedures, or by using other scientifically defensible methods.

1.2.4.1 Deriving a site-specific *EF* value from field measurements

The parameter *EF* can be expressed as the ratio of the concentration of selenium in particulate material to the concentration of selenium dissolved in water. Using the equation

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$$EF = \frac{C_{particulate}}{C_{water}} \qquad \textit{(Equation 11)}$$

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)

Deriving a site-specific *EF* value in this manner is a relatively straightforward and inexpensive procedure. An *EF* value for a particular aquatic system can be derived by collecting water samples, separating the particulate material from the water in each sample, measuring the concentration of selenium in the separated water and particulate material, computing the ratio of the two measurements from each sample, and then calculating the mean or median of all the ratios. Alternatively, a state or tribe could derive an *EF* value by verifying the statistical relationship between paired particulate and water concentrations through linear regression, calculating the ratios of paired $C_{particulate}$ to C_{water} values, and then taking the median ratio as the value of *EF*. This approach statistically evaluates the data representing the relationship between these two media. However, a sufficient quantity of data is necessary to calculate statistically significant fits.

Regardless of the method used to derive the value of *EF* from field measurements, field and analytical methods should be carefully planned and implemented when developing a site-specific, field-derived *EF* value. 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. Thus EPA recommends a sampling plan that prioritizes areas with these characteristics. Analytical methods to measure selenium in particulate material and in water are discussed in Appendix J.

1.2.4.2 Deriving an appropriate *EF* value from existing data

If suitable and sufficient site-specific measurements of $C_{particulate}$ and C_{water} are available, states and tribes could derive an *EF* value using these data. However, states and tribes should ensure that these data represent current conditions, are based on scientifically acceptable sampling techniques, and are obtained using proper quality assurance and quality control protocols to minimize uncertainty.

1.2.4.3 Extrapolating from *EF* values of similar waters

In circumstances where a site-specific, field-derived *EF* value is not available, an *EF* value from one or more aquatic systems with similar hydrological, geochemical, and biological characteristics could be used

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to approximate *EF*. However, states and tribes should carefully consider the possibility of introducing significant uncertainty into the calculation when using *EF* values extrapolated from other waterbodies. States and tribes should not use *EF* values derived from large-scale sites that encompass multiple water bodies or ecosystems, or that do not match the characteristics of the waterbody for which the water column concentration of selenium is being derived.

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 14 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 I-3).

Table I-3. Whole Body Se to Egg-Ovary Se Conversion Factors (*CF*)

Common name	Scientific name	CF
Cypriniformes		
bluehead sucker	Catostomus discobolus	1.82
Common carp	Cyprinus carpio	1.92
flannelmouth sucker	Catostomus latipinnis	1.41
razorback sucker	Xyrauchen texanus	1.43
roundtail chub	Gila robusta	2.07
White sucker	Catostomus commersonii	1.41
Esociformes		
northern pike	Esox lucius	2.39
Perciformes		
bluegill	Lepomis macrochirus	2.13
green sunfish	Lepomis cyanellus	1.45
smallmouth bass	Micropterus dolomieu	1.42
Salmoniformes		
brook trout	Salvelinus fontinalis	1.38
brown trout	Salmo trutta	1.45
cutthroat trout	Oncorhynchus clarkii	2.30
Dolly Varden	Salvelinus malma	1.61
mountain whitefish	Prosopium williamsoni	7.39

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Common name	Scientific name	CF
rainbow trout	Oncorhynchus mykiss	2.44

The data and methods used to derive *CF* for these species are described in Appendix B.

1.2.5.2 Deriving a *CF* value from existing data

The parameter *CF* is mathematically expressed as:

$$CF = \frac{C_{egg-ovary}}{C_{whole-body}}$$

(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 (mg/kg).

If suitable and sufficient data are available, a state or tribe could derive a species-specific *CF* value using the same numerical methods used to calculate the parameter *EF*. A state or tribe could calculate the ratio of the two concentrations in each sample tissue as defined in Equation 13, and then calculate the mean or median of all the ratios. Alternatively, a state or tribe could derive a *CF* value by verifying the statistical relationship between paired particulate and water concentrations through linear regression, calculating the ratios of paired $C_{egg\text{-}ovary}$ to $C_{whole\text{-}body}$, and then taking the median ratio of the paired values as the *CF* (see Appendix X). This approach statistically evaluates the data representing the relationship between these two tissue types. However, a sufficient quantity of data is necessary to calculate statistically significant fits. Regardless of the method used, care should be taken to ensure that the data used accurately represents current conditions, were based on scientifically acceptable sampling techniques, and were obtained using acceptable quality assurance and quality control protocols.

1.2.5.3 Deriving a *CF* value by conducting additional studies

States and tribes could perform additional studies to obtain the 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. Analytical methods to measure selenium in tissue are discussed in Appendix J. Where appropriate, additional data could be obtained as part of a NPDES permit application by invoking authority under CWA section 308 (or comparable State authority) to reasonably require NPDES-regulated facilities to collect information necessary to develop NPDES permit limits.

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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.

Because the pattern of selenium concentration in different body tissues varies between species, extrapolating a species-specific *CF* value from one or more surrogate species is not recommended. However, a *CF* value that is the average of all known species-specific *CF* values could be used when the *CF* value of the target species is not available, and the data needed to derive a species-specific *CF* value is not available or impractical to obtain. Using the average species *CF* value, however, lowers translation accuracy and should only be used when other species-specific options are not available.

1.2.6 Translate the selenium egg-ovary FCV into a site-specific water concentration value using Equation 18.

After determining the appropriate values of *CF*, $TTF^{composite}$ (derived from the product of the individual *TTF* values from each trophic level) and *EF*, a site-specific water concentration can be derived from the egg-ovary FCV using Equation 18. 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 18 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 is 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

Derivation of a water concentration from the egg-ovary FCV using the mechanistic bioaccumulation modeling approach (Equation 18) is subject to uncertainties from several sources. These include:

- Measurement error when deriving input parameters.
- Unaccounted factors affecting bioaccumulation.
- Inaccurate identification or proportions of trophic level 2 food-web organisms.
- Inaccurate or inappropriate *TTF*, *EF*, or *CF* values.
- Biological variability.
- Other unknown factors.

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Though not required, the effectiveness of effluent limits and waste load alsites of selenium that are based on water concentration values derived from the egg-ovary FCV should be confirmed whenever practical using appropriate fish tissue assessment methods. In addition, comparing estimated selenium concentrations in fish tissue with actual selenium concentrations obtained from small-scale field studies could also help evaluate the suitability of selected equation parameters.

1.4 Example calculations

Below are six hypothetical examples that demonstrate how to calculate a selenium water concentration from the egg-ovary FCV using Equation 18. These examples derive water concentration values for a variety of hypothetical aquatic systems with various fish species and food webs. For these hypothetical examples, species-specific *TTF* were taken from Tables 6 and 7 in the main text, and *CF* values were taken from Table 8 from the main text. 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 function for bluegill (TTF^{TL3})	1.48
Trophic transfer function for amphipods (TTF^{TL2})	1.22
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.2

EF = $\frac{C_{particulate}}{C_{water}}$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{combined}\ x\ EF\ x\ CF}\ (Equation\ 18)$$

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

$$= 1.48 \times 1.22$$

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= 1.81

$$C_{water} = \frac{15.2}{1.81 \times 0.85 \times 2.13}$$

= 4.64 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 function for fathead minnow (TTF^{TL3})	1.57
Trophic transfer function for copepods (TTF^{TL2})	1.41
Egg-ovary to whole-body conversion factor for fathead minnow (species-specific value not available, so median CF for family Cyprinidae is used) (CF)	2.00
Selenium egg-ovary FCV (mg/kg)	15.2

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

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{combined}\ x\ EF\ x\ CF} \quad (Equation\ 18)$$

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

= 1.57 × 1.41

= 2.21

$$C_{water} = \frac{15.2}{2.21 \times 0.85 \times 2.00}$$

= 4.14 µg/L

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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 function for Bluegill (TTF^{TL3})	1.48
Trophic transfer function for aquatic insects (median of Odonates, Water boatman, Midges, and Mayflies) (TTF^{TL2})	1.69
Egg-ovary to whole-body conversion factor for Bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.2

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

$$EF = \frac{8.75}{5.00}$$

= 1.75 L/g

$$\boldsymbol{C_{water} = \frac{C_{egg-ovary}}{TTF^{combined}\ x\ EF\ x\ CF}} \quad \textbf{(Equation 18)}$$

$TTF^{combined}$ = $TTF^{TL3} \times TTF^{TL2}$
= 1.48 x 1.69
= 2.50

$$\boldsymbol{C_{water} = \frac{15.2}{2.50 \times 1.75 \times 2.13}}$$

= 1.63 µg/L

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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 function for fathead minnow (TTF^{TL3})	1.57
Trophic transfer function for copepods and aquatic insects (TTF^{TL2}) Copepods = 1.41 Average of all aquatic insects = 1.69 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i \times w_i\right)$ = (1.41 × ⅔) + (1.69 × ⅓) = 1.50	1.50
Egg-ovary to whole-body conversion factor for fathead minnow (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	2.00
Selenium egg-ovary FCV (mg/kg)	15.2

$$\boldsymbol{EF = \frac{C_{particulate}}{C_{water}}} \quad \textbf{(Equation 12)}$$

$$\boldsymbol{EF = \frac{4.25}{5.00}}$$

= 0.85 L/g

$$\boldsymbol{C_{water} = \frac{C_{egg-ovary}}{TTF^{combined}\ x\ EF\ x\ CF}} \quad \textbf{(Equation 18)}$$

$TTF^{combined} = TTF^{TL3} \times TTF^{TL2}$
= 1.57 × 1.50
= 2.36

$$\boldsymbol{C_{water} = \frac{15.2}{2.36 \times 0.85 \times 2.00}}$$

= 3.79 µg/L

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1.5.5 Example 5

Fathead 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 function of fathead chub: Lowest matching taxon is the family Cyprinidae. Therefore, the TTF value of Cyprinidae is used (TTF^{TL3})	1.43
Trophic transfer function for insects (TTF^{TL2}) Average of all aquatic insects = 1.69	1.69
Egg-ovary to whole-body conversion factor for fathead chub (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	2.00
Selenium egg-ovary FCV (mg/kg)	15.2

$$\boldsymbol{EF = \frac{C_{particulate}}{C_{water}}} \quad \textbf{(Equation 12)}$$

$$\boldsymbol{EF = \frac{4.25}{5.00}}$$

= 0.85 L/g

$$\boldsymbol{TTF^{combined} = \left[TTF^{TL3} \times TTF^{TL2} \times w_1\right] + \left[TTF^{TL3} \times w_2\right]}$$

Where:
w_1 = Proportion of fathead chub diet from insects; and
w_2 = Proportion of fathead chub diet from algae

$$\boldsymbol{TTF^{comb} = [1.43 \times 1.69 \times 0.8] + [1.43 \times 0.2]}$$
= 2.22

$$\boldsymbol{C_{water} = \frac{15.2}{2.22 \times 0.85 \times 2.00}}$$
= 4.03 µg/L

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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 function of largemouth bass (TTF^{TL4})	1.27
Trophic transfer function of Western mosquitofish (TTF^{TL3})	1.25
Trophic transfer function for insects and crustaceans (TTF^{TL2}) Median all Insects – 1.69 Median all Crustaceans - $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i^{TL2} w_i\right)$ = (1.69 x ¾) + (1.41 x ¼) = 1.62	1.62
Egg-ovary to whole-body conversion factor for Largemouth bass (species-specific value not available, so median for the genus Micropterus used) (CF)	1.42
Selenium egg-ovary FCV (mg/kg)	15.2

$$\boldsymbol{EF = \frac{C_{particulate}}{C_{water}}} \quad \textbf{(Equation 12)}$$

$$\boldsymbol{EF = \frac{4.25}{5.00}}$$

= 0.85 L/g

$TTF^{combined} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$
$= 1.27 \times 1.25 \times 1.62$
$= 2.57$

$$\boldsymbol{C_{water} = \frac{15.2}{2.57 \times 0.85 \times 1.42}}$$

= 4.90 µg/L

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1.5.7 Example 7a.

Derivation of a site specific water column criterion for a river impacted by selenium.
Available data for a site indicates 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 15.2 mg/kg (dw). The allowable selenium water column criterion for this lotic waterbody is 4.8 ug/L. The following calculation shows how to derive a water column concentration that would achieve the 15.2 mg/kg (dw) egg/ovary tissue criterion.

Current selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.2
Selenium Water Column Criterion, Lotic Habitats (ug/L)	4.8
Allowable lotic water column concentration (ug/L)	X

1. Set up proportional equation to solve for allowable water column concentration

$$= \frac{\text{Lotic Water Column Criterion}}{\text{Allowable Water concentration (X)}} = \frac{\text{Current egg/ovary FT concentration}}{\text{Selenium egg/ovary criterion}}$$

$$= \frac{4.8 \text{ ug/L}}{X} = \frac{22 \text{ mg/kg dw}}{15.2 \text{ mg/kg dw}}$$

$$X = \frac{4.8 \times 15.2}{22} = \frac{72.96}{22}$$

$$X = 3.32 \text{ ug/L} = \text{Target Site specific Lotic Water Column Criterion}$$

1.5.7 Example 7b.

Derivation of a site specific water column criterion for a lake impacted by selenium.
Available data for a site indicates 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 15.2 mg/kg (dw). The allowable selenium water column criterion for this lentic

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waterbody is 1.3 ug/L. The following calculation shows how to derive a water column concentration that would achieve the 15.2 mg/kg (dw) egg/ovary tissue criterion.

Current selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.2
Selenium Water Column Criterion, Lentic Habitats (ug/L)	1.3
Allowable lotic water column concentration (ug/L)	X

2. Set up proportional equation to solve for allowable water colun concentration

$$= \frac{\text{Lentic Water Column Criterion}}{\text{Allowable Water concentration (X)}} = \frac{\text{Current egg/ovary FT concentration}}{\text{Selenium egg/ovary criterion}}$$

$$= \frac{1.3 \text{ ug/L}}{X} = \frac{22 \text{ mg/kg dw}}{15.2 \text{ mg/kg dw}}$$

$$X = \frac{1.3 \times 15.2}{22} = \frac{19.76}{22}$$

X = 0.89 ug/L = Target Site specific Lentic Water Column Criterion

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:

$$BAF = \frac{C_{tissue}}{C_{water}} \qquad \textit{(Equation I-2)}$$

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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)

Solving for C_{water}:

$$C_{water} = \frac{C_{tissue}}{BAF} \qquad \textit{(Equation I-3)}$$

To translate a fish tissue criterion to a water concentration value, states and tribes could develop a site-specific, field-measured BAF for the waterbody, and then calculate a water concentration criterion using Equation J-3. 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.

2.2 Managing uncertainty using the BAF approach

Considerable 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. 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.

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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.

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 time and cost necessary to collect sufficient data to establish the relationship between tissue and water concentrations. Costs increase as the spatial scale and complexity of the aquatic system

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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.

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 J-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

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	Site-specific
Fish tissue sampling not required for translation	Fish tissue sampling required

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APPENDIX J: Analytical Methods for measuring Selenium

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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.

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.

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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[3] for the purposes of implementing a selenium water quality criterion are listed below (Table J-1).

Table J-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

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:

3For 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.

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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.

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.

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.

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 3114 B 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:

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- 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.

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.

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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.

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.

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:

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- 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).

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APPENDIX K: Abbreviations

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Reference and site abbreviations

Reference	Site		Species	
Bi:	22	Miller's Lake, Wellington CO	FM	Fathead minnow
Birkner 1978	27	Sweltzer Lake, Delta CO	FM	Fathead minnow
	23	Twin Butter 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 Butter 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	Sweltzer Lake, Delta CO	NPK	Northern plains killfish
	23	Twin Butter 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
	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

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Reference	Site		Species	
	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 sunfish
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	RSh	Red sunfish
	SJ1	San Juan R. at Four Corners	RSh	Red sunfish
	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

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Reference	Site		Species	
	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 and	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
	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

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Reference	Site		Species	
HB: Hamilton and Buhl 2004	LEMC	Lower East Mill Creek	CT	Cutthroat trout
Le: Lemly 1985	BA	Badin Lake	BB	Black bullhead
	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
	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: Saiki and Lowe 1987	KP11	Kesterson Pond 11	WM	Western mosquitofish
	KP2	Kesterson Pond 2	WM	Western mosquitofish
	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: Saiki et al. 1993	GT4	Salt Slough at San Luis Wildlife Refuge	Bg	Bluegill
	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

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Reference	Site		Species	
	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

Reference and site abbreviations

Reference	Site		Species	
Bi:	22	Miller's Lake, Wellington CO	FM	Fathead minnow
Birkner 1978	27	Sweltzer Lake, Delta CO	FM	Fathead minnow
	23	Twin Butter 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 Butter 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	Sweltzer Lake, Delta CO	NPK	Northern plains killfish
	23	Twin Butter 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

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Reference	Site		Species	
Bu93: Butler et al. 1993	SP2	Spring Creek at La Boca	BhS	Bluehead sucker
	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
	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: Butler et al. 1995	ME2	McElmo Cr., downstream from Alkali Canyon	BhS	Bluehead sucker
	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
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	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

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Reference	Site		Species	
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	GnS	Green sunfish
	ME4	McElmo Cr., downstream from Yellow Jacket Canyon	RSh	Red sunfish
	ME3	McElmo Cr., upstream from Yellow Jacket Canyon	RSh	Red sunfish
	SJ1	San Juan R. at Four Corners	RSh	Red sunfish
	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
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Butler et al. 1997	MNP2	Large pond south of G Road, southern Mancos Valley	FM	Fathead minnow
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	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
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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
	CC-1A	Crow Creek – 1A	Sc	Sculpin
	CC-3A	Crow Creek – 3A	Sc	Sculpin

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Reference	Site		Species	
	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: Grasso et al. 1995	17	Arapahoe Wetlands Pond	FM	Fathead minnow
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	BA	Badin Lake	CC	Common carp
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	HR	High Rock Lake	CC	Common carp
	BA	Badin Lake	FM	Fathead minnow
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	BA	Badin Lake	GnS	Green sunfish
	BE	Belews Lake	GnS	Green sunfish
	HR	High Rock Lake	GnS	Green sunfish
	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

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Reference	Site		Species	
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
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Sa93:	GT4	Salt Slough at San Luis Wildlife Refuge	Bg	Bluegill
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	SJR2	San Joaquin R. above Hills Ferry Rd.	Bg	Bluegill
	SJR3	San Joaquin R. at Durham Ferry Recreation Area	Bg	Bluegill
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St:	M4720	Marsh 4720	BB	Black bullhead
Stephens et al. 1988	M4720	Marsh 4720	CC	Common carp